Concepts in Animal Parasitology

Scott L. Gardner and Sue Ann Gardner
Editors

Above: Students in the laboratory with teaching assistant Sebastian Botero-Cañola (center) during the University of Nebraska–Lincoln (UNL) Field Parasitology class taught each year at UNL's Cedar Point Biological Station in rural Ogallala, Keith County, Nebraska, United States, 2019. Source: S. L. Gardner. License: CC BY-NC-SA 4.0.

Front cover: Students from the University of Nebraska–Lincoln (UNL) Field Parasitology class taught each year at UNL's Cedar Point Biological Station in rural Ogallala, Keith County, Nebraska, United States as they search for intermediate host animals, such as snails and other invertebrates, in an around the cattle tanks at Arapahoe Prairie field site in nearby Arthur County, 2018. Source: S. L. Gardner. License: CC BY-NC-SA 4.0.

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University of Nebraska Lincoln

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Preface

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IMPETUS FOR PREPARING THIS BOOK

The United Nations (UN) has declared education as a basic human right. One of the UN's sustainable development goals is a call to ensure "inclusive and equitable quality education and promotion of lifelong learning opportunities for all" (United Nations, 2023; see also WOERC, 2012). Depending on the specifics of their implementation, financing, and dissemination models, open educational resources (OERs) have the potential to help in the effort to achieve equitable learning across the globe (Orr et al., 2015; Lee and Lee, 2021; see also Bali et al., 2020).

Open educational resources are "teaching, learning, and research materials in any medium that reside in the public domain or have been released under an open license that permits their free use and re-purposing by others" (Creative Commons, 2014). Wiley (2020) cites the Creative Commons' framing of OERs as providing explicit permission to "retain, re-use, revise, remix, and redistribute" openly-accessible educational material.

Aside from the obvious benefit of saving students money, OERs have been shown to promote equity among students. Their use has been shown to contribute to maintenance or improvement of student success, especially with respect to retention in school, course completion, grade point average, and subsequent educational attainment (Colvard et al., 2018; Griffiths et al., 2022; Fischer et al., 2015).

HOW TO USE THIS BOOK

Scope

This is a textbook covering concepts in animal parasitology. It is meant to be used by students, teachers, professors, researchers, and members of the public who are interested in learning about animal parasite biology, systematics, taxonomy, zoogeography, and ecology. The primary intended audience is upper-level undergraduate or graduate university students who have knowledge of basic biology and, particularly, basic animal biology.

Organization of the Book

This textbook was conceived to fill a gap in educational materials about parasitology. One of the main goals in both teaching and learning about parasites and parasitology is to understand the diversity of parasites and of parasitism as a way of life on Earth. With this in mind, the editors made a decision to treat the organization of the book as though led by the organisms themselves—a sort of bottom-up approach—and present the parasitic organisms as a parasitologist will first find them in nature, as in: Where they tend to exist in relation to their host, and more specifically, whether inside or outside the host animal. Therefore, the book includes sections covering a few taxonomic groups representing just some of the millions of extant endoparasite (Greek: **endo** = inside; **para** = beside; **sitos** = food) and ectoparasite (Greek: **ektos** = outside) species.

Examples of endoparasites are parasitic trematodes or nematodes that live inside the respiratory systems or gastrointestinal tracts of their hosts. Ectoparasites include lice and ticks, almost all fleas, many mites, a few platyhelminths that live on echinoderms, and even some chordates like the lamprey and vampire bat. Some groups of animals, such as monogeneans and mites, are not neatly categorized and may live part of their lives as endoparasites and part of their lives as ectoparasites or as free-living animals. Despite these myriad variations, the editors believe that the basic division between endo- and ecto- serves well enough to organize the chapters.

In approaching the organization in this way, the focus of the book is primarily at the level of species and other lower level taxonomy as opposed to higher-level groupings which are notoriously constantly in flux. The classification of parasites based on phylogenies is useful and necessary to understand the diversity, diversification, and evolution of parasites, but classification does not dictate the book's primary organization. Instead, the concept of biodiversity of parasites and their animal hosts is the main factor that motivates the research and teaching in the Harold W. Manter Laboratory of Parasitology (University of Nebraska State Museum, Lincoln, Nebraska, United States) where editor Scott L. Gardner conducts his work. It is this push toward understanding biological diversity of parasites that overarchingly informs the organization of this book.

Note about Bibliographical References

The citations in the book are formatted to promote finding usable copies, they are not meant to serve as an archival resource. As such, and to save space, only the first four authors are listed for each resource. A digital object identifier (doi) is included whenever one could be found; but the dois are not

hot linked since these links would often take readers to paywalled versions. Readers are encouraged instead to attempt to locate free, legal versions of the resources included in the references whenever possible. For example, free-to-read versions (and sometimes also open access versions) of the papers may be available in institutional repositories, on authors' personal websites, or from academic social media sites.

Note about Images

When selecting images, the editors relied on the guidelines included in Egloff et al. (2017) regarding copyrightability of images that serve as biodiversity data. Beyond this broad framework to guide selection, the images in the book were chosen ultimately based on the following criteria: Conceptual applicability, quality, allowable copyright and permissions, and (for human subject images) an acceptable declaration of informed consent (see Roguljić and Wager, 2020). Due to the constraints of these criteria, there are several sections in the book that are lightly illustrated. Where images are sparse or lacking, instructors are encouraged to insert their own images or select images from other sources, including those used under applicable fair use/fair dealing or educational use guidelines.

Accompanying Glossary

A supplemental glossary is in the process of preparation. Until the glossary is completed, a work that may be used in its stead for many of the terms found in the book is the Dictionary of Invertebrate Zoology (Maggenti et al., 2017) available online for free: https://digitalcommons.unl.edu/zeabook/61/

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Disclaimers

Although students of pre-medical studies, medical studies, or veterinary studies may use this text to learn foundational concepts in animal parasitology, it is not a medical or veterinary text. Further, it is not meant for any medical- or veterinary-related purposes whatsoever. When medical or veterinary topics are touched upon in the text, this is for educational purposes for those studying or interested in the biological sciences generally. *No medical or veterinary advice of any kind is offered or implied anywhere in this textbook*. *No* medical or veterinary diagnoses, treatments, or conclusions of any kind may be construed using the knowledge offered herein.

For studies specifically related to medical parasitology, readers may consult any of a number of qualified texts in the subject, including Medical Parasitology: A Textbook (Mahmud et al., 2017), Medical Parasitology (Satoskar, 2009), and Modern Parasitology: A Textbook of Parasitology, 2nd edition, (Cox et al., 2009), among others. Numerous medical periodicals are also appropriate sources of knowledge about medical parasitology. For medical diagnoses, qualified practitioners of medicine may be consulted directly.

For studies specifically related to veterinary parasitology, readers may consult any of a number of qualified texts in the subject, including Veterinary Parasitology, 4th edition, (Taylor et al., 2015) and Georgis' Parasitology for Veterinarians, 11th edition, (Bowman, 2020), among others. Numerous veterinary parasitology periodicals are also appropriate sources of knowledge about veterinary parasitology. For veterinary diagnoses, qualified practitioners of veterinary medicine may be consulted directly.

Invitation to Review and Give Feedback

If any qualified readers would like to serve as a reviewer for any of the sections, you are invited to please contact one of the editors to discuss the possibility of being assigned the task of reviewing. You will be credited in revisions if you

ultimately serve as a selected reviewer. In addition, if readers discover factual or typographical errors in the content, please contact one of the editors.

HOW THE BOOK WAS DEVELOPED

Origin of the Book

The concept for this book arose in 2018 around the time there was a concerted push to create open educational resources in universities (Austin, 2018; Sennott et al., 2015). This push seemed well-timed to the editors. In fact, the rising costs of textbooks has become a major problem for students to the point where it is basically untenable to expect students to pay for them anymore. The editors reasoned that it would be a good time to call on their esteemed and accomplished colleagues in academia to help create a new textbook in a massively collaborative endeavor, if they were willing to participate.

Also driving the idea of a new textbook, the seminal English-language parasitology textbook of our time, Gerald R. Schmidt and Larry S. Roberts' Foundations of Parasitology, 9th edition (Roberts et al., 2012), has recently gone out of print and there are no plans to update it. John J. Janovy, Jr., the lead author of the last several editions of the Schmidt and Roberts book, agreed that the creation of a new textbook was a good and timely idea.

Contributing to the decision to attempt the creation of a large-scale textbook project was the public access/open access platform available to the editors, namely, the Zea Books imprint of the University of Nebraska–Lincoln Libraries. In line with the OER ethos driving the creation of the content, this publishing imprint operates under a diamond open access model, such that neither the authors nor the readers have to pay to publish nor to read any work published as a Zea Book.

Development of the Book

At the time of the conception of the book idea, the editors capitalized on the availability of visiting scholars in the Harold W. Manter Laboratory of Parasitology (Lincoln, Nebraska, United States)—Griselda Pulido-Flores, Scott Monks, and Donald Gettinger, as well as local colleagues John J. Janovy, Jr. and Gabor Rácz, and student-colleagues Auggie Tsogtsaikhan Dursahinhan and Guin Drabik—and called together a couple of meetings to discuss their idea with the group. They asked them to envision what they would like to see in a new textbook, one that would be available online for anyone with a computer connection to access for free. Among many other good ideas they shared, they suggested that the book could possibly include numerous links to other sources and interactive modules, and pointed out that the information may be kept more current than was possible with a printed volume. Colleagues Paul Royster, Linnea Fredrickson, Catherine Fraser Riehle, and Mary Bolin in the University of Nebraska–Lincoln Libraries (Lincoln, Nebraska, United States) also provided encouragement and expertise that helped the project on its way.

When preparing to solicit manuscripts for this project, based on the preliminary conversations with colleagues, the editors first prepared an outline of the concepts desired to have covered and then created streamlined style requirements (the instructions for authors and references style guide are available online here: https://digitalcommons.unl.edu/parasittext/). They then asked numerous colleagues—all experts in their subareas of parasitology—to contribute one or more sections based on the outline. So many of them agreed to write sections that it seemed that it really might be possible to create a high-quality work with the input of so many fine experts. Every one of them submitted manuscripts quickly.

The editors gave the authors quite a bit of latitude regarding how to approach their assignment to write sections. They provided an optional template to work from (available here), but use of this format was optional. They wanted the authors to be able to express themselves in the way they each felt was best to demonstrate knowledge of their respective areas of interest within the larger subject of animal parasitology. This liberal approach naturally resulted in some variation in presentation styles, which is perhaps a plus for the reader. It breaks up the tone and emphases from section to section, and the reader gets a sense of each author's different voice and approach. The editors have worked to retain much of each author's preferred style of presentation, but with normalizing of typography and other style elements to help the manuscript finally cohere as a unified whole.

Some of the sections were sent out for review. This review process was open, so the authors knew who was reviewing their work and the reviewers were aware that the authors knew they were reviewing. Reviewed sections are marked as such with the reviewer's name and affiliation. Whether reviewed or not, all of the sections were editor-reviewed by both editors: Sue Ann Gardner edited primarily for bibliographic details and style elements, and Scott L. Gardner edited primarily for content.

Delayed Publication

With best-laid plans, the editors started to review and edit the sections as soon as they were submitted. Then a great number of both quite-dire and less-dire issues arose that interfered with the ability to complete the editing and production in as timely a manner as intended (selected challenges

include: The SARS-CoV-2 pandemic requiring remote teaching, a computer crash, a death in the family that then required weeks away from work and home, radical changes in administrations at the university, and other issues). With those issues finally receding in impact, five years after the project began, the book will be published at long last.

Demographic Data About the Authors

With editor Scott L. Gardner's large network of expert parasitologist colleagues, it was possible to seek out scholars who are experts in their field. While the first consideration when deciding who to invite to participate was expertise, the editors further worked toward the desired goal of equity and inclusion in the selection of authors. One result was a 1:2 ratio of women to men. While this does not represent parity, it is an improvement over days past when the majority of authors would likely have been men. Another result of efforts at equity and inclusion was the participation of many authors from outside the United States. Approximately 40% of authors are US-American and the remaining 60% are from one of 14 other countries (Argentina, Brazil, Australia, Japan, Mongolia, Bulgaria, Czechia, Germany, Hungary, Norway, Russia, Spain, Mexico, or Canada). Almost half of the authors (44%) do not have English as their first language.

Spanish-Language Version

In late 2018, the Office of the President at the University of Nebraska–Lincoln (Lincoln, Nebraska, United States) issued a call for proposals for Inclusive Excellence Development at the university. The editors were awarded funds to go toward translation of the textbook. With this, the editors partnered with a local professor of Spanish-language translation, Yoanna Esquivel Greenwood, who has created Spanish-language versions for numerous chapters in the book. Thanks to her work, and perhaps with the added input of some of the Spanish speakers among the authors, a comprehensive Spanish-language translation is forthcoming.

Acknowledgement of Authors' Contributions

From the Editors, Scott L. Gardner and Sue Ann Gardner

We sincerely thank all of the authors of this collaborative work. Your excellent contributions and dedication to the advancement of knowledge of animal parasitology have the potential to positively change the lives of countless students and teachers worldwide.

While we were grappling with challenges and distractions that delayed the editing of the manuscript of this book, we lost a few of our esteemed author colleagues. We wish to posthumously acknowledge Bernie Fried, Akira Ito, and Robin M. Overstreet for what turned out to be some of their truly late-career contributions. We miss them, and we feel so fortunate to have benefitted from their long-acquired knowledge and their willingness to join in on this project.

Dedication

From the Editors, Scott L. Gardner and Sue Ann Gardner

This book is dedicated to **all** of our academic forebears and mentors who made this effort possible—some of whom are authors* of sections of the book! We can't list everyone, but we can provide a truncated list to commemorate some people especially.

Sydney Anderson
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Part I

INTRODUCTORY CONCEPTS

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1

Introduction to Animal Parasitology

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Chapter 1

Introduction to Animal Parasitology

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Introduction

One of the most fascinating things that a person can experience in the complex realm of biology is the discovery of an animal living inside another animal. If this discovery takes place at an early enough stage in the development of a young person's view of the world, that is, before the rules and regulations of what of society thinks, and before what is good and what is bad are perfused into a learner's mind, the first discovery of living-motile trematode worms living inside the lungs of a frog or of tapeworms inhabiting the gut of a rodent can be exhilarating and a positively unforgettable experience. The questions that arise when these kinds of animals are encountered for the first time are innumerable and, if answered carefully and perhaps fully, may lead to more and more questions, and hopefully, more and more answers.

Many students of biology first begin to investigate parasites and parasitism via the initial study of the ecology, behavior, or systematics of a species of a free-living organism. That is, the free-living animal (pick your favorite species) is being studied for any of a myriad of reasons and during the investigations, those doing the work discover that there may be several species of parasites occurring in or on (or, more likely, both) their study animals. This discovery can occur for other reasons not related to **parasitology** at first, but then leads to investigation of parasitism.

True **parasitologists**—those who are intrigued with the intimate associations of parasites and are interested in the biology of the parasite itself—may become intensely focused on a single group, like tapeworms of rodents or gregarines of beetles or damselflies, for instance. Other students of parasitology may focus on the complete endoparasite fauna of a group of insects, fish, mammals, birds, amphibians, or reptiles. It is not unusual for a parasitologist to spend their whole career studying a single group of parasites pretty much to the exclusion of other parasites, as did Odile Bain, who worked on filarioid nematodes (phylum Nemata: superfamily Filarioidea) and Marie Claude Durette-Desset who works on trichostrongyloids (phylum Nemata: superfamily Trichostrongyloidea), both in the Laboratoire des vers, French National Museum of Natural History. Another example of a working parasitologist is Donald W. Duszynski from the University of New Mexico, who followed the path initially laid out by his mentor, William C. Marquardt at Colorado State University. Duszynski chose to focus the bulk of his entire career on protozoan parasites called the coccidia.

Humans—Including Scientists—Beginning to Notice Parasites

Even though the recognition of parasites and of parasitism had a recorded beginning in ancient Greece and China (Hoeppli, 1959), there is no doubt that parasites were known as part of the natural fauna by the earliest of peoples. For example, in the early 1950s, the nomadic Nunamiut Eskimo hunters in the Brooks Range of Alaska knew of and routinely recognized the strobilar (adult) stages of cestodes in the intestines of carnivores and other mammals and they recognized the larval stages of the cestodes in the viscera of the caribou that they prepared and used for food and shelter (Robert L. Rausch, personal communication; Rausch, 1993).

The first studies of parasites of animals and resulting scientific publications started during the late 1700s and early 1800s with formal publications by Johann Gottfried Bremser, Carl Asmund Rudolphi, Karl Moriz Diesing, Raphaele Molin, A. F. Schneider, R. von Drasche, Peter Simon Pallas (shown in Figure 1), Karl Theodor Ernst von Siebold, Johann August Ephraim Goeze, Karl Georg Friedrich Leuckart, Constantine Janicki, Otto von Linstow, and others. Much of the work that was originally published by Molin and Rudolphi originated from the collections made by Johann Natterer (see Guerrero, 2021) and Hermann von Ihering (see Klassen, 1992; Brooks and McLennan, 2002) during collecting expeditions into the

Figure 1. Portrait of Peter Simon Pallas. Source: Artist, Ambroise Tardieu; reproduced by Raikov, 1952; digitized by Kouprianov, 2006. Public domain.

Amazon region of Brazil. In the late 1800s, Leuckart trained many **helminthologists** in his parasitology laboratory in Leipzig, Germany including Henry Baldwin Ward and others.

As scientific knowledge of the natural world increased during the early 1800s, studies of the natural history of parasites produced increasing numbers of publications. Scientists wanted to know what these animals were and how they got where they were being discovered. It was soon revealed that some parasites had very complex **life cycles** and that parasites were extremely common in nature. Through time, as students of parasite diversity studied **transmission patterns**, **life histories**, and **pathologies**, and then much later, researchers put these together in **phylogenies**, more knowledge was generated that enabled new testable ideas to develop in ecology and evolution (Brooks and McLennan, 2002). The development of the ecological and evolutionary ideas that used parasites as indicators of both biogeographical and ecological relationships was aptly named **parascript** by Harold Winfred Manter (1966). Manter's research program in parasite **systematics** was foundational in the field of parasitology for the subsequent development of parasite phylogenetics and ecology which was ultimately articulated as a research program called **Historical Ecology** that was first outlined in a talk by Daniel R. Brooks (1985) at the Systematics Symposium of the Missouri Botanical Garden organized by Peter Raven. Brooks realized that Manter's insight was derived from his deep knowledge and understanding of the **biological diversity** of trematodes that occurred in marine fishes on both sides of the isthmus of Panama, even though at the time, there was not a firmly established method (in the English-speaking world) of consistent analysis of phylogeny (Manter, 1966). Subsequent groundbreaking work in the area of parasite phylogenetics and biodiversity was done in parasite systematics with the publication of the book Parascript: Parasites and the Language of Evolution by Brooks and McLennan (1993). For more information on the history of animal parasitology, see Janovy's chapter (Chapter 68) in this volume, as well as Sattmann (2002; in German) and Hoeppli (1959). As mentioned above, the parasitic way of life is one of the most common—if not the most common—way of protozoan and animal life that exists. It is likely that more than half of all species of organisms are parasites, and many are of very great economic and medical importance. Some of the most devastating diseases of humans, such as malaria, trypanosomiasis, and filariasis, are caused by parasites, and the economic loss caused by parasites of plants and animals worldwide reaches the equivalent of billions of United States dollars every year.

Definition of a Parasite

The concept of a **parasite** and its **host** essentially refers to the biological tension between 2 organisms that live physically adjacent to one another. With the classical definition of a **parasite** as **an organism living on or in another organism (the host) and usually causing some harm to the host**, the parasite sounds like it is merely a bad thing with respect to the host; and this definition works for the most part since most parasites probably *do* harm the host. In some species, the harm can be minuscule and undetectable, without causing discomfort to the host, or the damage can be significant, actually killing the host. For example, pinworm nematodes probably don't do very much to decrease the ability of their hosts to go about their daily lives or produce a normal number of offspring and live to old age. On the other hand, species of the phylum Acanthocephala, known as the thorny-headed worms, can cause a great deal of harm to their **definitive** or **final hosts** by penetrating the mucosal layer of the small intestine with their proboscis and sometimes the proboscis may penetrate the muscularis mucosa through the serosa into the peritoneal cavity, causing peritonitis, and when this occurs, the host usually dies.

Parasitism, beyond the classical definition provided above, can be defined in a very wide sense, that is, as **a close association between 2 organisms, in which a parasite depends on a host that provides some benefit to it** (usually nutrition or food, depending on the group of parasites), and the parasite does not always damage the host (as noted above, pinworms of rodents are good examples of this). A parasite can be very small relative to the size of the host—and most parasites *are* much smaller than the host; however, some parasites can reach huge sizes, and those that become numerous or are very large can even drain their host's blood of essential nutrients.

Parasitology is usually restricted to single celled eukaryotic, or protozoan (also called protistan) and multicellular or metazoan parasites, whereas many groups of organisms that lead a parasitic way of life, such as some fungi and bacteria, are usually instead included in the domain of microbiology, while viruses are studied in virology. However, it really depends on convention. In France, for example, fungi are often studied by parasitologists in addition to the helminths and protozoans or protistans.

Different authors use different definitions for parasitism, depending on their perspective or research interests. Thus, a medical parasitologist will stress that a parasite causes certain diseases and will exclude certain species from the definition which have no apparent ill effect on the host. A zoologist might be more interested in the physiological and morphological adaptations of a parasite to its host or of the host to its parasite. An ecologist may be more interested in the interactions of the parasite on its host and the animal populations with which parasites live, while an evolutionary biologist may be interested in the evolutionary interactions among parasites and their hosts without too much regard for the individual species of animals that are being studied. The definitions presented here are from the general perspective of a parasite **systematist**, one who is primarily concerned with the understanding of parasitism from the aspect of parasite biodiversity, how they evolved and are evolving, and any and all relationships among them (and their hosts).

Associations Related to Parasitism

Some types of ecological associations resemble parasitism in various aspects and cannot always be unambiguously distinguished from a parasitic relationship, either because little is known about a particular species or because intermediate forms exist. Such ecological associations include: Predation, commensalism, phoresis, mutualism, and symbiosis sensu stricto (meaning, in the strict sense). In the case of **predation**, the predator usually kills and eats another animal, the prey. In the case of **commensalism**, an organism associated with a host uses food found in the internal or external environment of the host and there may be no close phylogenetically determined relationship with the host or host group. For example, many species of barnacles and isopods can take up residence on the external surfaces of whales. These can then be termed **ectocommensals** (**ecto** = outside of the host). In **phoresis**, one organism uses another only for transport and/or protection. Barnacles can again serve as an example: Some species live attached to the skin of whales, by which they are carried around finding new sources of pelagic food (plankton). A **mutualistic** association is one in which both host organism and the associated species benefit. The Australian mistletoe bird *Dicaeum hirundinaceum* feeds on the seeds of mistletoes which are plants that derive most of their sustenance from their host plants, and the mistletoe depends on the bird for dispersal of its seeds through space. **Symbiosis** (sensu stricto) is an extreme form of mutualism, in which the association is compulsory, that is, both partners (symbionts) benefit and cannot live without each other. Very ancient examples of symbiosis are organelles (specialized cell components) of all protozoan (unicellular) and metazoan (multicellular) animals and plants, which are thought to have arisen by the joining of originally free-living organisms. However, the term symbiosis is also occasionally used in a wider sense that can include the phenomena of parasitism, commensalism, phoresis, and mutualism.

That a distinction between the various kinds of associations is sometimes difficult to make is shown by the observation that the same organism may sometimes be a parasite, commensal, mutualist, or predator, depending on the circumstances. Thus, oftentimes, the amoeba *Entamoeba histolytica* may feed on bacteria in the intestine of humans without causing any damage, or it may live as an often-fatal pathogenic parasite ingesting red blood cells and sometimes penetrating through the gut wall into the abdominal cavity, with fatal consequences. Some parasites may even improve the well-being of their hosts when infection intensities are low, but this is an understudied area.

Kinds of Parasitism

Lice, ticks, fleas, some monogeneans, and many crustaceans such as isopods and barnacles, as alluded to above, are **ectoparasites** that live on the surface of animals. Nematodes (such as species of Oxyurida or Oxyuroidea), tapeworms (such as fish, beef, and pork tapeworms), flukes (also known as trematodes, such as liver flukes, eye flukes, and blood flukes), and coccidian parasites (such as *Plasmodium*, which causes the disease malaria in humans) are examples of **endoparasites** found in the tissues or within the organs of their hosts. Cestodes and trematodes are **obligate**

parasites which cannot survive without a host at least for part of their life cycle, whereas some maggots (larvae of flies that usually feed on decaying organic matter) may be **facultative parasites**, which infect living animals only occasionally (note that there are plenty of species of flies in which their larval stages are parasitic in vertebrates and cannot live anywhere else). **Permanent parasites**, such as most parasitic helminths, including trematodes, cestodes, and nematodes, are organisms that are parasitic on or in a host over long time spans, whereas **temporary parasites,** such as most leeches, are parasitic only intermittently.

An example of a **sexually dimorphic** parasite is the chigoe flea *Tunga penetrans* Linnaeus in which only the female is a permanent parasite—usually on the toes of some hapless human or some other mammal—and the male may move around from toe to toe and from host to host. Some species of parasites are selective in their parasitic existence such as species of the phylum Arthropoda that range in diversity from marine gnathiid isopods (phylum Arthropoda: subphylum Crustacea: class Isopoda) to terrestrial chigger mites (class Acari: family Trombiculidae). Some species in these 2 groups are parasites only as larvae, thus they are referred to as **larval parasites**. In this example, the isopod larvae live on marine fish and suck their blood, yet when they molt to the adult stage they live the rest of their lives eating detritus in the benthic zone of the sea floor. The trombiculid mites (family Trombiculidae) exist as adults that eat detritus in the soil and they lay eggs there that hatch into larvae called chiggers that are the torment of humans and other mammals worldwide. Other larval parasites include the cysticercoids of hymenolepidid tapeworms (phylum Platyhelminthes: class Cestoda: family Hymenolepididae) that live in mites or beetles as larvae and mature to adults in their rodent final or definitive hosts. However, many organisms are **parasitic only as adults** and they are associated with a host for all, or at least part, of their sexually reproductive phase.

Female mosquitoes and some fly larvae like the Congo floor maggot (*Auchmeromyia luteola*; see Zumpt, 1965) are **periodic parasites** which visit a host periodically. In this example, the *A. luteola* maggot comes out of its daytime hiding place in the evening and fills up on the blood of a sleeping human, and then goes back into the floor to wait until the next feeding session. When individuals of the same species parasitize other individuals of the same species, they are referred to as **intraspecific parasites**. This type of parasitism is not very common but does occur. An example is that of males of some deep sea fish that live permanently attached to females of the same species, absorbing food and deriving physical protection from the female. **Hyperparasites** (of the primary, secondary, tertiary, etc. degrees) are parasites of other parasites. For example, some protozoans infect helminths (worms) in the intestine or tissues of fishes, and this also occurs in nematodes that have flagellated protozoa (*Histomonas meleagridis*) in the uterus of females that are actually transmitted to the next galliform bird host such as chickens and turkeys (class Aves: order Galliformes) and are protected in the eggs of the nematode. **Kleptoparasites** are animals which force others to regurgitate or drop their food and then steal and eat their prize, and this is an example of behavorial parasitism. Frigate birds and some hawks chase other birds in flight. Cowbirds and about 50 species of cuckoos are **brood parasites**, that is, they lay their eggs in the nests of other birds where they are incubated by and cared for by the parental birds of the nest they have invaded. **Microparasites** include viruses, bacteria, protozoans, and some small worms (helminths), which reproduce in or on the host, sometimes inducing immune responses in vertebrate hosts. **Macroparasites**, that is, large-bodied parasites, include most helminths and arthropods; most do not multiply within the host.

There are many species of hymenopterans (phylum Arthropoda: class Insecta: order Hymenoptera) that are considered **parasitoids.** These are animals that lay their eggs in insect or other arthropod hosts and the egg hatches and begins to feed on the host tissues. Here, the host may survive for some time before it is eventually killed by the feeding and growing larval parasitoid. In some cases, several levels of **hyperparasitism** have been identified in which parasitoids are parasitized, such as by a wasp.

Mechanisms of Infection

Specific **mechanisms of infection** are truly numerous and are well-studied in many species of parasites (Table 1). Some species of parasites possess conspicuous morphological adaptations that increase the probability that the life cycle will be completed. For example, eggs of some blood flukes of humans (namely, schistosomes causing schistosomiasis also known as bilharzia or bilharziasis) have spines which contribute, together with enzymes produced by the larva within the egg, to eroding the walls of blood vessels where the adults live, thus facilitating escape of eggs produced by the female directly into the bloodstream. The eggs then travel from the bloodstream through the walls of the blood vessels into the feces or urine, depending on the species of *Schistosoma* (adults of *S. haematobium* live in blood vessels around the urinary bladder while adults of *S. mansoni* live in the blood vessels of the intestines).

Adaptations to Parasitism

Each parasite species has adaptations that increase the probability of the parasite to infect, or make it to, a new host

Mechanism	Example organism(s)
Autoinfection (for example, eggs hatching and the larvae maturing in the host's intestine)	*Taenia solium* or *Strongyloides stercoralis*
Contact transfer	Mange mites of various species
Fecal contamination of wounds, mucosa, or lacrimal surfaces	*Trypanosoma cruzi* transmitted by reduviid bugs/kissing bugs
Ingestion of infected intermediate hosts	Trematodes and cestodes of various species
Ingestion of parasite eggs	*Trichuris*, *Ascaris*, *Taenia*, *Echinococcus*
Ingestion of parasite cysts from undercooked muscle of vertebrates	*Toxoplasma*, *Taenia*, *Echinococcus*
Ingestion of spores and trypomastigotes	Protozoans of various species
Ingestion of transport hosts, such the muscle of uncooked, never-frozen fish	Anasakine nematodes
Inhalation and swallowing of eggs	Phylum Nemata: Species *Enterobius vermicularis*
Inoculation	*Plasmodium* spp. transmitted by mosquitoes
Kissing	Flagellated protozoan *Trichomonas tenax*
Penetration into the nasal passage	Protozoan *Naegleria fowleri*
Penetration through skin	Phylum Nemata: Family Ancylostimatidae
Sexual intercourse	Flagellated protozoan *Trichomonas vaginalis*
Transmammary transmission via milk	Nematodes of various species
Transplacental transmission	*Plasmodium*, *Toxoplasma*, *Ancylostoma*, *Toxocara*

and increases the chance of survival in it. For example, *Plasmodium* species in birds cannot normally survive in primates, and the species of human pinworm (oxyurid nematode) *Enterobius vermicularis* is known only from humans, although other species of *Enterobius* occur in primates with 1 species being reported from rodents (Brooks and McLennan, 2002). In other words, each of these species possesses characteristics enabling it to complete its life cycle using these hosts. Such characteristics (in the very few cases analyzed in some detail) determine not only the species of host(s) used, but also the degree of **host range**, that is, how many host species a parasite can utilize (Brooks et al., 2022).

Like all animal species, parasites must be able to disperse, as populations with a small numerical density and limited geographic distribution may be at risk of extinction when environmental conditions become unfavorable or they may succumb to inbreeding depression via loss of genetic heterozygosity, and (perhaps) run the risk of overinfecting a local and restricted animal-host population. In parasites, dispersal may be mostly, or even entirely, passive; that is, the parasite is spread to new geographic areas and new hosts via the geographic dispersal of the host. Many parasites have elaborate dispersal mechanisms, such as flotation organs of larval flukes (cercariae), polar filaments on the eggs of some cestodes that live in water birds, and some parasites can even modify the behavior of their host to increase the probability that the parasite will make it to the next host.

Aggregation, Hermaphroditism, Parthenogenesis, and Asexual Reproduction

Surveys of the distribution of parasites in animal populations always find that not all potential host individuals are infected to the same degree. Most parasites are usually concentrated in a few individuals of the host population. This is what is meant by distributions being **aggregated** or **overdispersed**. There has been some debate about whether aggregation has a biological function, such as facilitating the finding of mates, or limiting the damage done overall to the host population. Statistically speaking, in the negative binomial distribution, the variance is greater than the mean, so the variance divided by the mean is greater than 1. Since these are counts of numbers of parasites in hosts that were examined, the fact that few hosts have many parasites shows an overdispersed or an aggregation distribution of the parasites in or on a few hosts. The parasites are not dispersed evenly throughout the host population. Whenever the variance/mean is greater than 1, it is said that the distribution is overdispersed or aggregated.

Overdispersion characterizes a phenomenon of aggregation of a majority of parasites in a minority of the host individuals in a certain population. Thus, the majority of hosts have no or few parasites. A very small number of hosts, however, carry a great number of parasites. Crofton (1971) first showed that overdispersion was present for parasite populations. Since then, overdispersion has been defined as axiomatic among parasites of a variety of vertebrate and invertebrate hosts (Knight et al., 1977; Anderson and May, 1985; Crompton et al., 1984). Patterns of overdispersion have also been discovered in populations of managed species of wildlife (Shaw et al., 1998; Wilson et al., 2002).

Additional research shows that the same general pattern occurs across several other species of animals. For example, cestodes of the species *Triaenophorus nodulosus* (class Cestoda: family Bothriocephalidea) in perch fish (*Perca fluviatilis*) show less aggregated distributions with only 54% of these worms occurring in 18.5% of hosts with 81.5% of fish

remaining uninfected or lightly infected. Data accumulated relative to infections by the nematode *Porrocaecum ensicaudatum* (phylum Nemata: superfamily Ascaridoidea) in populations of the European starling (*Sturnus vulgaris*) from 1 study, 89% of the hosts were uninfected or lightly infected, and 69% of the parasites were recorded in just a few (11%) of the hosts. In pond frogs *Rana nigromaculata* harboring nematodes of the species *Spiroxys japonica* (phylum Nemata: class Spirurata: family Gnathostomatidae), it was found that 70% of the parasites were recorded in just 4% of the frogs examined while 88% of the frogs were found to be uninfected and 8% had light infections (Shaw et al., 1998).

Overdispersion was also recorded for 4 species of the most common human-infecting **geohelminths** (Croll and Ghadirian, 1981) and a search of the literature shows that almost invariably, parasites are distributed through animal populations in a non-random way, but what determines this is still poorly understood. For summaries of this topic in helminth parasites, see Churcher et al. (2005) and Lester (2012).

General Reproductive Biology of Parasites

Common among parasites are the various methods of reproducing that are found in the Kingdom Animalia, including: **Hermaphroditism** (1 individual has fully functioning male and female organs), **parthenogenesis** (females are able to produce offspring without mating), and **asexual reproduction** (an individual reproduces by budding or spores in which there is no recombination of genes on the chromosomes). Thus, in asexual modes of reproduction, the resulting new individuals are clones of the original organism. Among most species of parasites, only a single individual or very few individuals will reach and successfully infect or colonize a new host. In this case, populations of parasites may establish and then increase in numerical density from just a few founder individuals, or even from a single founder individual, that makes it to a new animal that it can then utilize as a host. It is a paradigm of evolutionary theory that sexual reproduction creates new combinations of genes that provide the raw material for evolution via natural selection (Williams, 1966; Williams, 1992). However, in reproduction that requires no mating and thus no sexual recombination of genes via the mixing of chromosomes, the advantage of rapid population growth from a single propagule in a new environment may in the short term outweigh the advantages of sex (Ghiselin, 1969; Williams, 1992; Kearney, 2022).

An example of **asexual reproduction** in a parasite occurs in species of *Plasmodium* (the causative agent of malaria in people). This example illustrates the stage that occurs in the vertebrate intermediate host, in the red blood cells after the infective stages first multiply in liver hepatocytes and are released into the bloodstream. In the bloodstream, these parasites develop in the red blood cells (RBCs) and multiply by mitotic division of the nucleus and other cell organelles but not the cytoplasm. These then escape the RBCs into the bloodstream to invade more RBCs and undergo more cycles of development and multiplication (depending on the species).

Parasitic platyhelminths, including trematodes, cestodes, and monogeneans, with a few exceptions, are hermaphroditic and individuals can, if necessary (such as when there are no mates nearby), fertilize their own eggs, although they usually cross-fertilize due to several morphological and developmental stages that decrease probability of self-fertilization in these groups. Some species of nematodes, including those in the genera *Steinernema* and *Heterorhabditis* (entomopathogenic nematodes, namely, those that infect insects as part of their life history) also have hermaphroditic stages (Cao et al., 2022). Many other species have been shown to exhibit various methods besides sexual reproduction and some of these are reviewed in Triantaphyllou and Hirschmann (1964) and Maggenti (1981).

Parthenogenesis is the growth and development of an animal from an ovum without fertilization and this occurs commonly in species of *Strongyloides* (phylum Nemata: family Strongylidae) which infect mammals (see Cable, 1971; also see the definition of parthenogenesis in Maggenti, 1981).

Host Range

Some parasites are known to occur in or on a few or, in some cases, only 1 species of free-living animal. Definitions are always problematic, and defining species of parasites with limited host range (formerly, or at times still, referred to as host specificity) depends on vast knowledge that can only be based on extensive collections of animals conducted over broad geographic spaces and includes complete data for the specimens of both parasites and their hosts (note that if an animal is not parasitized, it is not a host, but is only a potential host). In order for these data to be useful, the specimens that are collected and processed and their associated data must be deposited in museums that maintain both specimens and their data in perpetuity. The reason that the host and parasite are both stored in museums after collection is to enable tests of the hypotheses of host-range by actually looking at, and using data for, both the host and parasite. Many times, the host group is misidentified in the field and the species name can only be positively known by comparative methods using museum collections (Brooks et al., 2015; Galbreath et al., 2019).

Most species of parasites show some level of limited host range, although the extent of limits among species is

variable. For example, the large human nematode *Ascaris lumbricoides* (phylum Nemata: order Ascaridida) has a direct life cycle and occurs in both humans and pigs (Araújo et al., 2015). The apicomplexan protozoan *Toxoplasma gondii* (phylum Apicomplexa: family Sarcocystidae) has been shown to occur in a wide range of mammals and birds and shows broad infectivity on those groups of potential hosts (Dubey, 2008).

As a more detailed example of host range, the nematode *Ransomus rodentorum* (phylum Nemata: superfamily Strongyloidea) had been reported to occur only in the cecum of pocket gophers while a related species of *Ransomus* occurs in species of mole rats in China and perhaps Mongolia. The pocket gophers are rodents with a subterranean lifestyle restricted to North America, Central America, and extreme northern South America (Nearctic). Chinese mole rats are also subterranean rodents, but they have a distribution in the Palearctic and northern Ethiopian regions with no known history of either the Chinese mole rats occurring in the Nearctic nor of the pocket gophers occurring in the Palearctic (Thenius, 1972). Relative to *R. rodentorum* in pocket gophers, this strongyloid species has never been reported from other sympatric species of rodents within the geographic ranges of the nematode, and despite intense field collecting in several areas in North America, this species has never been shown to occur in rodents that are phylogenetically close to gophers. It is interesting that no instances of infection with these nematodes have been reported from rodents that share burrow systems with pocket gophers, even from those that are phylogenetically related, such as the kangaroo rats or pocket mice. These groups are related at a basal level, all with a common ancestor linking the heteromyids (such as kangaroo rats) with the geomyids (pocket gophers) in the superfamily Geomyoidea, one major shared derived trait (**synapomorphy**) being external fur-lined cheek pouches. This is a case where the other species of rodents are both **sympatric** (meaning, occurring in the same geographic space; Brooks and McLennan, 2002) and **syntopic** (meaning, occurring in the same ecological space; see the definition of syntopic in Rivas, 1964. See also an explanation synapomorphy in Chapter 2.).

Attempts to understand patterns of diversity of parasites that have both wide and narrow host ranges have been ongoing with concentrated work and summaries presented first by Baer and Mayr (1957). This work has been one of the foundations of systematic and ecological parasitology since the beginning of the scientific study of parasites (Guerrero, 2021; Hoeppli, 1959); however, the collections of individual parasites from vertebrates representing myriad species and their deposition into museums (as well as depositing individual host animals) has not kept pace with the same work on the vertebrates themselves (Galbreath et al., 2019). In a summary of mammal collections in museums in the United States (Dunnum et al., 2018), there were estimated to be about 5,275,000 individual cataloged mammal specimens distributed through 395 active mammal collections. However, there are only a handful of major collections of parasites of mammals in the United States and, of those, only 2 collections have significantly large reciprocal collections of both mammals and the parasites that were found during geographically focused surveys and inventories of the mammals themselves. Thus, without excellent reciprocal collections of parasites and their hosts with their data available in museums, it is difficult to say very much about host range. Until more data are collected, certain questions will remain unanswered.

Rausch (personal communication) considered that the concept of host specificity was imprecise at best because the noun *specificity* implies an unvarying quality, and he considered that the degree of specificity cannot be easily expressed or measured and any experimental test of the concept would be biased in so many different ways that the results of tests would be invalid, or at best equivocal. Phylogenetic specificity was a term that was used by Baer (1951) to refer to helminths and their hosts that were shown to have coevolved. Baer considered ecological specificity to occur when opportunistic infections were involved. This is what is now called **ecological fitting** sensu Janzen (1985).

Species Richness of Parasites and Distribution of Parasites

Arndt (1940) was the first ecologist who counted the number of parasites as a proportion of a total fauna. In Germany, he found 10,000 parasitic species out of a total of 40,000 species, but did not include insects parasitizing plants, as he classified them as herbivores. Price (1977) included such species but excluded temporary parasites (for example, mosquitoes and leeches) in his survey of the British fauna. Price estimated that more than half of all British species are parasitic.

Thirteen large taxa (phyla, subphyla, or classes) consist entirely of parasites, and many other groups include a high proportion of parasitic species. Even among the vertebrates several species are parasitic, such as the sea lamprey *Petromyzon marinus*.

Virulence of Parasites

Virulence of parasites can be defined as the degree of damage done by the parasite to the host. There are 2 opposing trends which determine the degree of virulence: 1) Usually it is not a selective advantage to severely damage or kill its host, because this would also affect the fitness of the parasite;

2) parasite transmission to another host may be facilitated by such damage: A weak host may be easier prey for a predatory final host than a strong one. Therefore, evolution will lead to an increase or a decrease in the virulent nature of various parasites, depending on the circumstances.

Life Cycles

Many parasites have a **direct life cycle** (lice, fleas, monogenean flukes, and many nematodes) and they use a single host which harbors larval/juvenile stages as well as adults. Other parasites have **complex life cycles** and use a final (= **definitive**) host which harbors the mature stage, as well as 1 or several **intermediate hosts** which harbor the larvae, that is, they have **indirect life cycles** (for example all digenean flukes, all of which are from class Trematoda). An example of a trematode with 2 intermediate hosts is the lancoleate trematode *Dicrocoelium dendriticum*. In certain parasite species, alternative life cycles are possible. For example, in the aspidogastrean fluke *Aspidogaster conchicola*, both a direct and an indirect life cycle are possible: Adult worms in the mollusc produce eggs which are inhaled by other molluscs, but fish can also become infected by eating infected molluscs. In other aspidogastreans, and in the amphilinid tapeworm *Austramphiina elongata*, among many others, the life cycle is always indirect, involving both an intermediate and final host. In the amphilinid tapeworm, turtles serve as final hosts, eggs escape from the host in an unknown way, larvae hatch in freshwater and penetrate into a crayfish intermediate host, which is then eaten by a turtle.

Many species of parasites possess varied and diverse **behavioral adaptations** that facilitate completion of their life cycle and entrance into the next host in the cycle. Adult *Dicrocoelium dendriticum* (phylum Platyhelminthes: class Trematoda: subclass Digenea) infect the liver mainly of sheep, but other ungulates are also parasitized by these trematodes. These trematodes produce eggs which pass out of the host with the feces and are eaten by land snails, in which various larval stages are produced. The last stage is the tailed larva, or cercaria, many of which cluster in slime balls which are left behind in the mucus trail of the snail as it speeds to its objective, whatever that may be. If the trematode larvae are lucky, these slime balls are then eaten by ants. If not eaten, they dry up and die. After being ingested, the cercariae move from the intestinal tract to various parts of the ant. The first cercariae getting into an ant penetrate into the ant's subesophageal ganglion, inducing the ant to climb up a grass stem and, when the temperature drops, the cercaria induces cramp-like behavior in the ant, which consequently clings to the grass stem with its mandibles. This behavior increases the likelihood of the infected ant being eaten by a passing sheep or another ungulate.

Host-Parasite Interactions, Example: Cleaning Symbiosis

A considerable range of behavioral patterns leading to (or thought to lead to) the removal of parasites has been observed among animals. They include **preening and bathing of birds** in dust and water, and **passive and active anting**, where ants are allowed to passively crawl over the body, or where ants are actively squeezed over the plumage. Also, dogs rubbing their skin against rough surfaces, jumping of fish and whales out of water, and so on, may have a cleaning function. Best known is **cleaning symbiosis**, in which one animal (the cleaner) cleans another (the host) from parasites and diseased (necrotic) tissues. For example, cleaning behavior has been observed in birds which remove ectoparasites from cattle, hippopotamuses, large marine fish floating on the ocean surface, several species of shrimp, and some freshwater fish. Hosts are freshwater and marine fishes, whales and dolphins, and invertebrates, among others. Many cleaner fish possess special morphological adaptations which enable them to pick parasites off of the host skin or even gills (the mouth is located terminally to facilitate picking up of parasites, the anterior teeth are fused to form cutting plates, and color patterns are conspicuous, useful in signaling to hosts: "I am a cleaner!"). The cleaner fish *Labroides dimidiatus* even performs a cleaning dance to attract host fish. Invitation postures of hosts signal, in turn, to the cleaner that they are ready to be cleaned.

Generalization of Parasitism: Stockholm Paradigm

Parasites can be specialists or generalists depending on how much of their fundamental **host fitness space** is occupied in a population of animals (Agosta et al., 2010; Brooks et al., 2019). The smaller the fitness space being occupied by a parasite, the more specialized the parasite appears. The following is a short summary of the general ideas of the **Stockholm Paradigm** that deals with host-range and parasite use of animal populations. A more detailed explanation can be found in the book of the same title by Brooks et al. (2019). See also Agosta (2022) and Brooks et al. (2022).

The concept of host range infections has undergone rapid change in the past few years with the ideas of Brooks et al. (2015; 2019) forging new ground towards the interpretation of parasite-host relationships. It now appears that most parasites retain genetically deep phylogenetic signals of host or habitat exploitation that enable the parasites to cross potential host-species boundaries when ecological opportunities arise. Mutations or genetic modifications a priori *are not needed* as the underlying **symplesiomorphic** (meaning, shared ancestral) traits enable cross-species transmission to new hosts when they are available (that is, **syntopic**). These

opportunities arise due to climate and geographic range-oscillations (the **oscillation hypothesis**; Brooks et al., 2019), **taxon pulses**, manifested by both multiplication and extinction of species (Erwin, 1985), and ecological fitting in sloppy fitness space (Janzen, 1980; Agosta, 2006; Agosta and Klemens, 2008; Agosta et al., 2010). Putting all of these together, Brooks and his colleagues (2019) have termed this the Stockholm Paradigm in honor of the researchers at the University of Stockholm in Sweden who first put these synthetic ideas into the literature stream.

Capacity

What is meant by capacity? As noted earlier, every species, including all parasites, have specific environmental resources they need in order to survive and reproduce. In the case of parasites, those resources are specific attributes of their hosts. For a given parasite species, if only 1 host species has the required resources, the parasite can survive only in association with that species, and its survival is tied to the survival of that species. But the vast majority of inherited attributes of all species are evolutionarily conservative, meaning they occur in more than 1 species of host.

All parasites live in association with a restricted number of hosts, and some not so restricted, as seen in *Toxoplasma*, and sometimes only 1 host species is infected. Sometimes parasites are restricted to a potential or actual species of host by limited **capacity** but mostly parasites are restricted by limited **opportunity**. And so, when the conditions change—say, as a result of climate change or intrusion of humans and their domestic animals into previously uncut forest—new opportunities are created and the parasites move into hosts they had the capacity to infect but never before had the opportunity to (this could be the result of trophic changes locally or of geographic dispersal into new areas).

Ecological Fitting

Ecological fitting (sensu Janzen, 1985) refers to cases when a parasite has the opportunity to encounter a new potential host that has the required resources for survival, the parasite will then be expected to add that species to its repertoire. This, by the way, eliminates the need for the right mutation to show up at the right time to allow or enable the parasite to jump into a new species of animal to make it a new host.

Fundamental Host Fitness Space

For any given parasite, the range of all hosts that have the required resources is called the **fundamental host fitness space** (in accordance with Hutchinson's notion of fundamental niche space; Hutchinson, 1959), which Agosta called fundamental fitness space in order to relate it directly to evolution (Agosta, 2006; Agosta et al., 2010). The actual hosts inhabited by the parasite at any given time represent the **realized host fitness space** (in accordance with Hutchinson's realized niche space and Agosta's use of the term *fitness* rather than *niche*). One of the keys to the evolutionary success of parasites is that the fewer species of animals used as actual hosts (that is, the smaller the realized fitness space), the more potential opportunities to inhabit new species of hosts exist. In other words, given the opportunity to come into contact with a suitable but previously unexposed (potential) host species, a parasite would add the new host to its host range and survive even if the original species of host went extinct. The fewer hosts actually used, the smaller the proportion of actual host fitness space compared to fundamental host fitness space and consequently the sloppier (meaning, more filled with potential opportunities) the host fitness space. At the same time, the more restricted the realized host fitness space, the more specialized the parasite is within that fitness space. Alternatively, the more species of potential hosts used, the larger the proportion of actual host fitness space compared to fundamental hosts space, the less sloppy the fitness space, the fewer new potential hosts, and the more generalized the parasite is in fitness space.

This insight, developed by Agosta (2006) and elaborated by Agosta et al. (2010) and Brooks and Agosta (2020), obviates the need to define or even discuss host specificity since it is basically impossible to look at host specificity in an evolutionary sense. Conversely, the idea of fitness space has a Darwinian evolutionary origin that can be tested in an evolutionary context.

Oscillation Hypothesis

Periods of climatic/environmental stability are usually associated with events of local geographic isolation, hence, specialization of parasites occurring in limited geographic areas and many potential hosts unexposed in other similar but separate geographic areas; periods of environmental perturbations are usually associated with increased or expanded species-level geographic distribution, hence, generalization may occur with fewer potential hosts. Parasites thus tend to oscillate between specializing and generalizing in host fitness space, depending on environmental conditions; this is called the **oscillation hypothesis** that was developed by Janz and Nylin (1998).

Taxon Pulse

All species of parasites and their actual and potential hosts alternate between geographic isolation (geographic contraction in space) and geographic expansion through space via dispersal. This is called the **taxon pulse** (Erwin, 1985).

Environmental perturbations drive taxon pulses, which drive host range oscillations, which drive parasite diversification by ecological fitting in sloppy fitness space, reinforced by natural selection (Agosta et al., 2010). Well worked-out examples that show these various parts of the Stockholm Paradigm include those presented in Brooks et al. (2006; 2015; 2019) and Malicka et al. (2015).

Ecological Fitting Example

Surveys and inventories are the primary ways that large scale and complete collections of parasites and their actual and potential hosts are accumulated over large geographic scales in short periods of time (Gardner, 1996; Gardner and Jiménez-Ruiz, 2009; Gardner et al., 2012; Galbreath et al., 2019). A final example of ecological fitting presented here stems from survey and inventory work on mammals and their parasites funded by the National Science Foundation (grant numbers BSR-9024816 and DEB-9496263), from a collection locality labeled 7 km S, 4 km E Cruce Ventilla in the Department of Oruro, Bolivia (read as "7 kilometers south and 4 kilometers east of Cruce Ventilla in the Department of Oruro, Bolivia"). The specific locality, referred to here as near Cruce Ventilla, was visited by a field team from the Museum of Southwestern Biology (Albuquerque, New Mexico, United States) and the American Museum of Natural History (New York, New York, United States), September 29–30, 1986 (Anderson, 1997).

Several species of mammals and their parasites were obtained at this locality. Of particular interest, 3 of the species of mammals were collected from the same burrow systems that had been constructed and were being actively used and maintained by subterranean rodents called highland tuco-tucos; species name *Ctenomys opimus* (Wagner). At this locality, several specimens of *C. opimus* were collected from the burrows, as well as several individuals of yellow-toothed cavy, species name *Galea musteloides* Meyen, and many individuals of 1 species of leaf eared mice, species name *Auliscomys boliviensis* (Waterhouse, 1846). Specimens of the mammals were collected sequentially or simultaneously, and all of the mammals were recorded as using the same burrow systems using the same entrances and exits. Great care was taken in performing the collections and necropsy on the specimens at this site because it appeared to be an opportune chance to identify any parasites that potentially could be shared among the 3 syntopic species of rodents that were occurring in the same micro-geographic space, using the same ecological space, and using the same resources (Rivas, 1964).

After collections were made using standard methods and necropsies performed (see Gardner and Jiménez-Ruiz, 2009; Galbreath et al., 2019), it was immediately evident that a single species of parasite was shared among 2 of the species of rodents but not all 3. The metacestodes were found only in *Ctenomys* and *Auliscomys*. This cestode was identified later as the larval form of *Taenia talicei* Dollfus, 1960, a polycephalic (meaning, having many scolexes) taeniid (order Cyclophyllidea: family Taeniidae: genus *Taenia*) identified by the morphology of the hooks and the multi-strobilate (many strobila associated with a single infection) nature of the larvae. Pinworms of the genus *Helminthoxys* were found in the cecum of the *Galea* but not in the cecum of individuals of *C. opimus*. However, many individuals of *C. opimus* were infected with a species of *Paraspidodera* that occurred in their cecae and large intestines. The individuals of *A. boliviensis* that were examined were shown to be infected with trichostrongylid nematodes (phylum Nemata: superfamily Trichostrongyloidea) in the small intestine and pinworms of the genus *Syphacia* (phylum Nemata: order Oxyurata) in the cecum. Current investigations are under way on both the endoparasites and the ectoparasites of this same host assemblage near Cruce Ventilla, Bolivia. This sharing of metacestodes among several species of rodents of widely divergent phylogenetic lineages illustrates the phenomenon of **ecological fitting** and the fact that metacestodes of *Taenia talicei* have broad host-range tolerances while the adults probably are more restricted (although no carnivores were collected and examined at or near this locality). It is generally observed that adult cestodes in the genus *Taenia* show host range use that is somewhat narrow, and this may partly be due to the effects of sympatric or syntopic species of intermediate hosts.

Economic and Hygienic Importance of Parasites

Some of the most important tropical diseases of humans are caused by parasites, such as schistosomiasis (bilharziasis) (caused by the blood fluke *Schistosoma*), filariasis (caused by several different species of filarioid nematodes), amebic dysentery (the protozoan *Entamoeba histolytica* is the causative agent of this one), and, in particular, malaria (at least 5 species of the protozoan *Plasmodium*). Annually, more than 247 million people are infected with various species of *Plasmodium*, the causative agent of malaria, and around 619,000 people die from it every year worldwide, particularly children in sub-Saharan Africa. The webpages of the World Health Organization (WHO), Division of Tropical Diseases and of the United States Centers for Disease Control and Prevention (CDC) contain information about the current status of the important parasitic diseases, which is continually updated. Information on prevalences of infection with various parasites and their geographical distribution are available at the CDC web site (https://cdc.gov) and at the WHO site (https://platform.

who.int/mortality/themes/theme-details/topics/topic-details/MDB/infectious-and-parasitic-diseases).

Global warming will lead to a spread of parasitic infections into some countries and increase prevalences of parasites in others that already have high parasite loads in their populations, especially in tropical and subtropical regions that will continue to warm over the next few hundred years (Brooks et al., 2019).

Parting Thought

The rest of this book provides an in-depth overview of many species of parasites, how they are related to one another, their adaptations, effects on hosts, and their importance as fellow inhabitants on Earth.

This introduction is fittingly ended with a quote from Harold W. Manter (Figure 2), one of the leaders in parasitology from the late 1920s through 1970 and the namesake of the Harold W. Manter Laboratory of Parasitology, one of the world's leading laboratories of systematic parasitology. Manter was an early proponent of the mutability of continents and plate tectonics and worked to provide evidence of continental movement with data from parasites and their hosts. From this work, he proposed the idea of **parascript** (Brooks and McLennan, 1993). Extracted from the book Host-Parasite Relationships (McCauley, 1966), Manter stated:

> Thus, parasites reflect both current environmental conditions and also the influences of ancient times—both ecology and phylogeny … Parasites of fishes, particularly such an abundant and diverse group as the Trematoda, furnish information about present-day habits and ecology of their individual hosts. These same parasites also hold promise of telling us something about host and geographical connections of long ago. They are simultaneously the product of an immediate environment and of a long ancestry reflecting associations of millions of years. The messages they carry are thus always bilingual and usually garbled. Today, we know only a few selected pieces of the code. As our knowledge grows, studies based on adequate collections, correctly classified and correlated with knowledge of the hosts and life cycles involved should lead to a deciphering of the messages now so obscure. Eventually there may be enough pieces to form a meaningful language which could be called ***PARASCRIPT: The language of parasites which tells of themselves and their hosts both of today and yesteryear***.

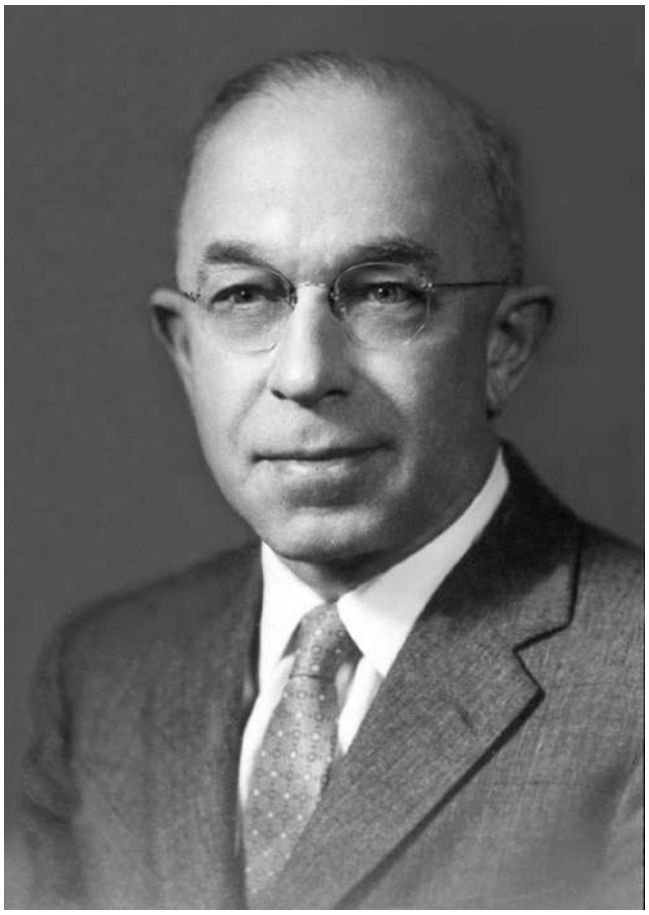

Figure 2. Harold Winfred Manter (1898–1971), circa 1960. Manter was a professor in the Department of Zoology, University of Nebraska (Lincoln campus; Lincoln, Nebraska, United States) from 1925 to 1971. He worked on systematics and biogeography of parasites of fishes, although during his tenure at Nebraska, he trained dozens of students in other areas of parasitology. The Harold W. Manter Laboratory of Parasitology (HWML) was named after him, having been established after his death in 1971 by Curator of the Parasitology Division of the University of Nebraska State Museum, Mary Lou Hanson Pritchard. Source: HWML. License: CC BY.

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2

Phylogenetic Systematics in Parasitology

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Chapter 2

Phylogenetic Systematics in Parasitology

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Connection Between Phylogenetic Systematics, Taxonomy, and Classification: A Review

Every species, whether it is the bacterium *Escherichia coli*, the malaria-causing *Plasmodium falciparum*, or the blue whale, *Balaenoptera musculus*, has a formal, given scientific name. Each name is in 2 parts, and in Latin, hence this formal name is also called the organism's Latin binomen (**bi** = 2-part, **nomen** = name). The first part of this 2-part name is the genus of the organism (for example, *Plasmodium*) and the second one, the specific or species name (for example, *falciparum*). However, it is conventional (and important) to use both parts of the scientific name (for example, *P. falciparum*), together when referring to the species. Because these names are in Latin, it is also conventional to *italicize* the scientific name in print or underline them when they are hand-written. The practice of giving each organism a formal name is **taxonomy** and relies on a set of methods.

Taxonomy goes hand in hand with, and is part of, a related scientific practice of placing organisms into formally named sets using a hierarchical system. This system of grouping, familiar to all of us since our biology classes in high school (Figure 1a), is **classification**. Early naturalists placed organisms that broadly shared common features first in larger groups and then ones that shared a smaller subset of features into progressively smaller groups, so there could be some order in describing and cataloging the vast diversity of life on Earth. A common formal classification scheme was devised (Figure 1a) and originally began with the category kingdom. It is a scheme that we follow to this day. Deciding what to name a species (taxonomy) when describing it for the first time or revising/changing the name depends on correctly classifying that organism. The ranks or categories of classification called the **genus** and **species** come at the very end of formal classification (Figure 1a). Here are examples of 2 species, the human broad tapeworm (*Dibothriocephalus latus*) (Figure 1b) and humans (*Homo sapiens*) (Figure 1c), formally classified.

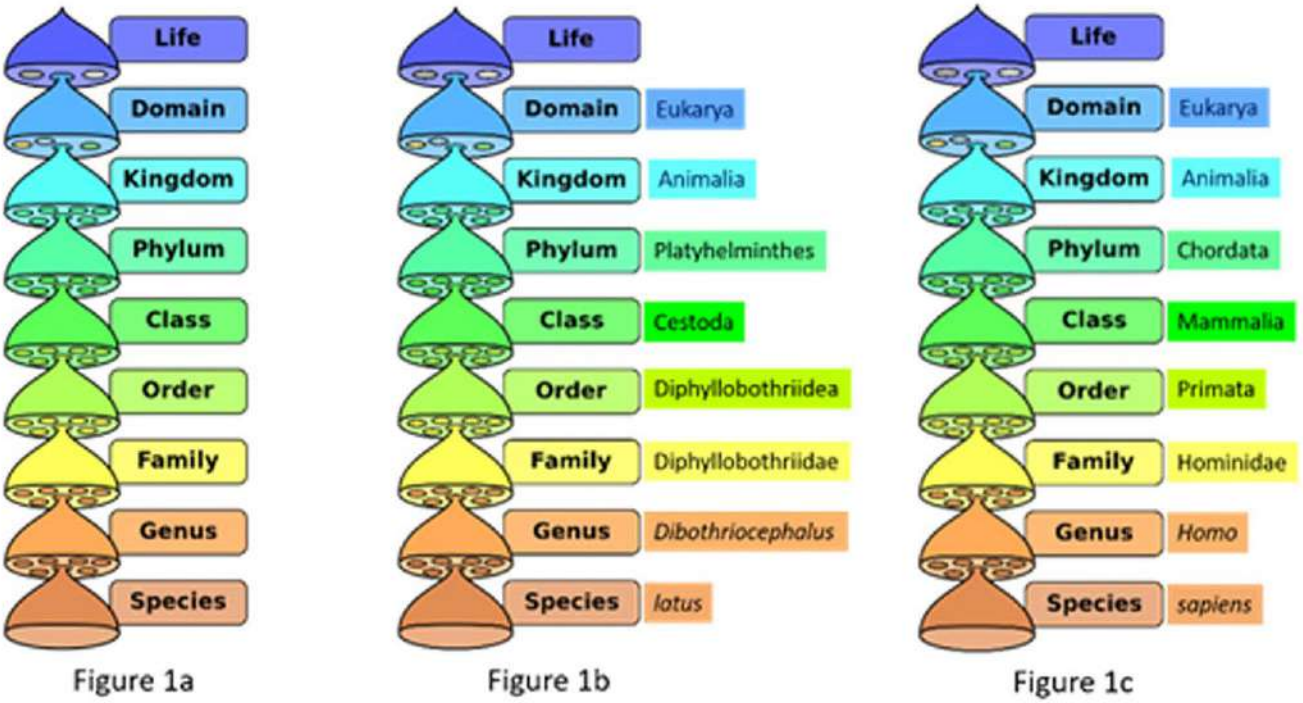

Figure 1a, b, c. Names of taxonomic classification of organisms. Source: Adapted from Ideophagous, 2021. License: CC BY-SA 4.0 International.

Between these major categories or ranks (kingdom, phylum, class, order, family, genus, and species), taxonomists created subgroups to further fine-tune the classification. Examples of other categories are ranks such as subphylum, lower in rank than phylum but higher than class, or suborder, lower in rank than order but higher than family. In other words, a phylum could contain several subphyla, each with their own set of classes, and orders with their own set of suborders, etc. Note that this does not change the actual hierarchical nature of the classification but refines it. For example, the phylum Chordata is subdivided into 3 subphyla; 1 subphylum is the very familiar Vertebrata (vertebrates, a group to which we humans belong). The word vertebrate is used more often than the word chordate (for the phylum Chordata) because the other non-vertebrate chordates are rarely encountered in nature.

Taxonomy and classification fall under a broader branch of science called **systematics**, and scientists engaged in this research are called **systematists**. The following sections will include a brief review how systematics developed and flourished in the 20th century, and what impact it had on parasitology.

Cultivating a Deeper Understanding

What does **classification**—the formal grouping of organisms—imply and what methods are used to classify and place organisms in their correct groups and give them their appropriate scientific names?

Before scientists knew about evolution and genetics, organisms were classified based on their similarities. More common and general similarities were used for higher ranks or categories (for example, phylum) and similarities that were more limited to particular groups were used for lower ranks, such as class. For example, naturalists and anatomists noted that a large group of animals, including lampreys, jawed fishes, amphibians, lizards, snakes, turtles, mammals, birds, crocodilians, and even varieties of extinct fossil animals such as dinosaurs, pterosaurs, and others, possessed a stiff rod-like structure in their backs. Anatomists proposed that this structure, called the notochord or its modified version, a bony vertebral column, could be used as a unifying feature to group all organisms that possessed it, so they established the phylum Chordata (chordates). For chordates that possessed a bony spine, the vertebral column, they established the subphylum Vertebrata (vertebrates) to distinguish them at the time from chordates such as hagfish and lampreys that only had an unmodified notochord, which they considered primitive. The notion that some organisms and their features were ancient or primitive was well established because of the fossil record and the work of paleontologists. Naturalists also noted that only a subset of vertebrates possessed hair and mammary glands, so they grouped the ones that did into the next available lower taxonomic category, class, and named them Mammalia (mammals). Similarly, only a subset of vertebrates, birds, possess feathers, so for those vertebrates, naturalists established the formal class Aves. They also did this for amphibians (class Amphibia) and reptiles (class Reptilia). It is worth noting, albeit obviously, that naturalists were basing their classification on **comparative anatomy**.

Soon after formal classification was established in the 18th century, naturalists began thinking about the diversity of life on Earth as the product of **evolution**. Evolution proposed that all natural kinds of organisms–species–originated from previously existing natural kinds by modification, which led to the inevitable conclusion that all of life on Earth is related in the form of a giant family tree. As a result, taxonomists recognized that **similarity** among species was because of **evolutionary relatedness**. In other words, evolution provided, and for the first time, a unifying basis for understanding *why* species were more or less similar to one another.

Once evolutionary biology became widely accepted as the unifying theory in biology, taxonomists strove to produce **natural classifications**, that is, classifications that reflected the evolutionary, or genealogical, relationships of organisms. What this meant for the formal classification scheme (Figure 1a) was that when taxonomists examined the existing classification of species, or placed organisms they were discovering and describing in a particular class or family or genus, they needed to be reasonably confident that the placement reflected the evolutionary relationships of the species in question.

For several decades since the widespread acceptance of evolution in the early 1900s, taxonomists continued to use a combination of anatomical features, often newly discovered ones, to propose or revise the existing classifications of a wide range of organisms. Nevertheless, the practice suffered from the lack of a clear and objective methodology that could challenge or supplant the expert opinions and assertions made by leading taxonomists and systematists of the time. In other words, there was no consistent method of producing new classifications or testing existing ones. This problem was true for higher classifications, whether a species belonged to a particular order or family, as well as for lower-level classification, for example deciding whether a species belonged in one genus or another.

In 1963, Robert R. Sokal and Peter H. A. Sneath provided the first detailed objective method: **Numerical taxonomy**. In this once widely-used method, taxonomists tabulated data from as many morphological features of the species they were studying as they could and then analyzed those data using a particular mathematical algorithm (a set of computational

rules). This was akin to a **cluster analysis**, whereby species sharing the greatest number of characteristics would be grouped together. In other words, the method produced groupings based on overall similarity. The method in which groupings of species was based on such overall similarity came to be known as **phenetics**. The method had the advantage that both data and analyses were explicit, and hence, repeatable. Furthermore, the analyses could be improved by adding more data.

Phylogenetic Systematics

German entomologist Willi Hennig developed a fundamentally different method, called **phylogenetic systematics**, first published in German in 1950. Once it was translated into English in 1966 and became more widely accessible, it fundamentally transformed the practice of systematics, including how taxonomy and classification are practiced. Describing Hennig's approach, Brooks (1985), who first introduced phylogenetic systematics to parasitology, put it succinctly (emphases and word in brackets added):

> [Hennig] asserted that all species are composites of **ancestral** and **derived** traits; therefore, there are no such things as archetypes that, by definition, are all-primitive. This assertion led directly to Hennig's proposed methodology. If the traits exhibited by any species are a combination of primitive and derived features, then the traits shared by two or more species will be indicators of phylogenetic relationship. **Shared primitive traits** indicate general phylogenetic relationships while **shared derived traits** indicate more particular phylogenetic relationships. Two species that share a derived trait or traits that are unique to them are each other's closest relatives.

The idea in the last sentence from Brooks (1985) can also be applied to any taxon, whether it is a species or genus or any rank higher than that. For example, if 2 genera share a **derived** trait *unique* to them, the genera are each other's closest relatives.

In the technical language of phylogenetic systematics, relatively primitive or ancestral traits are called **plesiomorphies** (singular: plesiomorphy) or **plesiomorphic traits**, whereas relatively derived, that is, more recently evolved traits, are called **apomorphies** (singular: apomorphy) or **apomorphic traits**. Shared plesiomorphic traits are called **symplesiomorphies**, whereas shared derived traits are called **synapomorphies**. In phylogenetic systematics, synapomorphies are all important, and finding synapomorphies is a critical step in discovering true relationships among taxa.

The effect of phylogenetic systematics on classification was profound. Henceforth, valid natural groups could only be recognized or diagnosed by their synapomorphies, not by shared plesiomorphy. For example, if we want to examine the relationships among tetrapods, then the vertebral column is not a useful trait because all tetrapods have one, so it can't be used to distinguish some tetrapods from others. The vertebral column is a plesiomorphic trait for tetrapods. It is plesiomorphic because the common ancestor of tetrapods possessed this feature. Similarly, the presence of 4 limbs with digits is also not useful when trying to find out which tetrapods are related to which others either because the condition of having 4 limbs with digits is the ancestral tetrapod condition. On the other hand, an amniotic egg, found only in a subset of tetrapods, is a relatively more recently evolved type of egg compared to the ancestral egg of tetrapods that did not have an amnion surrounding the developing embryo. So, an amniotic egg can be used as a synapomorphy to relate mammals and sauropsids (birds, crocodilians, lizards, snakes, and turtles). Going a step further, within this amniote group, only a subset of amniotes have hair and mammary glands. Hair and mammary glands must then have evolved after the amniotic egg, and so can be used as synapomorphies for this group called mammals. Using phylogenetic systematics, the evolutionary relationships of the major groups of vertebrates can be depicted in the form of a branching diagram or **phylogenetic tree** and the synapomorphies placed on it (Figure 2).

In this phylogenetic tree (Figure 2), each group that is diagnosed by at least 1 synapomorphy is called a **monophyletic group**, often referred to as a **clade**. Thus, amniotes form a monophyletic group, comprising the common ancestor of all amniotes and all of the group's descendants. The clade amniotes is nested within a larger clade, the tetrapods, which in turn is nested within an even larger clade, the osteichthyans, and so on. Note the hierarchical nature of the relationship; there are groups within groups. This hierarchical relationship can be used to develop natural classifications, that is, classifications that reflect evolutionary relationships rather than arbitrary criteria or overall similarity.

What this foregoing example also illustrates is that every species, indeed every organism, is a mixture of very ancient anatomical (and biochemical) features, some that are not so ancient, and others that are quite recent. Humans, *Homo sapiens*, are able to produce collagen, a trait that is shared by every animal, including sponges. Collagen production actually defines what it means to be an animal; and, as such, it is one of humans' oldest traits. Humans' bony spine is ancient too, but not as ancient as our ability to produce collagen. Human jaws are also ancient, but not as ancient as the spine. Humans'

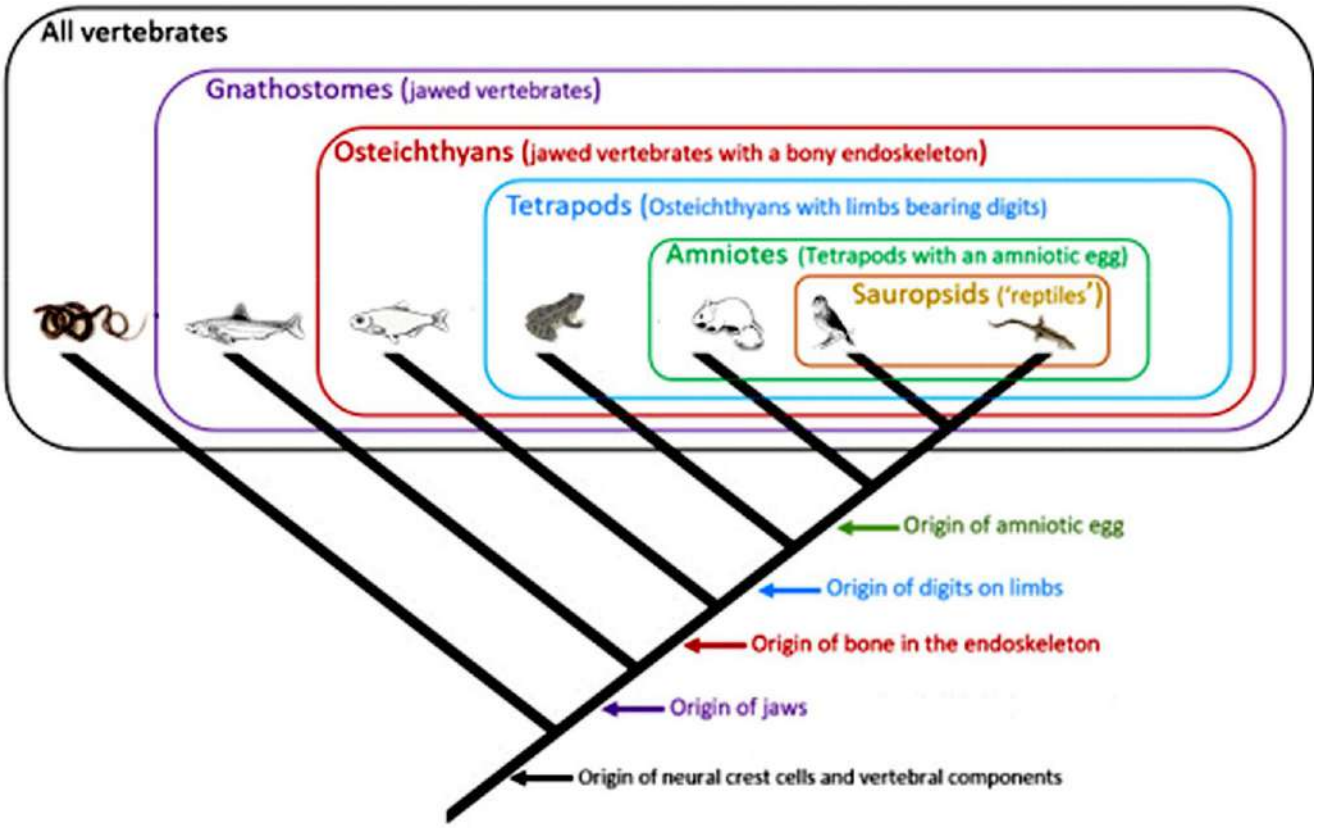

Figure 2. Basic phylogenetic tree of vertebrates. Snake image source: S. Stone, ca. 1789–1790, from the State Library of New South Wales, Australia. Public domain. Shark image source: P. S. Foresman, 2020. Public domain. Fish image source: Mrmw, 2021. Public domain. Frog image source: Z. Thompson, 1842. Public domain. Mouse image source: Gwilz, 2013. License: CC BY-SA 4.0. Bird image source: P. S. Foreman, 2020. Public domain. Lizard image source: J. de Graag, 1954. Public domain.

4 limbs with digits (fingers and toes) are also old, but not as old as the jaws that arose in distant ancestors some 450 million years ago. Human hair is a relative newcomer, only about 135 million years old. Humans' opposable thumb is much more recent, perhaps only about 2 million years old, and humans developed the ability for speech and, subsequently, language less than 200,000 years ago. These (and many other) traits can be **ordered**, ranging from the most ancient (earliest evolved; also called **plesiomorphic**) to the most recently evolved (**apomorphic**) as follows:

Collagen → vertebral column → jaws → limbs with digits → hair → opposable thumb → speech/language

Notice, too, that any feature/trait can be plesiomorphic or apomorphic relative to another feature/trait in this ordered series. For example, jaws are apomorphic relative to the vertebral column, but plesiomorphic relative to the tetrapod limb. Understanding the order of traits is an important part of phylogenetic thinking and practice. A word of caution here; sometimes the same traits/features may evolve independently in species that are distantly related by convergent evolution. These instances can be confusing; ornithischian dinosaurs have hip bones like those of birds hence the name Ornithischia (from the Greek **ornith** = of a bird). But birds share a greater number of synapomorphies with the theropod dinosaurs even though those dinosaurs have a hip that is unspecialized and is unlike that of birds and ornithischians. Therefore, the phylogeny of birds places them with theropod dinosaurs rather than with ornithischians.

There are online resources that provide useful overviews of phylogenetic systematics and related topics. The University of California, Berkeley hosts one such easily accessible and user-friendly resource, available at https://evolution.berkeley.edu/evolibrary/article/phylogenetics_01.

With advances in biotechnology and the ability to obtain DNA (deoxyribonucleic acid) and amino acid sequences, a new and rich source of data has become available. In molecular datasets, individual bases (nucleotides) or amino acids serve as characters and changes in these components (bases or amino acids) are conceptually treated in the same way as

changes in morphological characters. These data can thus be used for phylogenetic analyses. Molecular phylogenetics has now superseded morphology-based phylogenetic systematics in most areas, with the obvious exception of paleontology. Although both morphological and molecular data can be combined in an analysis, molecular data by their very nature (hundreds or thousands of bases or amino acids as characters) vastly outnumber morphological data.

Methods for Constructing Phylogenetic Trees

Several methods are currently used to analyze the relationships of taxa. These include Neighbor Joining (NJ), Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). Each method has its own set of assumptions. Neighbor Joining is considered a phenetic method by many because it uses a distance matrix of characters, and although computationally fast, is often inaccurate. It serves as an adequate first pass in an analysis and can be used in an exploratory manner, but has been supplanted by other, more powerful phylogenetic methods. Maximum Parsimony was originally developed for morphological data and is the oldest of the true phylogenetic methods. It is still preferred by some systematists on philosophical grounds. Maximum Parsimony uses an age-old principle in science–Occam's razor–whereby the tree that requires the least number of steps, that is, the fewest evolutionary changes, is the preferred phylogeny. Whereas MP makes fewer assumptions than other, probability-based methods that followed, it has been shown to have limitations in certain circumstances. Currently, probabilistic methods, such as ML and BI, are more commonly used to infer phylogenies. Of the several books available, Hall's (2018) book makes phylogenetic analyses accessible to all biologists by combining the basic theory of the methods mentioned above with a stepwise guide to doing basic analyses with a user friendly and popular phylogenetics software package, MEGA 7.

Reading a Phylogenetic Tree

Consider the phylogenetic tree in Figure 3 that shows the relationships of the 2 species of human lice *Pediculus humanus* and *Pthirus pubis* (modified from Reed et al., 2007).

This tree was generated by Reed and his colleagues (2004; 2007) who analyzed a combined dataset of DNA sequences

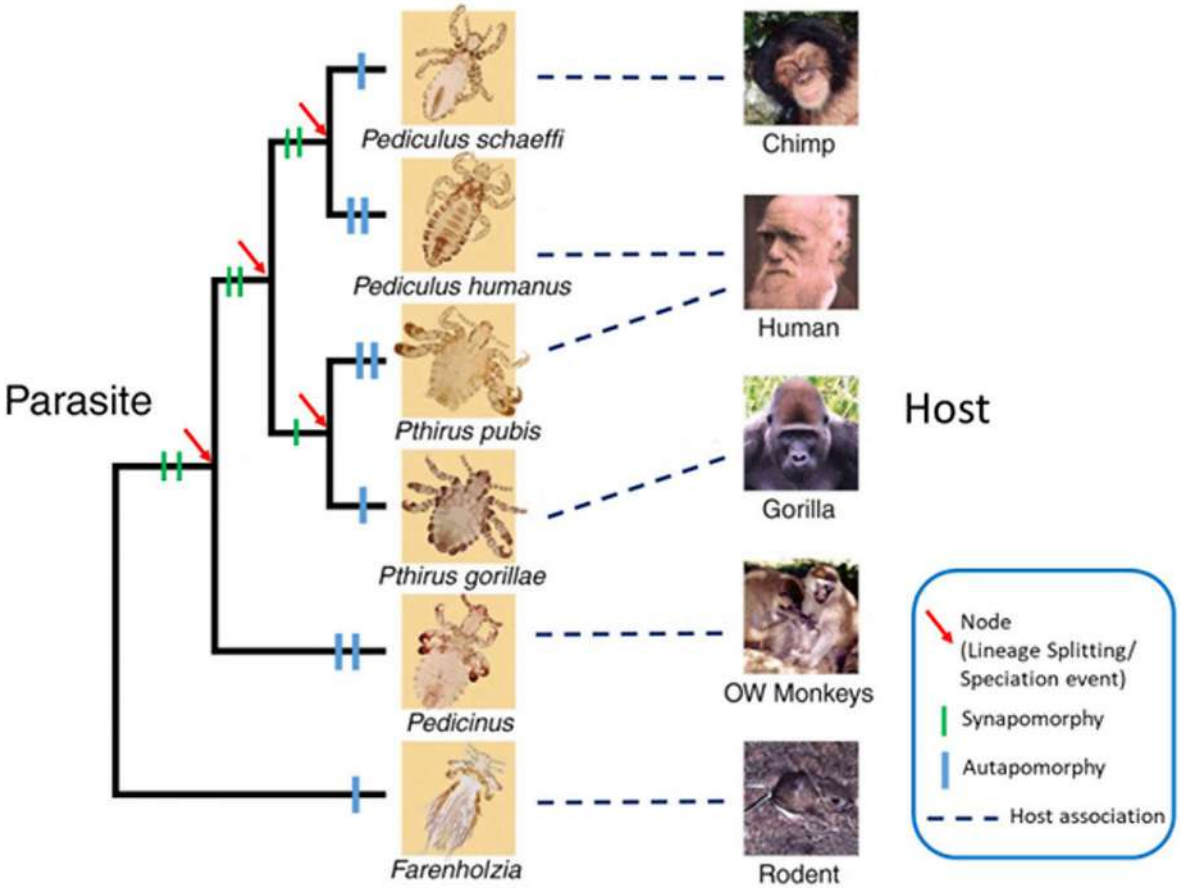

Figure 3. Phylogenetic tree for primate lice and their vertebrate hosts showing nodes, synapomorphies, autapomorphies, and host associations. The number of lines shows the number of synapomorphies and autapomorphies. Source: Adapted from Reed at al. 2004; 2007. Photo credits: J. W. Demastes, T. Choe, and V. Smith, 2004. License: CC BY 2.0.

of genes for cytochrome *c* oxidase subunit 1 (*cox1*) and cytochrome *b* (*cytb*). What do the various parts of the tree mean? First, locate the taxa (in this case species of lice) placed terminally at the end of the branches. The branching pattern reveals the relationships among these lice species. *Pediculus humanus* is most closely related to *Pe. schaeffi* from chimpanzees. Because *Pe. humanus* and *Pe. schaeffi* are each other's closest relatives, they are called **sister species**. *Pthirus pubis* is most closely related to *Pt. gorillae* from gorillas, so they are sister species as well. The red arrows point to the **nodes** of the tree. Nodes signify the splitting of the ancestral lineage into 2 daughter lineages, and in this tree denote the **speciation events** that produced the daughter species. The green bars on the internodes denote the **synapomorphies** based on which the relationships are established. Blue bars on the branches denote apomorphic features unique to each species; such traits are called **autapomorphies** (plural; singular autapomorphy) and are useful for diagnosing or identifying individual species but are not useful for uncovering relationships (recall that only synapomorphies can reveal relationships; see Figure 4). The letters **A, B**, **C**, and **D** are the ancestors of their daughter lineages or species. This is where we have to be cautious in our interpretation. **C** is the ancestor of *Pediculus schaeffi* and *Pe. humanus*. **D** is the ancestor of *Pt. pubis* and *Pt. gorillae*. **B** is the common ancestor of **C** and **D**. Going down to the base of the tree, one finds **A**, the common ancestor of **B** and the lineage that produced the genus *Pedicinus* in Old World monkeys. Another genus of lice, *Farenholzia*, found in rodents, serves as the **outgroup** to the group of lice being analyzed (the **ingroup**). The outgroup is used to root the tree, which is used to establish the order of change in the characters used in the analysis. The relative position of the different branches of the tree produce the tree's **topology** or shape. Note that this phylogenetic analysis indicates that *Pe. humanus* and *Pt. pubis*, are *not* each other's closest relatives, even though they are both found in humans.

Further Applications of Phylogenetic Systematics in Parasitology: Some Examples

Phylogenetic systematics can change our understanding of parasite relationships. Consider the case of the parasitic flatworms; they are grouped into 3 classes: Trematoda, Monogenea, and Cestoda. For much of the 20th century, and despite some opinions to the contrary, the monogeneans were considered trematodes. However, molecular phylogenetics indicated that the monogeneans are actually more closely related to cestodes than to trematodes, which in retrospect was suggested by the presence of the cercomer, a larval structure that some considered homologous to the monogenean haptor (see Figure 5).

A multi-gene phylogenetic analysis (Laumer et al., 2015) corroborates the inference that all parasitic trematodes had a common ancestor and that monogeneans are likely more

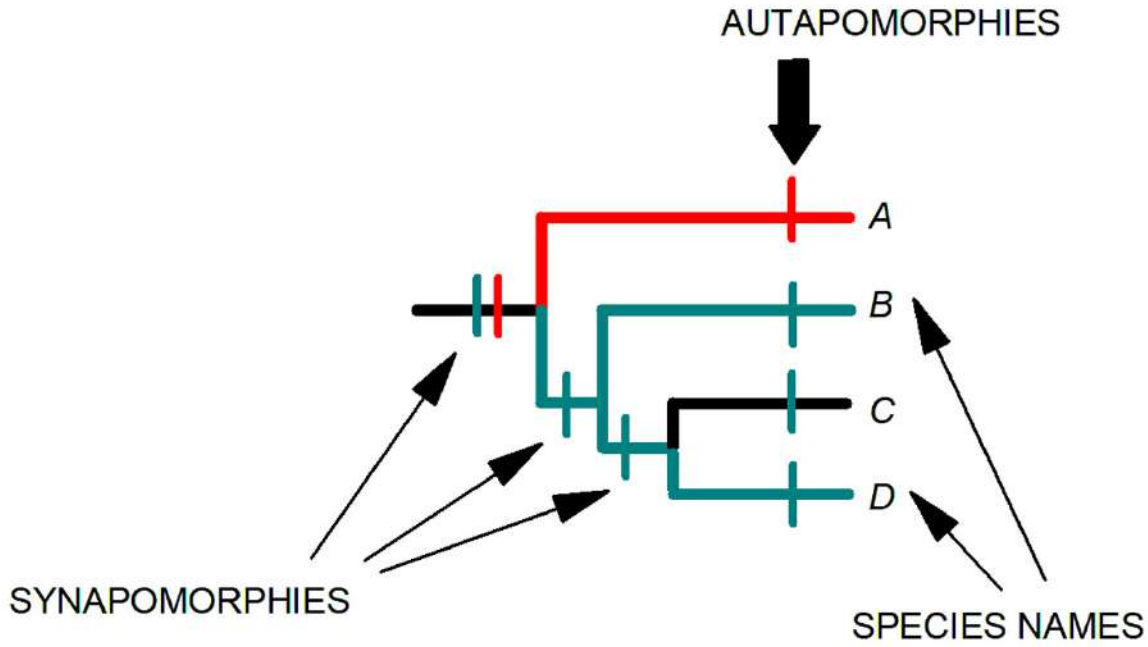

Figure 4. A phylogenetic tree showing distribution of characters. Characters that are shared by species are called **synapomorphies** (meaning, shared derived characters). A character that occurs only in 1 species is called an **autapomorphy**, which, more generally speaking, is a trait that is unique to a taxon. Source: S. L. Gardner, HWML. License: CC BY.

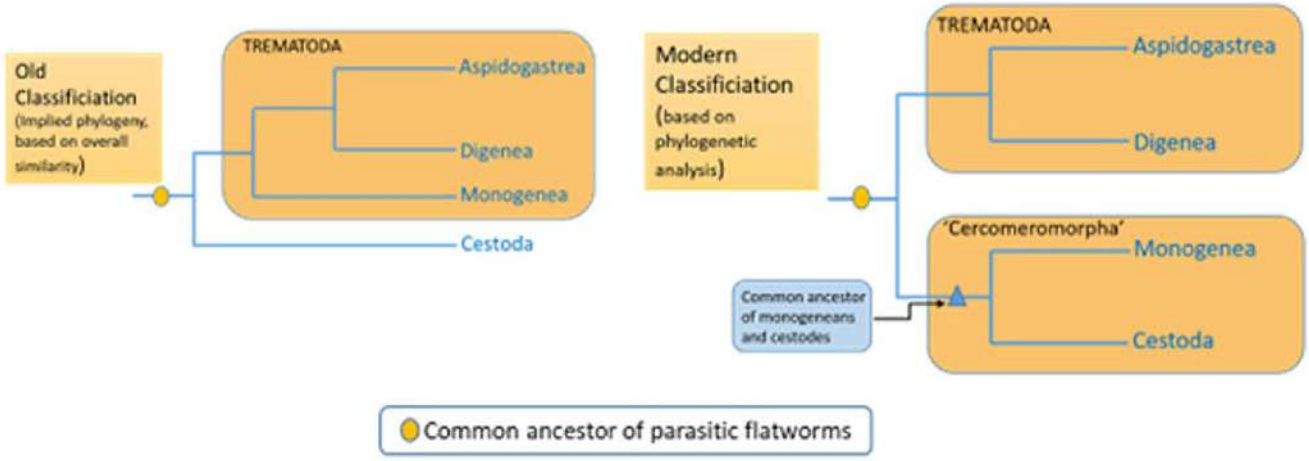

Figure 5. Cladograms showing the common ancestor of parasitic trematodes (flatworms) under an old classification and under a more modern classification. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

closely related to cestodes, but they found weaker support for the Cercomeromorpha than previous analyses suggested (see Figure 6). Laumer et al. (2015) also found that, as was previously proposed, parasitism evolved once in the flatworms and that all parasitic flatworms had a common ancestor.

Several conclusions can be drawn from this tree: 1) The parasitic flatworms form a strongly supported clade called the Neodermata, that is, the 3 groups of parasitic flatworms had a common ancestor in the distant past, 2) the Neodermata is a relatively late branching (recently evolved) clade of flatworms and sister to the free living Bothrioplanida, 3) the tapeworms and monogeneans form a clade called the Cercomeromorpha, and are therefore are more closely related to each other than either of them is to the Trematoda.

As methods improve and become more rigorous and sophisticated, phylogenetic reconstructions/hypotheses change and become arguably more robust. Let us examine how this happened using the case of a group of blood cell infecting parasitic organisms, the haemosporidians, that includes the human malarial parasites. In other words, a question that arises is: What are the relationships of the human malarial parasites, *Plasmodium falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*, and has that understanding changed over time?

One of the early studies on phylogenetic relationships of *Plasmodium* spp. was by Escalante et al. (1998) who used the Neighbor Joining (NJ) method to analyze sequences of the cytochrome *b* gene. They found that the 5 human infecting malarial species *did not* form a clade by themselves; instead, these species were in different parts of the tree (Figure 7). This suggested that humans became hosts of *Plasmodium* at different times in the evolutionary history of these parasites. In addition, there is strong nodal (statistical) support for the relationship of *P. falciparum* and *P. reichenowi*, statistically unsupported evidence of a sister relationship between *P. malariae* and *P. ovale*, and for a well-supported clade that contains *P. knowlesi* and *P. vivax*, as well as with 8 other species that infect a variety of other animals. The analysis also shows that an unknown species of *Hepatocystis* falls within a clade of *Plasmodium* spp.

The tree generated by Escalante et al. (1998) may be compared with a more recent, large, multigene study of human malarial species, using a maximum likelihood (ML) approach (Rutledge et al., 2017; see Figure 8). First, note that there is a difference in the number of species used in the 2 studies. Several species present in the earlier study by Escalante et al. (1998) are absent in this more recent analysis. Having different species in various analyses is not unusual when different datasets are used; not all species may have been available and the focus of the studies are different. Nevertheless, all of the human malarial species and several other species are present, which allows us to compare the interrelationships of the human *Plasmodium* species in the two studies.

Two clades are highlighted with colors, the *Plasmodium malariae* clade in red and the *P. ovale* clade in blue. The *P. ovale* clade contains the 2 subspecies of *P. ovale* and the *P. malariae* clade contains an additional form (possibly species) that the researchers uncovered in their analysis. Note that the human malarial species are in different clades. In several aspects this tree is similar in topology to the one by Escalante et al. (1998): 1) The human malarial species don't form an exclusive clade by themselves but are spread across the tree in different clades, 2) *P. falciparum* is closely related to *P. reichenowi*, and 3) *P. vivax* and *P. knowlesi* are in the same clade. A notable difference is the relationship between *P. malariae* and *P. ovale*, although note that the node showing the

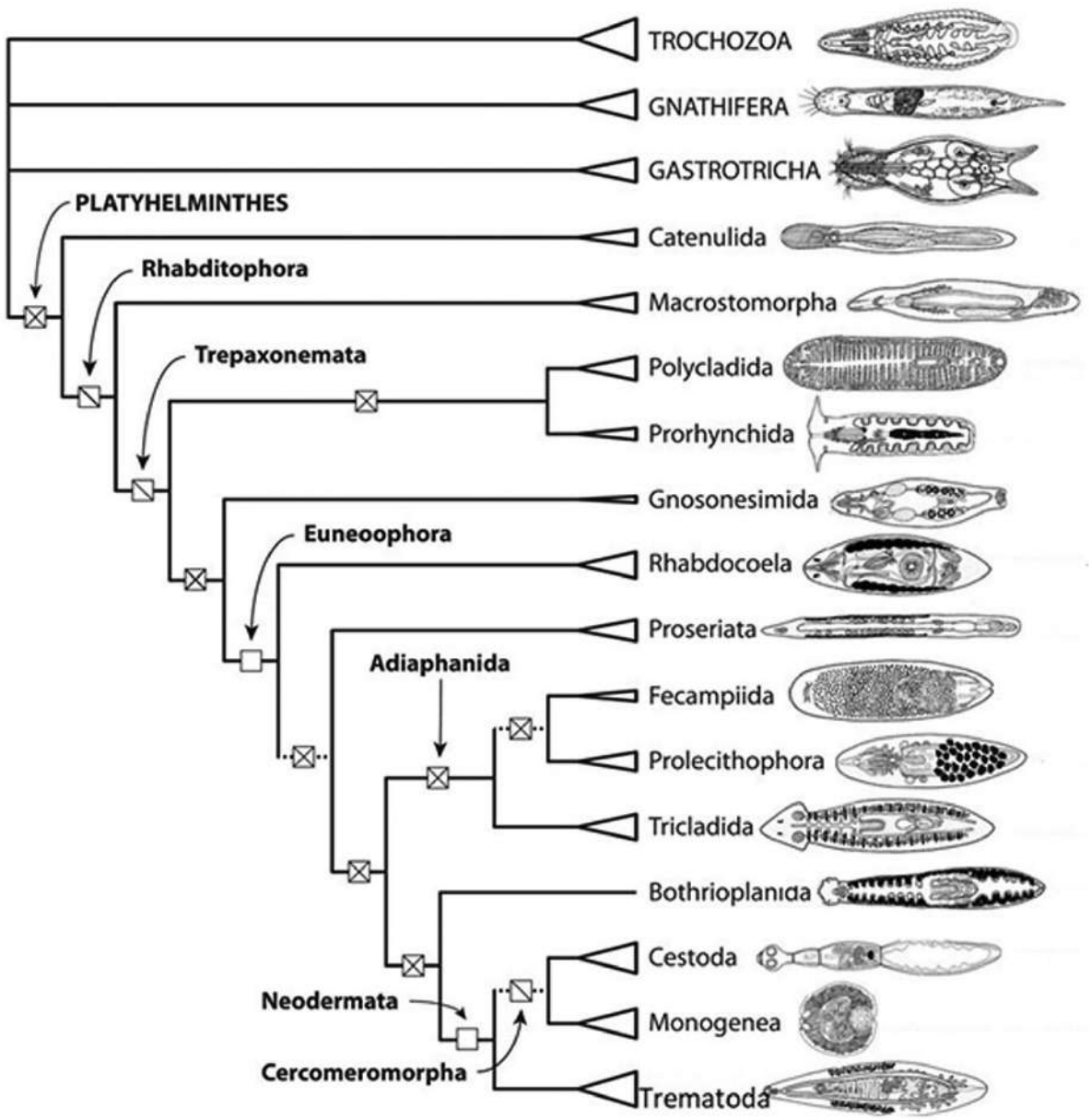

Figure 6. A multi-gene phylogenetic analysis by Laumer et al. (2015) corroborates the inference that all parasitic trematodes had a common ancestor and that monogeneans are likely more closely related to cestodes, but they found weaker support for the Cercomeromorpha than previous analyses suggested. Source: Laumer et al., 2015. License: CC BY.

relationship of these 2 species in the NJ tree by Escalante et al. (1998) has no statistical nodal support. The more sophisticated phylogenetic method used by Rutledge et al. (2017) has resulted in a better (meaning, more robust) tree with very high nodal support for the clades.

Rutledge et al. (2017) also used a molecular clock model to estimate the divergence levels of the species as calibrated to the *Plasmodium falciparum* and *P. reichenowi* split (×). They used a previously published date of 3.0–5.5 Ma (= million years ago) for the *P. falciparum* and *P. reichenow*i split. Calibrating the other splits to this date, they dated the *P. ovale* split to 20.3 Ma and the *P. malariae* split to 3.5 Ma. Cartoon silhouettes show the typical hosts of the different species.

Galen et al. (2018) improved upon previous studies. They analyzed a combined dataset of sequences from 21 protein coding nuclear genes and produced a comprehensive phylogenetic analysis of haemosporidians (see Figure 9). How should the tree be interpreted? Does it change the relationships of human malaria causing *Plasmodium* spp. inferred from previous analyses?

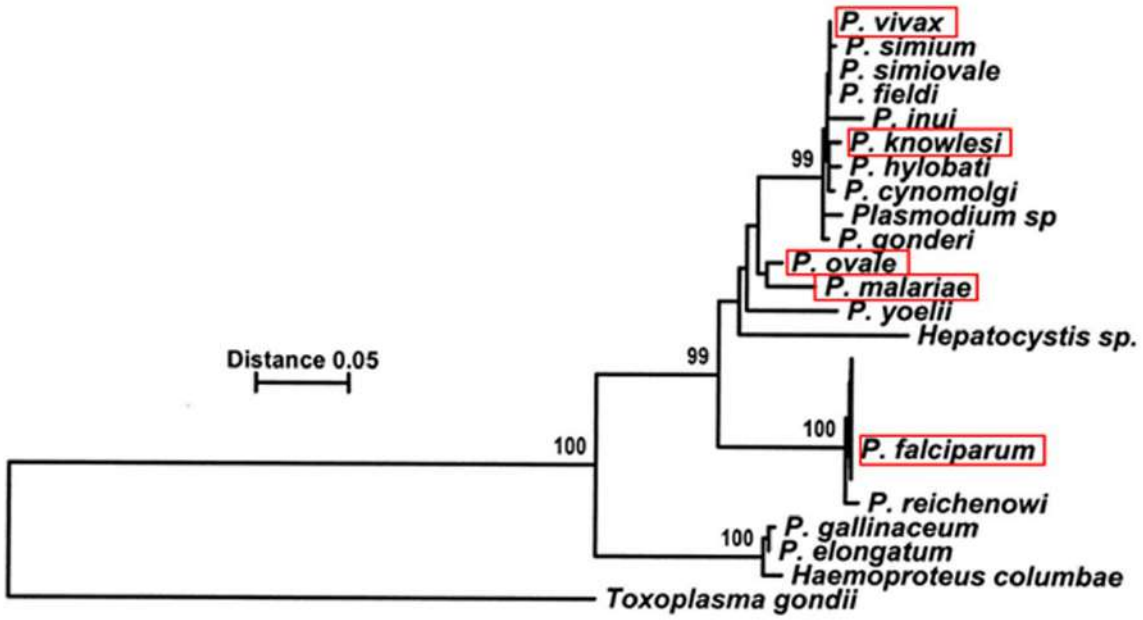

Figure 7. Relationships of the different *Plasmodium* spp., including the human malarial species *P. falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax*. Neighbor joining (NJ) analysis. Source: Escalante et al., 1998. Public domain.

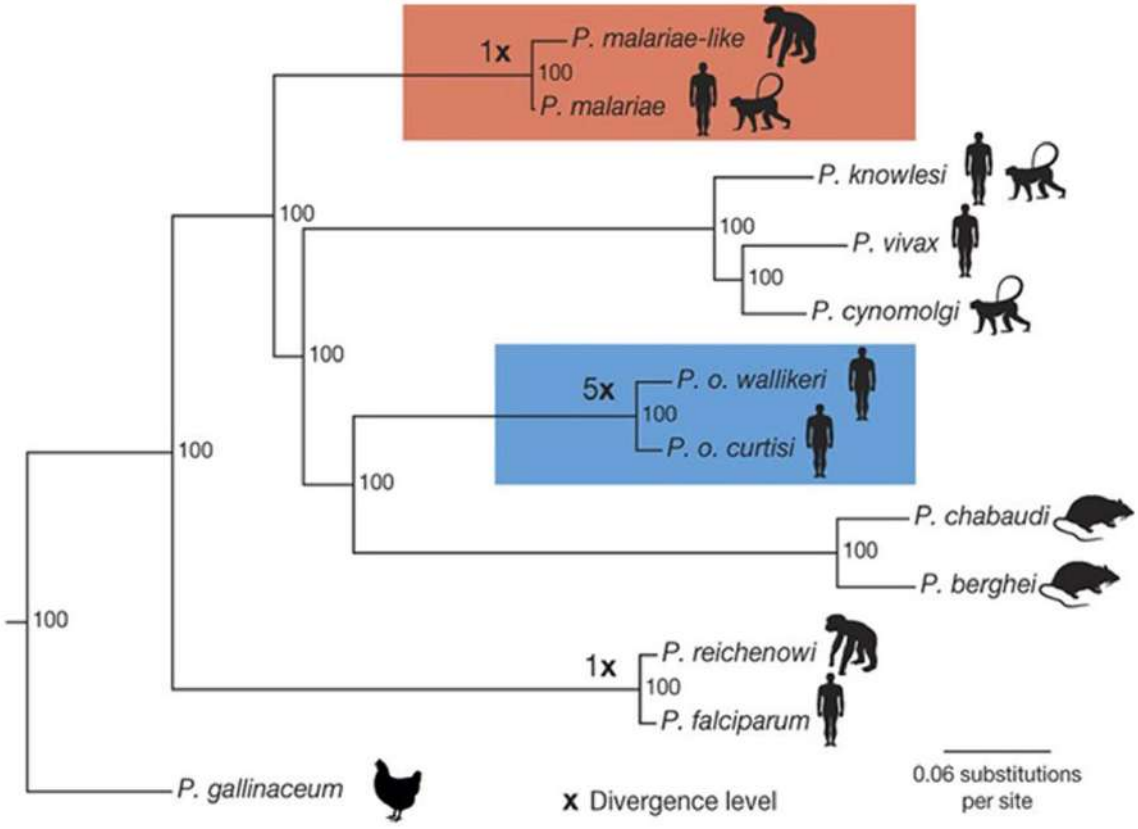

Figure 8. The more sophisticated phylogenetic method used by Rutledge et al. (2017) compared to the one employed in the study by Escalante et al. (1998) has resulted in a better (meaning, more robust) tree with very high nodal support for the clades. Source: Rutledge et al., 1998. License: CC BY 4.0.

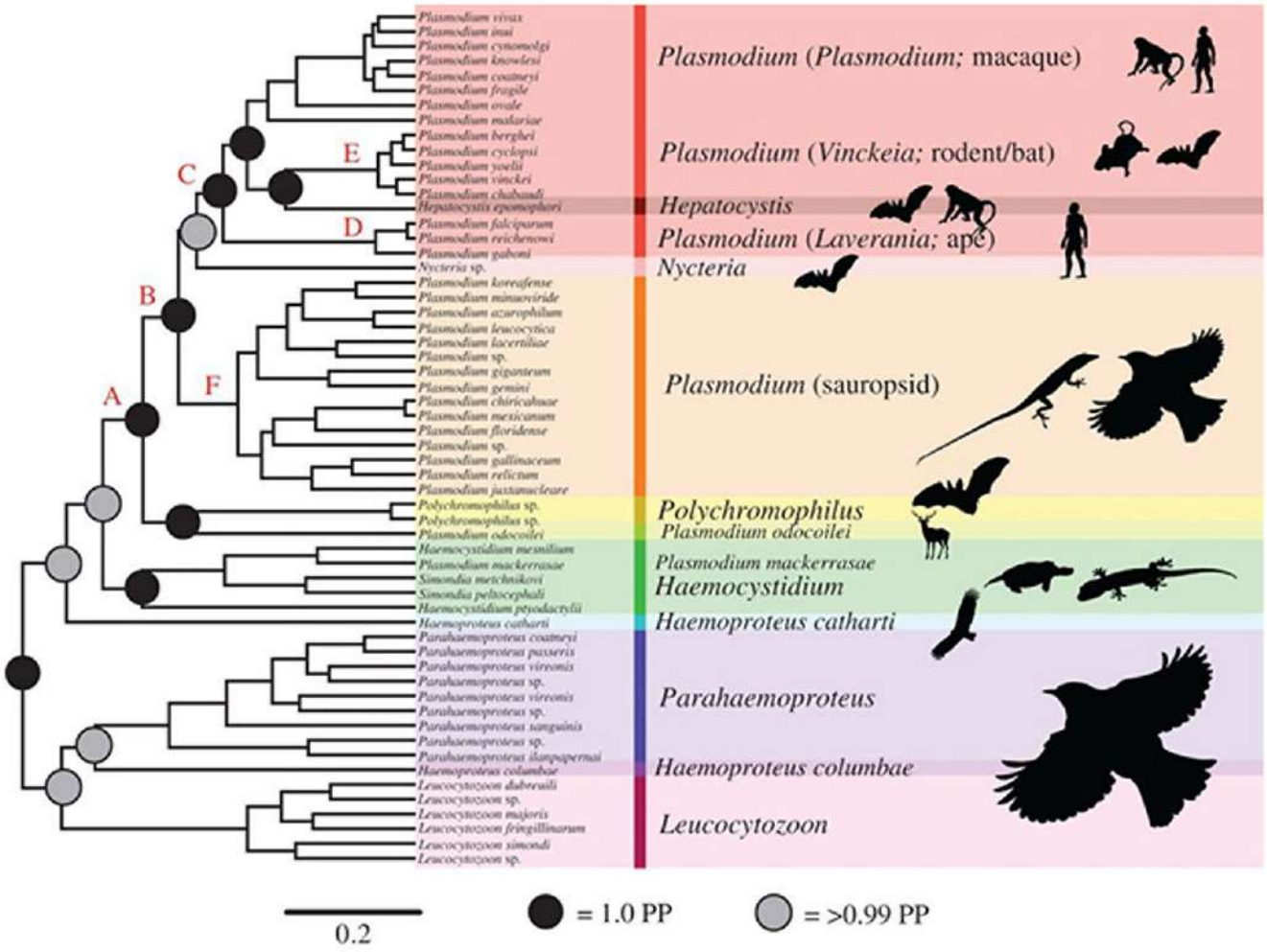

Figure 9. This is the favored haemosporidian phylogeny according to Galen at al. (2018). Shown as silhouettes are representatives of the vertebrate host group for each haemosporidian lineage. Source: Galen et al., 2018. License: CC BY 4.0.

If the tree generated by Galen et al. (2018) is compared with the tree of Rutledge et al. (2017), it is evident that the branching relationships of the human *Plasmodium* spp. are generally consistent. *Plasmodium falciparum* is related to *P. reichenowi*, a relationship that appears in both Escalante et al. (1998) and Rutledge et al. (2017). Thus, it appears that *P. falciparum* had a very separate origin than the other human *Plasmodium* species. *Plasmodium vivax* and *P. knowlesi* still belong to the same clade, albeit without strong support, which corroborates both previous studies. However, this tree goes far beyond analyzing the relationships of the human malarial species. By analyzing all the known haemosporidians, the authors have provided a tantalizing deep historical view of these parasites. It appears that the original ancestral hosts of the haemosporidians are birds (and other sauropsids) of the past.

Coevolution and Host Shifting (Host Switching)

One of the fundamental questions that parasitologists often ask is: How did a particular species of parasite come to be associated with a particular species of host? For example, how did humans become hosts of their 2 louse species, *Pediculus humanus* and *Pthirus pubis*? Comparing the phylogenies of the lice and humans allows exploration of that question. The example shown in Figure 10 is taken from the work of Reed and colleagues (2007). Note that Janzen (1985) considered a more strict definition of coevolution to be reciprocal evolution of host and parasite

When comparing the phylogeny of the lice (on the left) with the phylogeny of their primate hosts (on the right), there is a congruence (topological similarity) between portions of the louse phylogeny and the primate host phylogeny. This suggests that the parasites evolved along with their hosts; this is considered by some researchers to represent

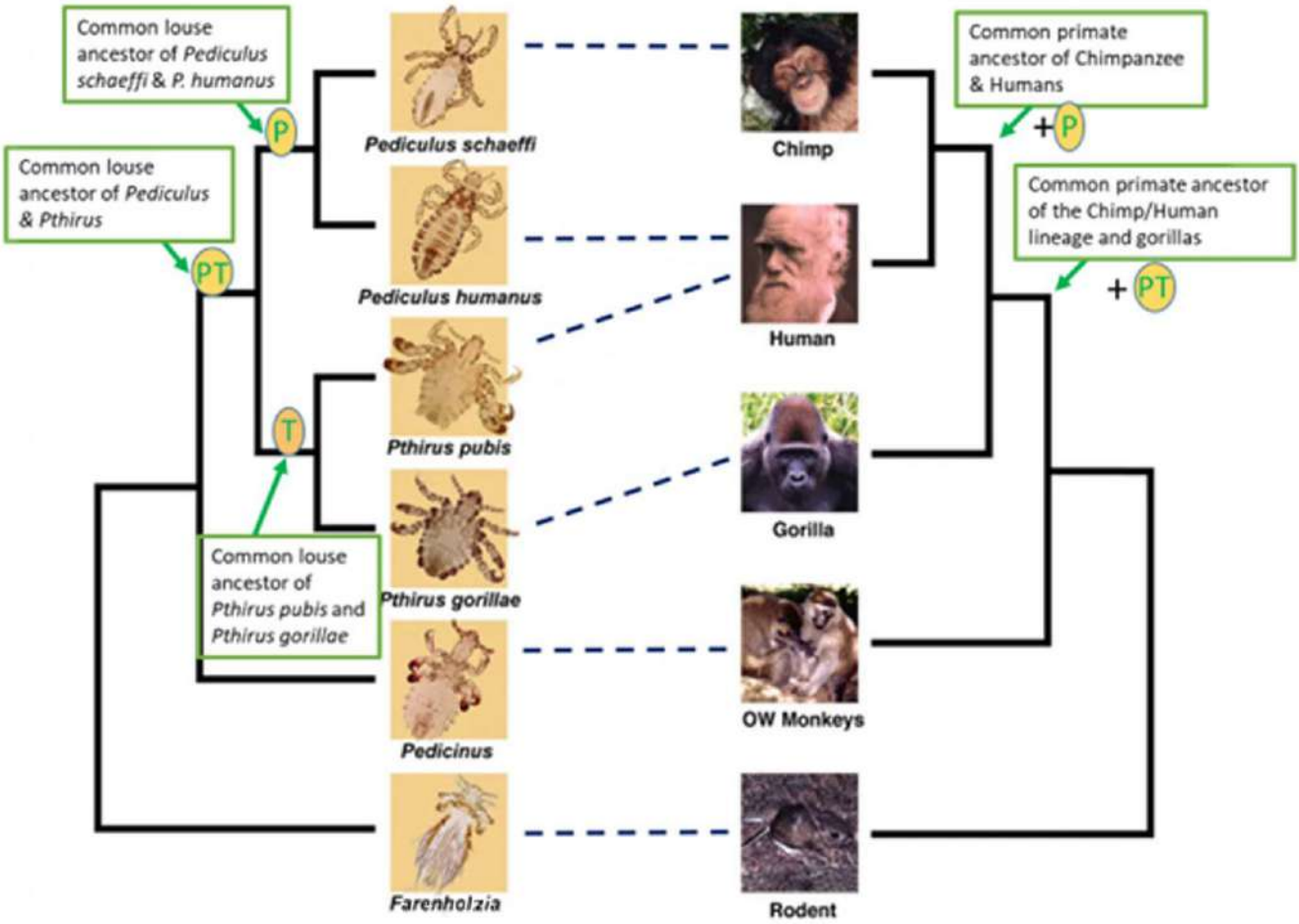

Figure 10. Phylogenetic trees for primate lice and their vertebrate hosts. Trees shown as a cladogram with no branch length information and based on molecular and morphological data. Dashed lines represent host-parasite associations. Humans are unique in being parasitized by 2 genera (*Pediculus* and *Pthirus*). Source: Adapted from Reed at al., 2007. Photo credits: J. W. Demastes, T. Choe, and V. Smith, 2004. License: CC BY 2.0.

coevolution. For example, the 2 sister species of *Pediculus* occur on hosts (chimps and humans) that are also each other's closest relatives. Logically, it may be inferred that the common ancestor **P** of the 2 *Pediculus* sister species was present in the common ancestor of chimps and humans. This type of coevolution, where there is a tight congruence between parasite and host phylogeny, that is, where the parasite phylogeny mirrors the host phylogeny, is called **cospeciation**. Similarly, by further comparing the phylogenies of the louse and primate hosts, we can infer that because the genus *Pediculus* is sister to the genus *Pthirus,* the common louse ancestor **PT** of *Pediculus* and *Pthirus* must have occurred in the common primate ancestor of the chimp-human lineage *and* gorillas. However, upon further scrutiny, it becomes evident that there is an incongruence between the phylogeny of the 2 species of *Pthirus* and their hosts. *Pt. gorillae* is a gorilla parasite and *Pt. pubis* is a human parasite, but the gorilla and humans are not sister host species, while chimps and humans are. So, while the 2 species of *Pediculus* show cospeciation, the 2 species of *Pthirus* do not. How could this have happened? What is the explanation for the current associations of the 2 species of *Pthirus* in gorillas and humans?

There are 2 explanations for the association of *Pthirus* lice. In order to understand the alternate explanations, it will help to first simplify the trees and superimpose the louse and primate host phylogenies (Figure 11).

The incongruence between the phylogeny of the hosts and *Pthirus* (dashed lines, Figure 10) becomes apparent. One explanation for this is that the ancestral *Pthirus* and the ancestral *Pediculus* both originated on the common ancestor of the chimps, humans, and gorillas, that is, there was **duplication** of lineages in that ancestor (see coevolutionary hypothesis 1, Figure 12). Our human hominid ancestors retained

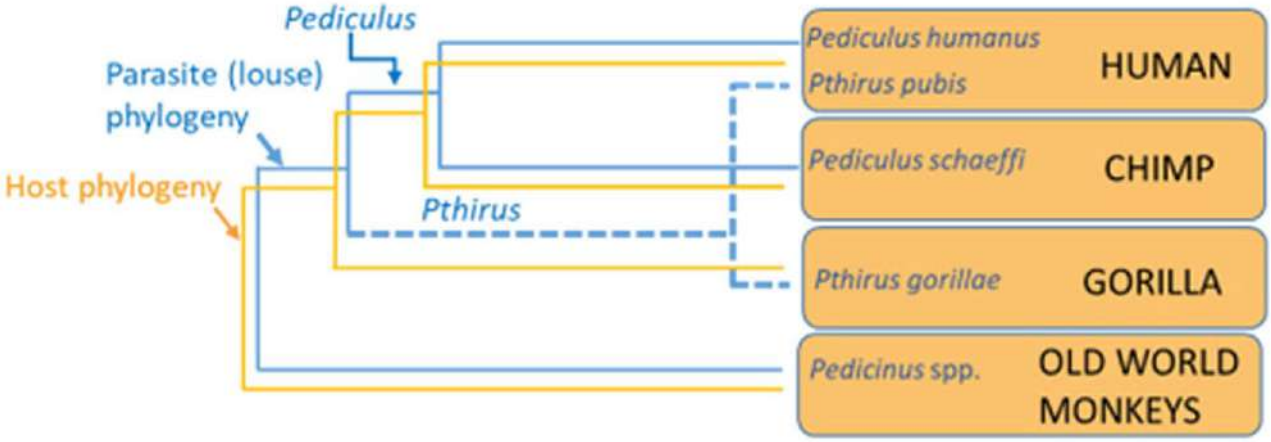

Figure 11. A simplification of the trees with the superimposition of the louse and primate host phylogenies. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

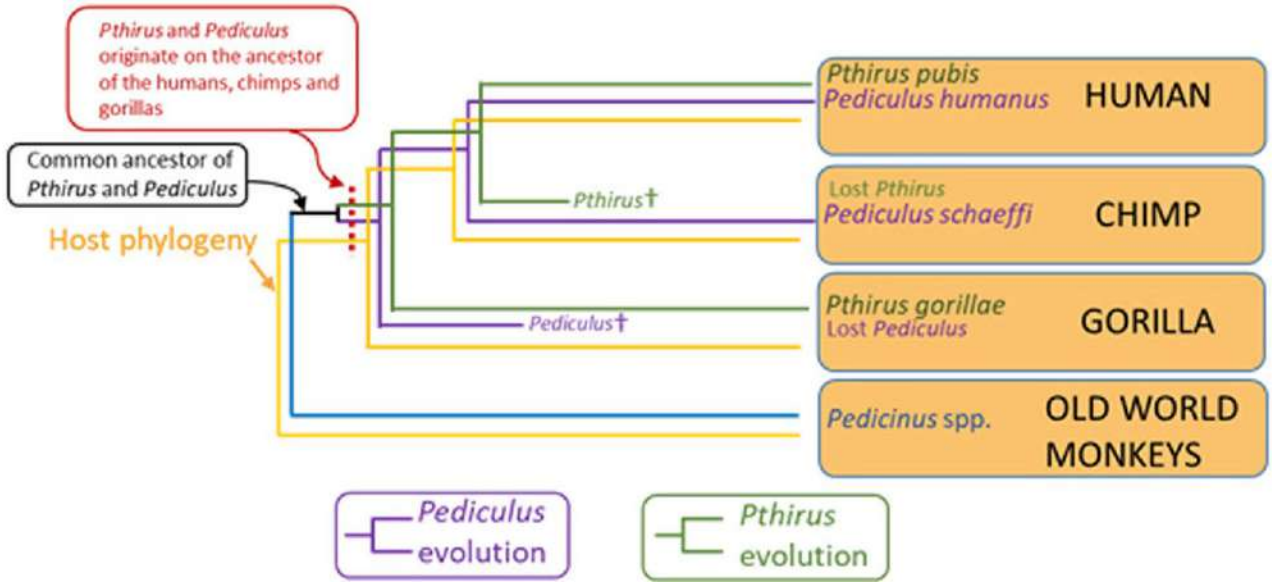

Figure 12. Coevolutionary hypothesis 1. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

both lineages (*Pediculus* and *Pthirus*) but the chimpanzee lineage lost *Pthirus,* while the gorilla lineage lost *Pediculus*. In other words, in this reconstruction, a neat pattern of co-speciation is altered by extinction events in 2 host lineages (chimps and gorillas) that resulted in the louse-host associations seen today.

There is, however, a simpler explanation that does not require the elaborate extinction events proposed in the previous hypothesis. Instead, it may be proposed that human hominid ancestors acquired the louse ancestor of humans' *Pthirus pubis* from some ancient ape of the gorilla lineage, that is, by **host-shifting**, also known as **host-switching** (see coevolutionary hypothesis 2, Figure 13) or **ecological fitting** (see Janzen, 1985).

How to choose between these 2 alternate reconstructions or hypotheses? The principle of parsimony may be applied and then it may be argued that the second hypothesis requires only **1** step, 1 instance of host-shifting, to explain the incongruence between the louse and primate phylogenies. In contrast, the first hypothesis required a lineage duplication, followed by 2 separate, independent, instances of lineage extinction. Therefore, hypothesis 2 is the more parsimonious explanation and in the absence of the any other evidence to the contrary, is the preferred working hypothesis. In this

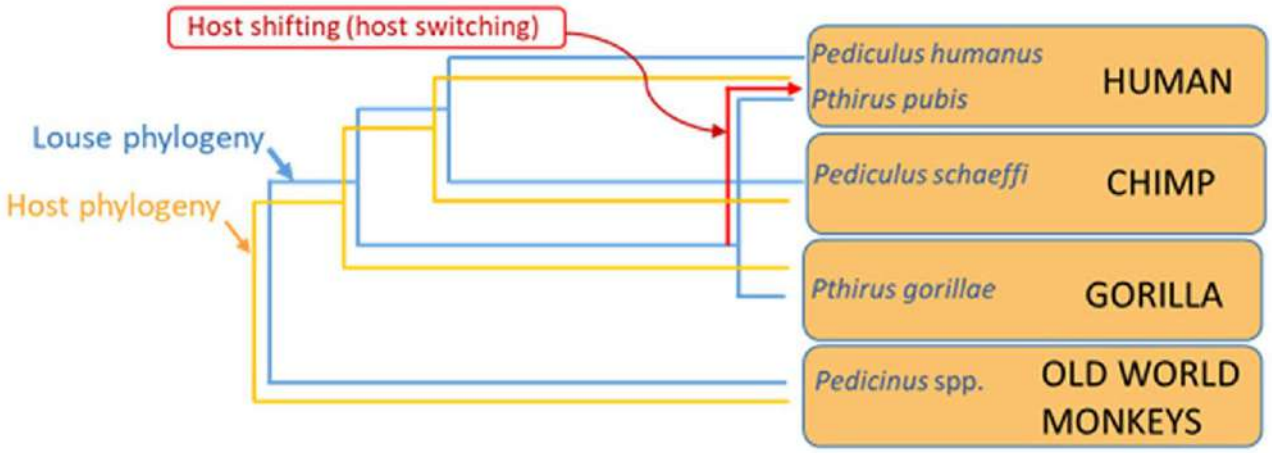

Figure 13. Coevolutionary hypothesis 2. Source: A. Choudhury, 2019. License: CC BY-NC-SA 4.0.

particular case, however, the authors were able to apply evidence from estimated divergence times of the primate host lineages to show that *neither* hypothesis on its own was consistent with the known evolutionary history of the hosts. Their final analysis indicated that both duplication and extinction, followed by host-shifting likely occurred to produce the present-day associations between the lice and their primate hosts.

Phylogenetic Systematics, Coevolution, and Biogeography

Phylogenetic systematics not only allows the examination and exploration of the coevolution of parasites and hosts but also their historical biogeography, that is, how and where they came to be associated with their hosts. Here is a simple example that illustrates this application. The trematode genus *Bunodera* comprises species that are found in fishes belonging to the family Percidae (perches) and Gasterosteidae (sticklebacks). Three of the species occur in sticklebacks. By mapping the hosts and their distribution on the phylogeny of these trematodes (Figure 14), both the coevolutionary history as well as the history of the host-parasite associations

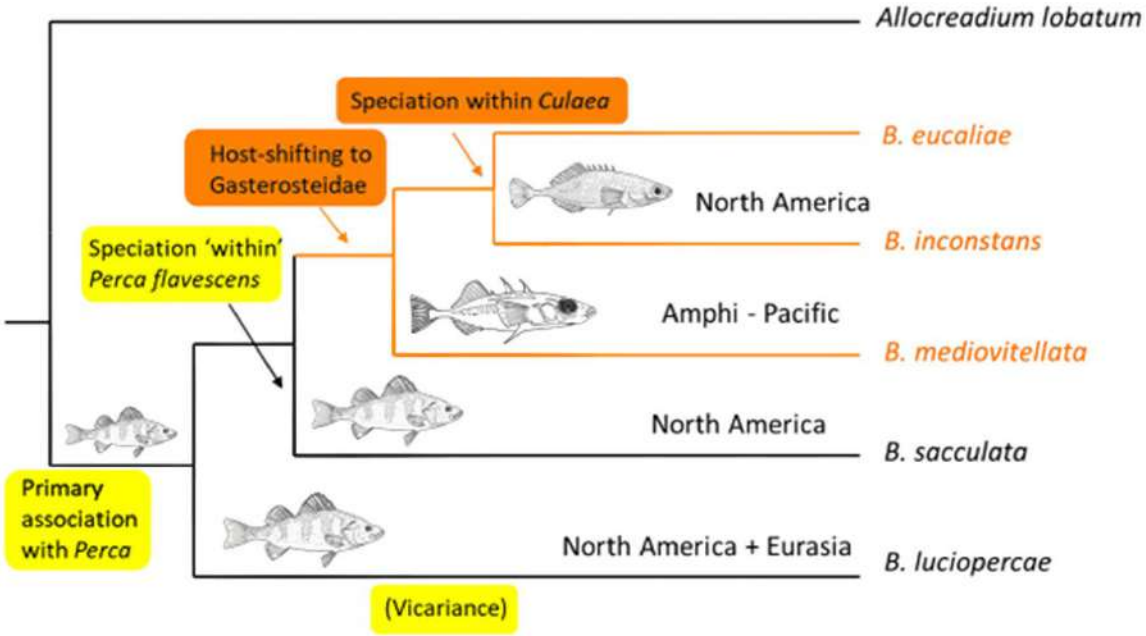

Figure 14. The trematode genus *Bunodera* likely originated in percid fishes in the northern latitudes and became associated with sticklebacks in North America via ecological fitting in the distant past. There appears to have been further speciation in the freshwater brook stickleback, *Culaea inconstans*, a stickleback species endemic to the freshwaters of North America. Source: A. Choudhury and V. León-Règagnon, 2005. License: CC BY-NC-SA 4.0.

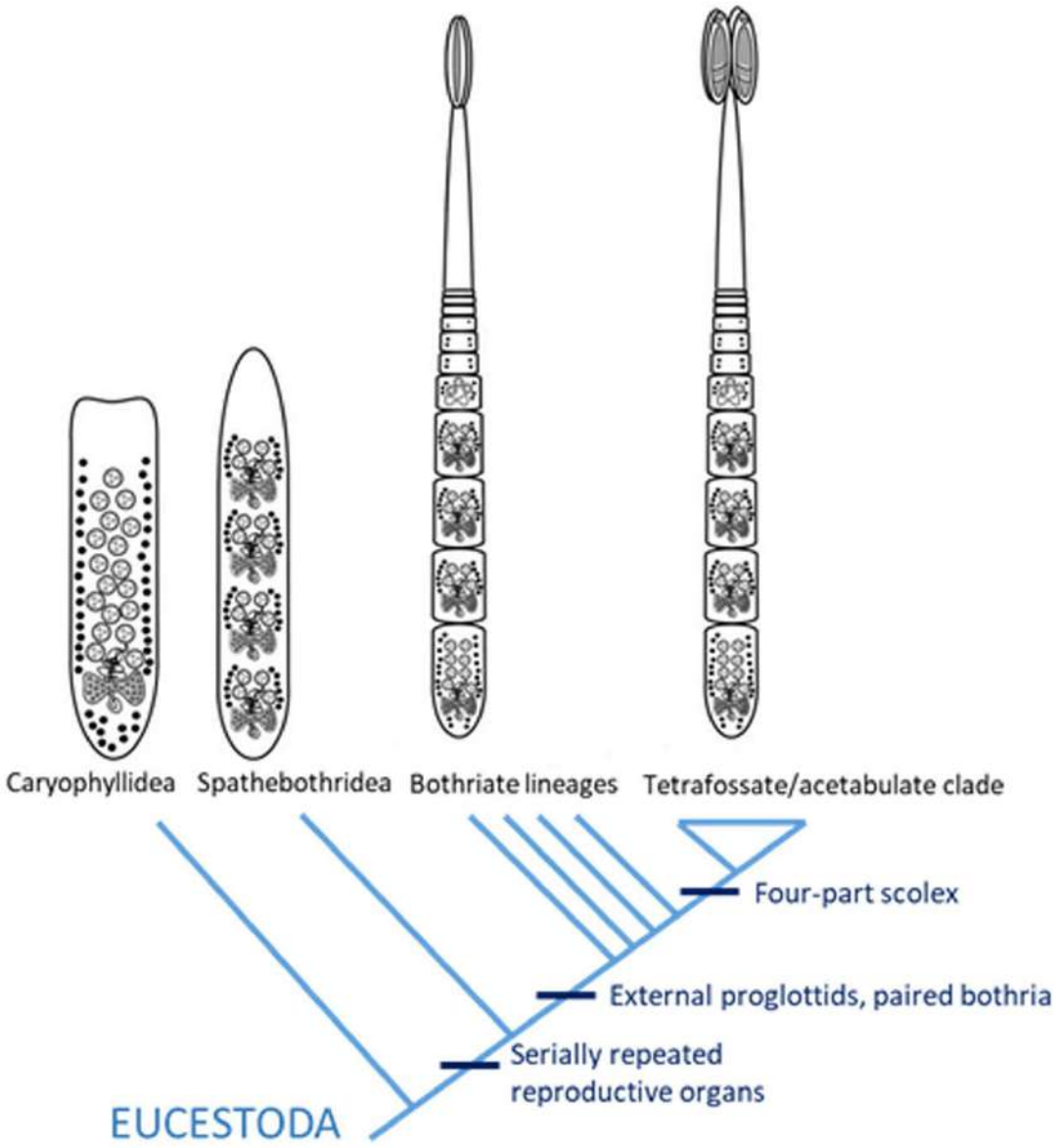

Figure 15. A phylogenetic representation of the evolution of strobilization as a derived character in some cestodes. Image source: A. Choudhury modified after Olson et al. (2001), 2019. License: CC BY-NC-SA 4.0.

may be deduced. Doing so reveals that the genus likely originated in percid fishes in the northern latitudes and became associated with sticklebacks in North America via ecological fitting in the distant past. There appears to have been further speciation in the freshwater brook stickleback, *Culaea inconstans*, a stickleback species endemic to the freshwaters of North America.

Phylogenetic Systematics and Mapping Traits

Phylogenetic trees also can help elucidate the evolution of body plans and a variety of morphological, biological, and behavioral traits. Consider, for example, the bewildering diversity of tapeworms, the Cestoda. The vast majority of tapeworms belong to a subgroup called the Eucestoda. Among the eucestodes are an order of unsegmented tapeworms with a single set of reproductive organs, Caryophyllidea. Another order, Spathebothridea, also comprises unsegmented tapeworms, but they possess serially-repeating sets of reproductive structures. The vast majority of the remaining eucestodes have a strobila with externally-visible segments called proglottids. Is the unsegmented condition with a single set of reproductive structures as seen in Caryophyllidea a primitive feature? Are the caryophyllideans an early branching lineage of tapeworms or is their morphology highly

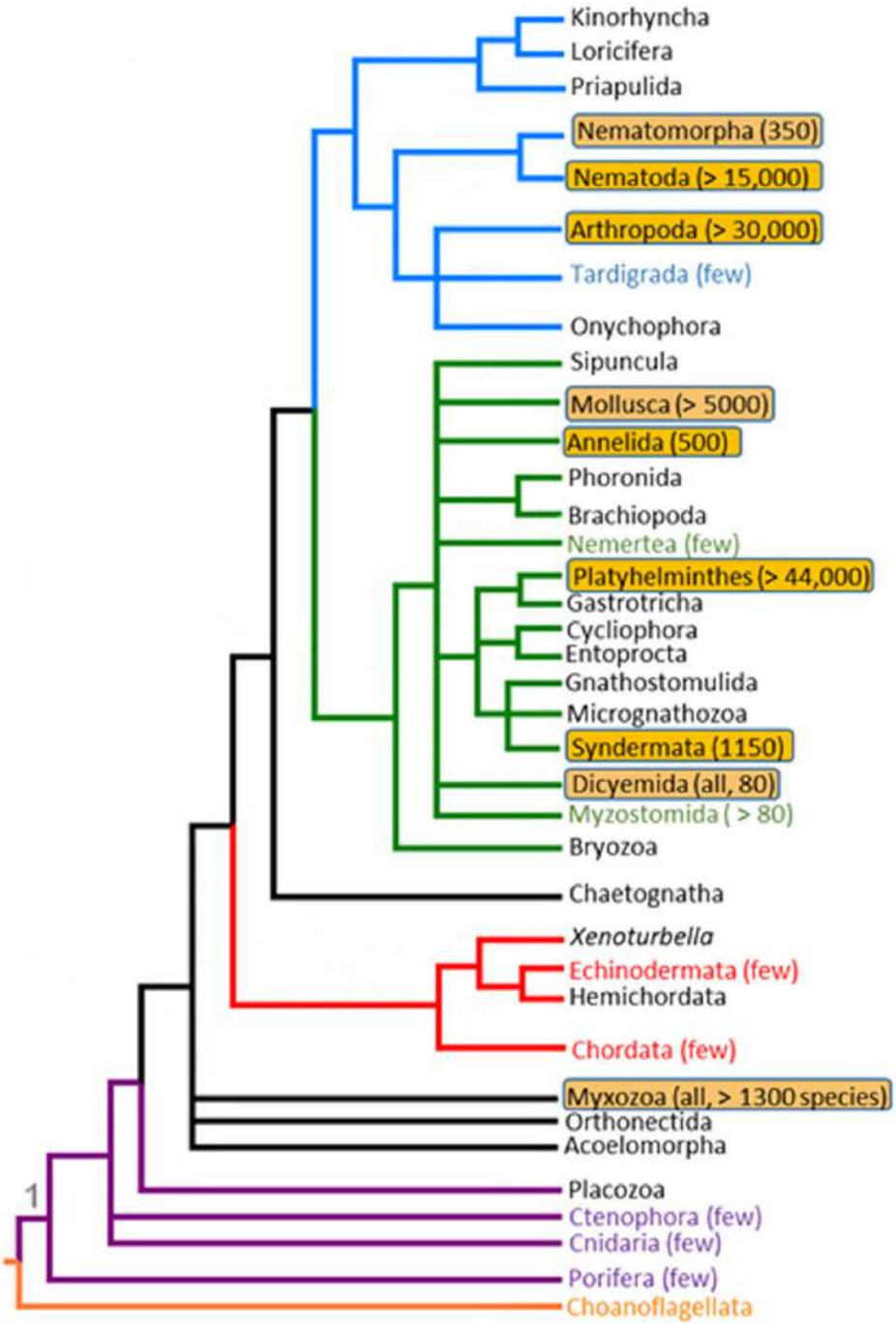

Figure 16. Metazoan phylogeny showing the wide-ranging polyphyly of parasites. Source: A. Choudhury modified after Wlodzimierz (2006), 2019. Public domain.

modified from strobilate segmented cestodes? Mapping the morphology of tapeworms on their phylogenetic tree allows us to address these questions.

A phylogenetic analysis of the Eucestoda by Olson and his colleagues (Olson et al., 2001; see Figure 15) shows that Caryophyllidea is an early-branching group and further reveals that the condition seen in Caryophyllidea is primitive and not highly modified and reduced from strobilate ancestors. The phylogenetic tree also reveals that the superficial external segmentation (proglottisation) of cestodes is a more derived condition and that a scolex with 4 attachment structures (plural bothridia, singular bothridium) may have evolved from a scolex with 2 attachment structures (plural bothria, singular bothrium).

Parasites Are a Polyphyletic Assemblage with a Common Lifestyle

Parasitology is unique in the field of organismal biology since most other subjects in organismal biology are developed and organized around monophyletic organismal groups; ornithology is the study of birds, entomology the study of insects, acarology the study of mites and ticks, mammalogy the study of mammals, and so on. Unlike these other subjects that deal with monophyletic groups of organisms, parasitology is the study of certain organisms, in this case, parasites—all of which share a common **lifestyle** (parasitism), rather than a unique common ancestry as a group. In other words, there is no unique common ancestor only for all parasites. If the phylogenetic tree of animals is examined, parasitic species will be found in a wide range of phyla, highlighted in the tree above (Figure 16), along with their free-living relatives. Parasitic nematodes are related to free-living nematodes, parasitic trematodes to free-living trematodes, parasitic annelids to free-living annelids, and so on. The approximate number of parasitic species in each phylum is in parentheses. This clearly shows that parasitism evolved independently many times in the evolution of life on Earth, and that parasites evolved from pre-existing, closely related, free-living ancestors.

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Supplemental Reading

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Helminth Identification and Diagnostics: Basic Molecular Techniques

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Chapter 3

Helminth Identification and Diagnostics: Basic Molecular Techniques

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Introduction

Molecular systematics, that is, the use of DNA sequences to address a variety of questions on the identity, species boundaries, and relationships of organisms has now become a powerful and useful approach that complements traditional systematics based on morphology. A perusal of the literature on parasite systematics suggests that much but not all recent understanding and hypotheses of parasite identification and phylogenetic relationships have been obtained through the application of molecular methods (for example, Olson et al., 2003; Nadler et al., 2010). This review summarizes some key protocols in molecular systematics as are used for studying helminth parasites.

Collection of Specimens

The first step in doing molecular systematics is the proper recovery of helminths from the host. Although the specimens used for DNA extraction and subsequent processing need not be handled in the same gentle manner as specimens for morphological studies, they should be collected live, cleaned in 0.6% saline or PBS (phosphate buffered saline) by gentle pipetting or agitation in a petri dish to wash off adhering debris, and then preserved and stored for subsequent processing. Specimens that are to be used for DNA work should be stored directly in 95% or 100% ethanol, making sure that the ethanol does not contain denaturing agents such as ketones, aldehydes, methanol, or kerosene, which are harmful to DNA. A careful reading of the label on the ethanol bottle will indicate what denaturing agents were used. Often, commercially available 95% ethanol is preferred because it may not contain any denaturing agents. Isopropanol can be allowed as a denaturing agent. The sample should be stored in ethanol in a cryovial or in a similar suitable vial and should be kept chilled in a regular freezer (at –20 °C) if possible or in a regular refrigerator (approximately 4 to 8 °C) until use. As a cautionary note, formalin is very harmful for DNA work and the worms being used for DNA analysis should never be brought in contact with formalin. See Gardner and Jiménez-Ruiz (2009) for details on collection methods.

Note that each time a sample of worms is collected with the intention of doing molecular work, a small subsample of worms from the same batch should also be separately fixed for a corresponding voucher sample to confirm the identity of the worms being studied using morphological examination. These specimens should be fixed by the proper techniques that will allow good stained whole mounts to be produced and be suitable for histology or scanning electron microscopy (SEM). For certain helminths (cestodes, trematodes, nematodes), using hot (steaming) 5% or 10% neutral buffered formalin is an easy way of producing relaxed and well-fixed specimens for subsequent stained whole-mounts. If a fume hood or proper ventilation is not available, killing helminths with hot PBS (or saline) and then placing them in unheated fixatives (formalin alcohol acetic acid (FAA) and so on) will suffice for producing adequate stained whole mounts, but worms fixed in this way are not suitable for histology and not ideal for SEM work.

In certain cases, for example, in the case of cestodes, a piece of the worm may be collected in ethanol for DNA analysis and the rest of the worm fixed for morphology, which now allows the specimen to be treated as a **hologenophore** (meaning, a vouchered specimen for which there is corresponding DNA sequenced data) (Pleijel et al., 2008). Occasionally, acanthocephalans, nematodes, monogeneans, and larger trematodes can also be treated in this manner (Gardner and Jiménez-Ruiz, 2009).

Another technique that is now often used is killing the worms in hot water or hot PBS and immediately placing them in 95% ethanol. This saves time and desired portions of the worms can be later excised in the lab for DNA extraction. The disadvantage of this method is that ethanol is only a preservative and is not a fixative, and 95% ethanol can cause worms to shrink, become rubbery, and collapse.

While collecting and fixing specimens for morphological and molecular studies, it is important that vials, Petri dishes, and pipettes that have come in contact with formalin or other fixatives such as Bouin's or FAA (AFA) be kept separate from instruments and glassware used for handling worms being collected for DNA work.

Several specimens should be collected for molecular analysis but even 1 specimen is better than none. For worms that are less than 0.5 mm in length, 2–5 specimens are usually enough to guarantee sufficient DNA on extraction. For specimens 3–5 mm in length, 1 or 2 specimens is/are usually sufficient. DNA can be even extracted from single worms as small as 0.2 mm. Specimens can be stored in 100% molecular grade ethanol in a refrigerator or freezer for years but the quality of the DNA does decline with length of storage time unless the sample is stored at less than −85º C.

Another important aspect is the proper recording of data and the proper labeling of tubes. Tubes or vials that contain specimens for DNA work should be labeled on the outside with paint markers or in other ways that will not be erased by freezing and thawing. Paper labels are often used for labeling specimens inside the vial but should not be used for specimens being stored for DNA analysis because the labels may introduce contaminants.

DNA Extraction

DNA can be extracted from collected worms using standard techniques, such as phenol-cholorform extraction or a variety of commercially available kits. The phenol-chloroform extraction is a standard extraction technique, but phenol is a harmful chemical and the procedures have to be conducted with the proper precautions. As a result, scientists have switched to less toxic methods or easier and less toxic alternatives such as commercially available and fast extraction kits such as Qiagen's DNEasy DNA extraction kit. Other companies, such as Invitrogen, Promega, and others, also manufacture extraction kits. Such kits combine extraction with a subsequent cleaning step and each company provides a booklet with its kit that outlines the protocol. The extracted DNA can be stored in the freezer at −20 °C or at colder temperatures of −85 °C (or even lower).

DNA Amplification

The next step in the process is the amplification of the desired genes of the specimens from which the DNA is extracted. In helminth systematics, the ribosomal RNA gene array (sometimes referred to as rRNA) and the cytochrome *c* oxidase subunit 1 gene (*CO1*) are commonly targeted for obtaining sequences. The most common regions of the rRNA gene array are usually parts of the small subunit (18S) and large subunit (28S) but also portions of the internal transcribed spacers (ITS-1 and ITS-2) as well as the 5.8S region. In the absence of full-length sequences, partial sequences of certain regions of these genomes are still useful. The method that is most widely used for amplifying portions of the target genes is the polymerase chain reaction (PCR). The **PCR reaction** requires several key ingredients:

1) A **polymerase enzyme** that will not denature at high temperatures. The successful isolation and commercial production of a polymerase from thermophilic prokaryotes allowed such enzymes to be used in the high temperature conditions encountered in the reaction. Several types of polymerase enzymes are available of which the polymerase isolated from the hot springs bacterium *Thermophilus aquaticus* (Taq polymerase) is the most common. This enzyme can be purchased from a variety of biotech companies.
2) **Primers**: These are small (usually 20–30 bp long; bp = base pairs) strands of DNA with sequences that are identical to portions of the genes that are being targeted for amplification. In a PCR reaction, primers are used in pairs (a forward primer and a reverse primer), and prescribed quantities of each primer are used. The forward primer binds upstream on the target gene and the reverse primer binds downstream and they work in opposite directions on each of the 2 complementary single strands of the double stranded DNA (ds DNA); the denaturing of DNA is part of the PCR reaction. Primers are usually made to order by supplying the biotechnology company that manufactures primers the letter sequences needed. There are several standard primer sequences that have been published in the literature.
3) **Magnesium buffer**: A special buffer that contains the required amount of magnesium for the enzyme to work adequately is supplied by the company that supplies the polymerase enzyme.
4) **DNA substrate**: This is the DNA that was extracted from the parasites using the protocol outlined before.

The reagents listed above are mixed in prescribed amounts in special PCR tubes and the reaction mixture is placed in a thermocycler. Numerous models of thermocyclers are commercially available from biotech companies, such as the ones made by Perkin-Elmer. Thermocyclers can be programmed and users have to specify the reaction conditions. Most published papers specify the PCR conditions. The PCR method, once standardized for a certain pair of primers, can be repeatedly used with success. Once amplification is completed, the PCR tube is removed from the thermocycler and the amplified DNA is first tested by running (electrophoresis) a small

aliquot (~ 5 µl) on a mini gel along with a DNA ladder appropriate for PCR products. PCR products can range anywhere between 300 to 2,000 bp, depending on the primers, the gene being targeted for amplification, etc. If the electrophoresis gives positive results and there is no evidence of mispriming (multiple amplified products on the gel), the remaining PCR product is purified by passing it through a membrane or column which binds the amplified DNA, which is then eluted out in a buffer or sterile deinonized water. There are standard kits for purification that are available commercially from biotech companies. This amplified and purified DNA sample can be stored in −20 °C or −80 °C (or lower) and a small amount of this is usually used for sequencing.

When the sample is ready for sequencing, it is thawed and a small aliquot of the purified PCR product is sent along with an aliquot of the primers but separately (unlike the PCR reaction, the sequencing reaction only uses one of the primers at a time). The sequencing reaction usually requires ~ 40 ng of purified amplified DNA and so the purified DNA has to be quantified first. Quantification can be done using DNA quantification ladders in a mini gel electrophoresis.

Sometimes the sequencing primers may be different from the PCR primers but most times the PCR primers are also used for the sequencing reaction. The sequencing can be done manually but this is time-consuming and no longer cost effective. Instead, most sequencing is now done on automated sequencers but due to the high cost of purchasing, maintaining, and operating automated sequencers (both material and personnel costs), many labs send their PCR products and primers in a standardized mixture to biotech labs that offer sequencing services. The turn-around time is usually fast. In the United States, many such sequencing facilities are able to send back the sequences within 2–3 days of receiving the samples.

In summary, here are the steps in PCR-based identification and systematics:

1) DNA extraction
2) PCR-based amplification
3) Purification of PCR product
4) Sequencing
5) Retrieval and evaluation of DNA sequences
6) Alignment of sequences
7) Comparisons and phylogenetic analyses

Working with the Sequences

Sequence data are usually received in 2 formats: As chromatograms and as actual nucleotide (letter) sequences. Each sequence is first manually checked for accuracy by checking the chromatogram, using a viewing or editing software package such as FinchTV (Geospiza, Inc.) or ABI EditView, or any number of other packages for manipulation of molecular sequences. These programs can generally be downloaded from the web. Undetermined nucleotides in the sequences to be examined are either left as "N" or are replaced by the correct nucleotide if this is apparent from the chromatogram. Careful examination and proper judgment are necessary to determine how much of the sequence is usable. The usable portion is extracted and copied and pasted into a sequence manipulation program. Such a program allows the assembly of a database of sequences for further comparison and analysis.

Often, one of the first steps in using any DNA sequence that is generated is finding what that sequence is most similar to among the vast number available in GenBank. GenBank is a repository of sequences deposited by researchers from published and unpublished studies (https://www.ncbi.nlm.nih.gov/genbank/). The search is done using the Basic Local Alignment Search Tool (BLAST) through the NCBI BLAST portal (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This search, which provides results usually in a few seconds to minutes, allows one to see a list of taxa with sequences that match the sequence that has been generated. The BLAST search also shows pairwise comparisons between the sequence submitted and the sequences that match it as well as other details of the comparisons.

One popular program that allows working with the sequences is MEGA (Molecular Evolutionary Genetic Analysis). It is also updated in a timely fashion by the authors, the latest version being MEGA 11.0. This program can be downloaded without cost from https://www.megasoftware.net. In MEGA, sequences from GenBank can be downloaded into an alignment file for additional comparisons. Once an alignment file with the sequences of various species of interest has been compiled, the next step is to align these sequences, that is, to have the nucleotide bases lined up in a homologous corresponding manner (since we do not know the exact position of the sequences in the genome); different sequences may start and end at different base positions in a gene or genome. There are several stand-alone programs that can also be used to align sequences, such as 'ClustalX' (Thompson et al., 1997). In MEGA, the sequence alignment programs 'ClustalW,' and 'Muscle' are embedded within the MEGA software. Alignments are performed on the assembled sequences from the various species using parameters that are set by the program or by manipulating certain parameters depending on the nature of the sequences (Hall, 2001). A copy of the unaligned raw sequences should always be saved and not overwritten by the aligned file because if a new sequence is added to the database, it must be added to the unaligned (meaning, raw) sequence database and the alignment performed again.

Systematic Applications

Once the sequences have been aligned, the unaligned extra overhanging portions on either side are pruned or trimmed and this new dataset can now be used for a variety of purposes, including:

1) The sequences of species can be compared to determine the similarity. This may provide clues as to whether or not 2 samples belong to the same species or can be used to study variation between populations. For example, if consistent molecular differences among isolates from the same geographical area correlate with morphological differences and/or different levels of host range, then a case can be made for different species.
2) The aligned sequences can be used for identification purposes or to determine the evolutionary relationships among the species being studied. There are several programs that can be used for such analyses and several are available in MEGA. There are various settings that can be chosen while doing a phylogenetic analysis, and there are various methods to evaluate how robust the resulting tree of relationships is; the bootstrap analysis is perhaps the most common.

Examples of Explanations about How to Identify Particular Species

Correct application of species names to specimens by biologists is critically important, because species are named according to the agreed-upon rules of scientific naming using the system of **binomial nomenclature** developed by Linnaeus (1758) with the publication of the 10th edition of Systema Naturae. Each species with a unique binomial (**bi** = 2; **nom** = name, from Greek; in this case, **genus** and **species**) provides an instant means to know what species are being referred to anywhere in the world (ICZN, 2024). Following are descriptions of a few sources of methods for species identification.

A useful example of the application of molecular techniques to address questions of helminth systematics is a paper by Hernández-Mena et al. (2019) that examines the relationships of species in the family Allocreadiidae. Pertinent references as well as details of the methods used can be found there.

Methods for collecting and processing mammals for museum collections can be found in Wilson et al. (1996). Specific techniques for collecting parasites from vertebrates can be found in Gardner and Jiménez-Ruiz (2009), which is focused on obtaining and processing parasites from bats; however, the methods can be applied to collections of helminths, ectoparasites, protozoans, and blood parasites from any of the vertebrate classes. Additional methods are found in a book chapter specifically written for reptiles by Gardner et al. (2012), and for mammals in general by Gardner (1996) and Galbreath et al. (2019).

Examples of descriptions of species of *Eimeria* (phylum Apicomplexa: family Eimeriidae) include Jensen et al. (2015) and Tinnin et al. (2012). Some examples of descriptions of nematodes (phylum Nemata) can be found in Drabik and Gardner (2019) and Rodrigues et al. (2020). For descriptions of some of the phylum Platyhelminthes including cestodes, see Caira et al. (2017), and for those in the family Arostrilepididae, see Dursahinhan et al. (2022). For descriptions of trematodes of the family Dicrocoeliidae, see Gardner and Pérez-Ponce de León (2002). This is just a small sampling of available valid descriptive literature.

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4

Parasites in Relation to Other Organisms

Hosts, Reservoirs, and Vectors

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Chapter 4

Hosts, Reservoirs, and Vectors

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Introduction

From the parasite's perspective, a **host** represents a resource and a habitat where the parasite can grow and reproduce. Once produced, reproductive stages of the parasite must find their way back to infect another host. Unlike most free-living organisms, one of the major challenges for a parasite is to continuously encounter and colonize suitable hosts for the propagation of the next generation in the life cycle. From a statistical point of view, any individual parasitic organism has an exceedingly low probability of transferring from one host to another. Indeed, the spatial and temporal difficulties parasites face to complete their life cycle must be overcome by enormous reproductive outputs and/or by exploiting complex ecological associations between successive hosts (Tinsley, 1990).

For any parasite transmission event to occur, an infective stage of a parasite has to first encounter a potential host. This challenge can be considered an **encounter filter** (Euzet and Combes, 1980). For example, ecological conditions will affect the spatial and temporal overlap of host and parasite populations and species-specific behavioral characteristics can bridge or reduce encounters between parasites and their hosts. Adaptations that increase encounter rates with potential hosts will likely lead to higher infection probabilities (Combes, 2005). Following the encounter; however, another hurdle must be cleared which can be thought of as a **compatibility filter**, and this must be overcome for a parasite infection to become established. In this case, and after encountering a potential host, the compatibility filter determines whether the parasite is able to survive, grow, or reproduce in the host. For example, a parasite might be able to infect a variety of different species of potential hosts, but most of those species would not possess the necessary resources for the parasite to survive. Even when appropriate hosts are encountered, host susceptibility to the parasite is controlled by a variety of host factors such as genetics, immunity, and physiology, among others (Combes, 2005). To overcome these challenges, parasites have evolved various types of life cycles, which include different types and combinations of hosts used for multiplication, growth, reproduction, and/or transmission.

The Role of Hosts in Life Cycles and Transmission of Parasites

Parasitologists differentiate among various types of hosts based on the specific roles those hosts play in the development, reproduction, and transmission of the parasite. In a typical life cycle, a host in which a parasite reaches sexual maturity and reproduces is known as the **definitive host**. In contrast, an **intermediate host** is one that is required for parasite development, but one in which the parasite does not reach sexual maturity. In most cases, the parasite goes through morphological and developmental changes in an intermediate host. In some cases, the parasite increases in numbers within an intermediate host. For example, all species of digenetic trematodes and some species of cestodes increase in number in the intermediate host through an asexual process known as **polyembryony**, the formation of more than one embryo from a single zygote (Craig et al., 1997). As a result of polyembryony, intermediate hosts can play a major role in increasing the probability of parasites encountering the next host in the life cycle.

A **paratenic host**, or **transport host**, is one in which the parasite does not undergo any development. However, in many cases, a paratenic host is essential for the transmission of the parasite and acts as a trophic bridge between the intermediate and definitive host (Baer, 1951). For example, some species of trematodes found as adults in the Eustachian tubes of frogs, use frogs as definitive hosts and aquatic microcrustaceans as intermediate hosts in their life cycles. However, because frogs do not generally consume microcrustaceans, a paratenic host must be involved in bridging the gap in trophic transmission. In this case, aquatic insects that commonly feed on microcrustaceans, such as damselflies and dragonflies, accumulate large numbers of these trematodes in their digestive

tracts, and the trematodes do not develop in the microcrustacean. Frogs then eat the damselfly and dragonfly paratenic hosts and, in the process, become heavily infected (Bolek et al., 2010; Stigge and Bolek, 2015).

Most parasites must complete at least part of their life cycle by infecting 1 or more **obligate** or **required hosts**. In contrast, **facultative** parasites are usually not parasitic, but become so, opportunistically, when they encounter a potential host. For example, when certain species of free-living amoebas, such as *Naegleria fowleri* or species of free-living nematodes in the genus *Halicephalobus* are accidentally ingested or enter an opening of a novel host, they can establish within the host, and in some case cause serious and many times fatal conditions (Anderson et al., 1998; Kinde et al., 2000; Visvesvara et al., 2007). Similarly, when an obligate parasite infects a host which is different from its normal host, that host is called an **accidental** or **incidental host**. A number of cases have been reported of humans serving as accidental hosts for the nematode *Angiostrongylus cantonensis*, a species that normally resides in the lungs of various species of rats. Humans become infected with *A. cantonensis* by ingesting terrestrial gastropod intermediate hosts that are living on raw vegetables, such as lettuce (Pien and Pien, 1999). In humans, the nematodes migrate to the brain where they cause abscesses, brain swellings, and hemorrhages. Eventually, the juvenile nematodes die and degenerate. In this situation, humans can also be considered a **dead-end host** for *A. cantonensis*, because the parasite is not transmitted to functional hosts to continue its life cycle (Pien and Pien, 1999).

It should be noted that most, if not all, free-living species on our planet serve as hosts for many species of parasites. As a result, those free-living animals can serve different roles in the life cycles of different parasite species. One group of free-living animals that commonly serve as intermediate or paratenic hosts for numerous species of parasites are the gastropods (phylum Mollusca: class Gastropoda). Terrestrial, freshwater, and marine snails have been reported as intermediate and/or paratenic hosts for most species of digenetic trematodes, as well as various species of nematodes, tapeworms, and even acanthocephalans (Hopp, 1954; Dollfus, 1974; Rysavý, 1986; Lockyer et al., 2004; Lu et al., 2018). As an example, a single species of freshwater snail, *Physa acuta*, collected from various streams and wetlands across north-central Oklahoma, United States, serves as the first or second intermediate host for at least 9 species of flukes, and as a paratenic or accidental host for 3 species of horsehair worms, 1 species of nematode, and 1 species of thorny-headed worms, all of which infect various insects or vertebrates as definitive hosts (Gustafson and Bolek, 2016; Harkins et al., 2016; Koch, 2018; Figure 1).

Reservoir Hosts and Vectors

Another definition commonly used in the parasitology literature is the concept of **reservoir host**. Broadly defined, a reservoir species maintains a parasite infection in nature and serves as a source of infection for other species of animals. From a medical perspective, the definition of a **reservoir host** is usually restricted to any animal that maintains parasites as a source of infection for humans or domestic animals. In addition, many parasites that infect humans, domestic animals, and wildlife are transmitted by **biological vectors**. The term **vector** has been applied to a diverse group of potential animal hosts, and when used broadly in parasitology, can include any animal that transmits parasites from one host to another (Wilson et al., 2017). However, from a medical, ecological, and evolutionary perspective, a **vector** is defined as a mobile micropredator (for example, mosquito, leech, or vampire bat) that feeds on the blood or other bodily fluids of vertebrates and in some cases invertebrates (Figure 2). (Lafferty and Kuris, 2002; Wilson et al., 2017).

In most sanguinivorous species of animals that can also act as vectors of parasites, blood and/or tissue parasites from an infected animal may be ingested in 2 main ways; 1) Through **telmophagy**, in which the ectoparasitic animal abrades the skin and capillary beds of a vertebrate and a small hemorrhage forms, from which the animal vector then feeds, and 2) via **solenophagy**, in which a vector directly pierces blood vessels of its host to feed. For example, female horse flies and deer flies use telmophagy and when they feed, they lacerate the skin of their host with specialized cutting bladelike maxillae and then suck up the blood with sponge-like labellae (Matheson, 1950). In contrast, female mosquitoes are solenophagic feeders with mouthparts that are adapted to piercing vertebrate skin with their cutting maxillae and then suck blood with the hypopharynx (Choo et al., 2015; Mullen and Durden, 2009). Note that males do the same thing but with plants.

Based on their relationship with the parasite, vector-hosts can be assigned to 2 groups, including either mechanical or biological vectors. **Mechanical vectors** merely transmit the parasite between and among vertebrates, but without any multiplication or development of the parasite within the vector-host. Although not necessary for the multiplication or development of the parasite, mechanical vectors are essential for the **transmission** of various parasite species among its vertebrate hosts. A typical example includes flies (order Diptera) of the family Tabanidae (horse flies and deer flies) which are mechanical vectors for *Trypanosoma evansi* (order Kinetoplastida: Trypanosomatidae) of horses and other vertebrates (Bowman, 2013). Because female tabanids are not subtle and may cause pain when they bite their victim, they are usually quickly dislodged by defensive movements of the

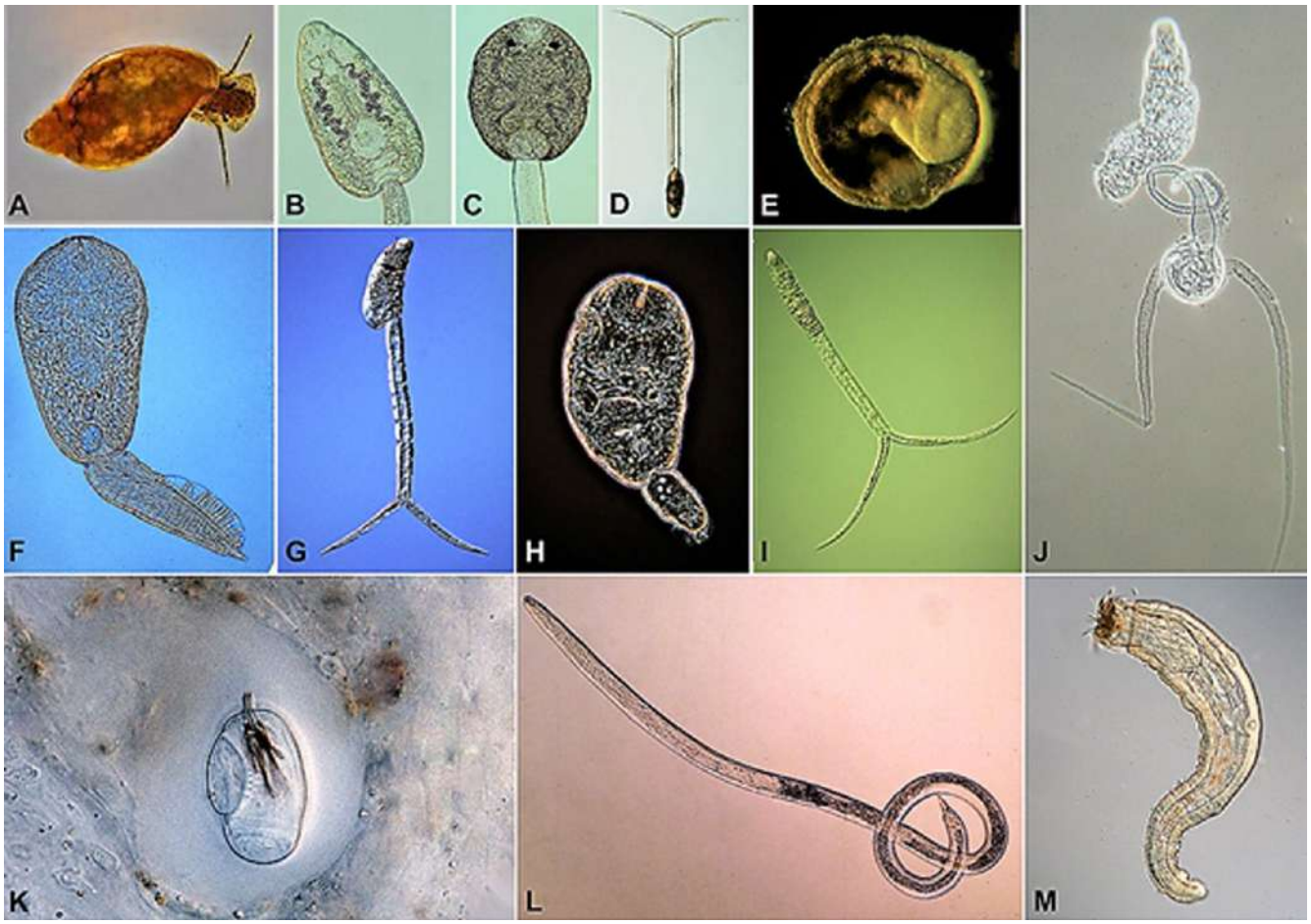

Figure 1. An example of a common North American freshwater snail, *Physa acuta* (A) and 12 species of parasites from 4 phyla representing different types of host associations. B–D, F–J) Show the cercarial stages of 8 species of digenetic trematodes which develop within the snail host and are released into the water column, to infect a second intermediate host. *Physa acuta* serves as the first intermediate host in the life cycles of these parasites. E) A metacercarial stage of the digenetic trematode *Allassostomoides parvus* which is infective to turtle definitive hosts. *Physa acuta* serves as the second intermediate host in the life cycle of this parasite. K) A cyst of a horsehair worm, *Paragoridus varius* in the tissue of *P. acuta*. Horsehair worms infect crickets and other arthropods as definitive hosts and they can use aquatic insects as paratenic hosts. Because crickets do not usually feed on aquatic snails, *Physa acuta* is considered an accidental host for this parasite. L–M) A juvenile *Spiroxys contortus* (nematode) and a juvenile *Neoechinorhynchus emydis* (acanthocephalan). Both of these parasites use microcrustaceans as first intermediate hosts and aquatic turtles as definitive hosts. *Physa acuta* may act as a an accidental/paratenic host for these parasites when individuals ingest infected microcrustacean first intermediate hosts and which are then eaten by the turtle definitive host. Source: M. Bolek. License: CC BY-NC-SA 4.0.

host and rarely remain on a host long enough to become fully engorged with blood. Instead, the tabanid quickly flies off the infected host and lands on another animal to feed again. In essence, it ingests blood frequently from multiple hosts and, in the process, it can mechanically and rapidly transmit *T. evansi* from one horse to another. In contrast to mechanical vectors, a **biological vector** is one in which the parasite multiplies and/or develops within organs and/or tissues of the vector host. Often, there is a time lag between acquisition of the parasite by the biological vector and the ability of the parasite to be transmitted by that vector to a new definitive host. This has been called the **extrinsic incubation period**.

Within biological vectors, and during the extrinsic incubation period, 3 types of multiplication and/or developmental patterns of the parasite can occur (Figure 3). **Propagative transmission**, involves simple amplification of a parasite within the vector-host. In this case, the same form of the parasite taken up by the vector multiplies within the vector and is then transmitted to a new vertebrate host. Examples include various species of bacteria, and some trypanosomatid protozoans, where the parasite multiplies within the vector-host but does not change morphologically. In contrast, **cyclopropagative transmission**, involves asexual and/or sexual multiplication of the parasite, and hence amplification of

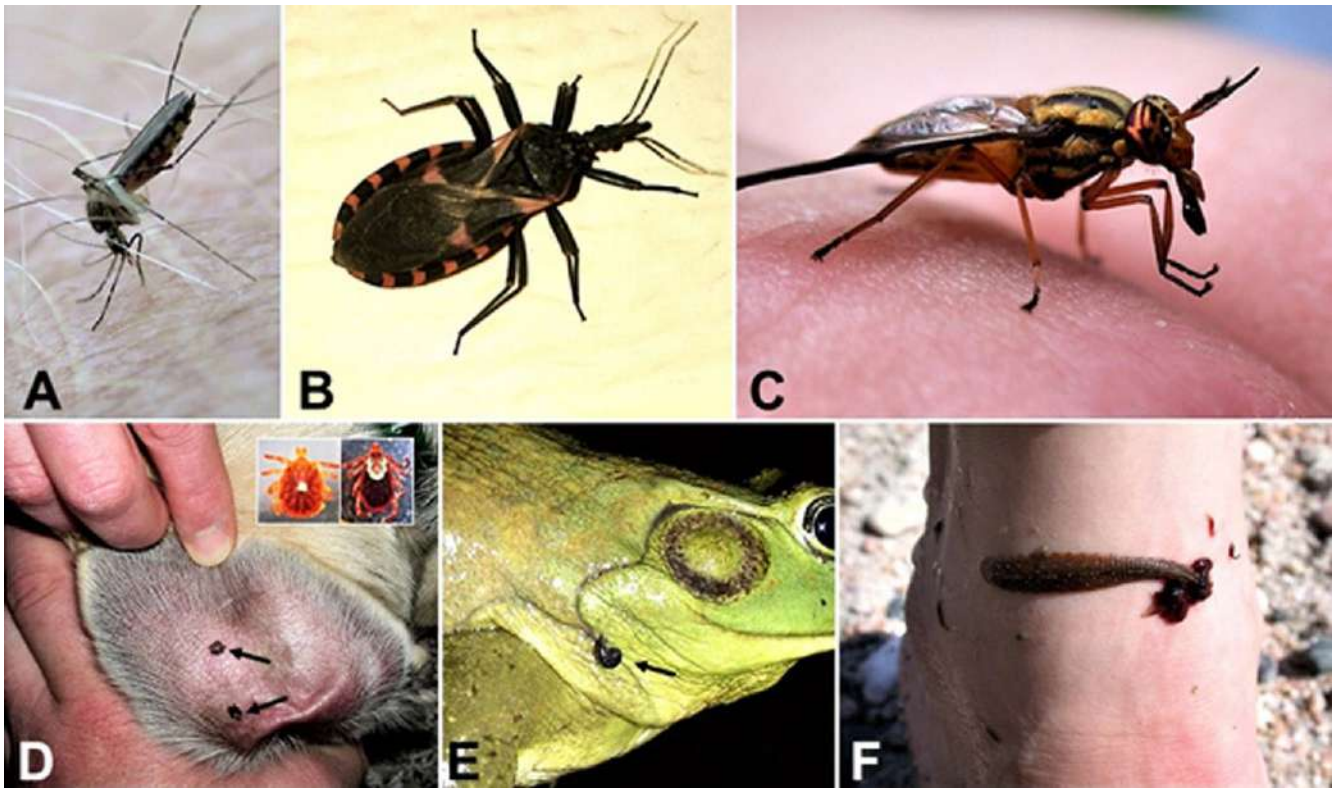

Figure 2. Examples of typical vector hosts. A) Female mosquito in the genus *Aedes* in the process of taking a blood meal. Note the specialized sucking mouth part injected into the skin of author M. Bolek. B) A reduviid bug. This is one of the primary biological vectors of *Trypanosoma cruzi,* the causative agent of Chagas disease. This species of bug can transmit the infective parasite stage to the vertebrate host through its feces. C) A female striped-backed deer fly, *Chrysops vittatus*. Because of their blood feeding habits, many species of deer flies serve as mechanical vectors for parasites. Note the complex mouth parts, used to slice open the skin of the victim, after which the fly sips blood from the pooling blood on the surface of the skin. D) Females of 2 species of hard ticks, *Amblyomma americanum* and *Dermacentor variabilis* (arrows), attached and feeding on the ear of a stray dog, *Canis lupus familiaris*. Ticks are common biological vectors for various parasites including protozoa and various helminths. E–F) Leeches (order Rhynchobdellida: family Glossiphoniidae) *Placobdella picta* (arrow) and *P. rugosa* feeding on a bullfrog, *Lithobates catesbeianus*, and the leg of Melissa Bolek (order Primates: family Hominidae), respectively. Leeches are common biological vectors for protozoan parasites of amphibians and reptiles. Note, in E the numerous young leeches feeding from the same bite wound as the mother leech. Source: M. Bolek. Informed consent obtained from all human subjects. License: CC BY-NC-SA 4.0.

the parasite within the vector-host. Importantly, in cyclopropagative transmission, the form of the parasite transmitted to the next vertebrate host is morphologically distinct from the initial form taken up by the vector-host.

An example of asexual cyclopropagative development occurs in the trypanosomatid *Trypansoma cruzi* within its reduviid bug vector-host; sequential cycles of asexual and sexual reproduction within mosquito and tick vector-hosts occur in various genera of apicomplexans such as *Plasmodium* and *Babesia*, respectively. As a result—and depending on the specific vector-host and parasite reproductive relationship within the vector—some biological vectors can be classified as **definitive** or **intermediate hosts**. In the case of *Plasmodium* in vertebrates and their mosquito host, the **vertebrate is the intermediate host** while the **mosquito is the definitive host because sexual reproduction occurs in the stomach wall of the mosquito**. Finally, **cyclodevelopmental transmission** involves no multiplication of the parasite, but instead, the parasite develops within the vector to the next stage which is infective to the vertebrate host.

In cyclodevelopmental transmission, there is usually mortality and reduction in the number of parasites that are initially ingested by the vector relative to the number that are available when transmitted to the vertebrate host. Hence there is no amplification of the parasite in vector-hosts with cyclodevelopmental transmission. Examples of vector-borne parasites with cyclodevelopmental transmission include filarioid nematodes such as *Litomosoides* spp. (superfamily Filarioidea: family Onchocercidae), which depending on the particular species, reside in various tissues of vertebrate definitive

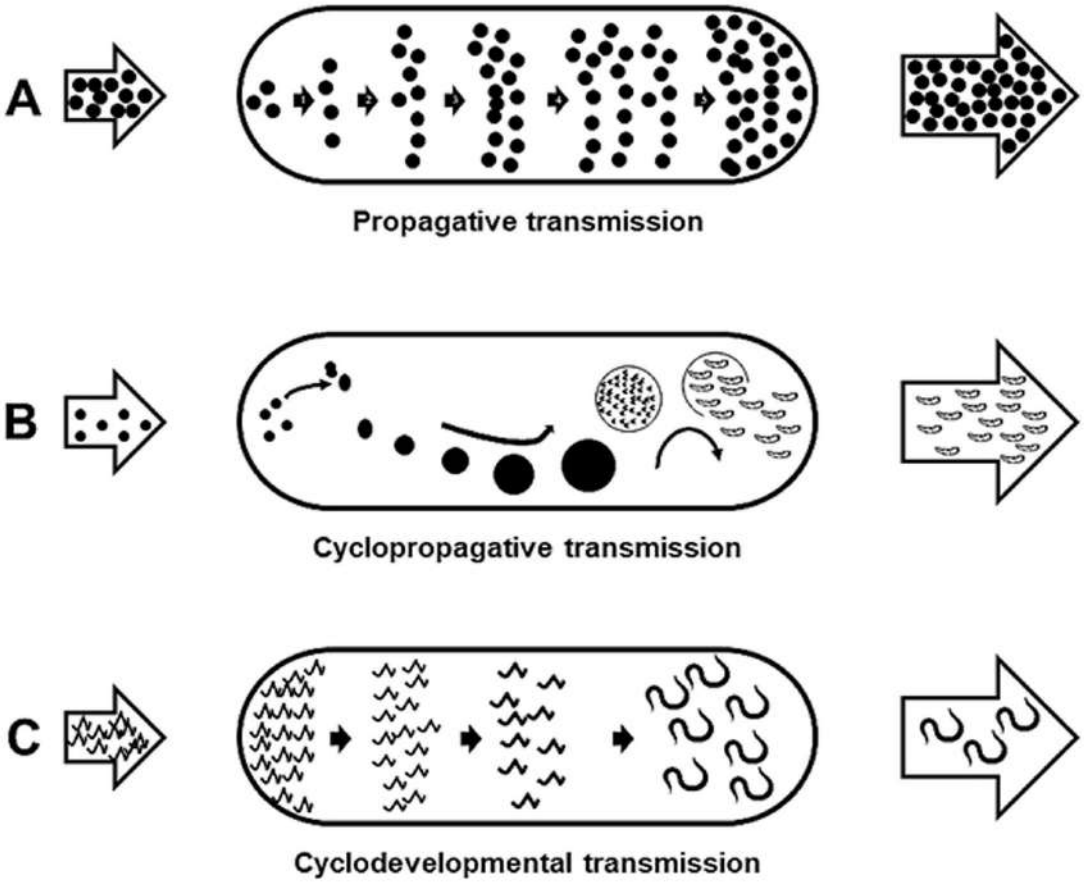

Figure 3. Types of biological associations between parasites and their vector hosts, represented by ovals. The arrows on the left indicate blood ingested by the vector from an infected vertebrate host, and the arrows on the right represent the infective parasite stage transmitted to another vertebrate host after a sufficient incubation period. A) Propagative transmission, the parasite multiplies within the vector, usually by an indefinite number of generations of binary fission. The stages transmitted are the same but far more numerous than originally acquired during the vector's original blood meal. Examples of parasites with propagative transmission include some species of trypanosomatid protozoans. B) Cyclopropagative transmission, the parasite undergoes 1 or more cycles of asexual and/or sexual reproduction where it increases in numbers. The infective stage to the vertebrate host, is morphologically distinct from the form originally acquired during the vector's original blood meal. Examples of parasites with cyclopropagative transmission include the causative agents of malaria and Chagas disease in humans. C) Cyclodevelopmental transmission, the parasite develops from the stage acquired by the vector host to an infective stage to the next vertebrate host, without any multiplication or reproduction. There is usually a loss of parasites from the original number acquired by the vector, and the final number that develop to the infective stage to the next host. Common examples of cyclodevelopmental parasites include filarioid nematodes. Source: Adapted from McClelland (1992), 2019. License: CC BY-NC-SA 4.0.

hosts and release infected stages known as microfilariae into the blood, connective tissues, or skin. Once ingested by their mosquito intermediate vector-host the microfilariae develop to the next stage that is infective to the vertebrate host (Anderson, 2000).

Any parasites within the body of a vector-host must eventually exit the vector to be transmitted to a new host. Many vectors transmit parasites between successive vertebrate hosts during blood feeding. In some mechanical vectors, the parasites may be regurgitated back into the mouthparts and subsequently transmitted to a new vertebrate host during a blood feeding session. Similarly, in many biological vectors, the parasite is transmitted to a vertebrate host through inoculation or contaminated mouthparts during blood feeding. It is important to note, however, that not all vector-hosts transmit parasites between successive vertebrate hosts while taking a blood meal. This is particularly true for parasites that develop to the infective stage within the hindgut, or in the hemocoel of their vector-hosts and, as a result, cannot be transmitted through inoculation via contaminated mouthparts (Figure 2).

The causative agent of Chagas disease *Trypanosoma cruzi* is one such example. *Trypanosoma cruzi* protozoans develop to the infective stage in the hindgut of their kissing bug vector, which includes various species of kissing bugs, such as *Triatoma sanguisuga*, and is then transmitted to the vertebrate host in the feces, when the bug defecates while feeding. Humans become infected when they scratch the bite wound, rub their eyes, or move the feces of the bug into the mucus membranes of the mouth or nose. These actions inadvertently inoculate the infective stages of *T. cruzi* in the bug's feces into the various infection portals. Similarly, the apicomplexan parasite *Hepatozoon americanum* infects dogs as the intermediate host, and the lone star tick, *Amblyomma americanum*, as the vector definitive host. In this case, the parasite develops to the infective stage in the hemocoel of the tick vector and dogs become infected when they ingest infected ticks while grooming (Ewing and Panciera, 2003).

Finally, species-specific interactions between parasites and the type of reservoir and vector-hosts they employ in their life cycles can become extremely convoluted. In some cases, both mechanical and biological vectors can transmit a single parasite species. As mentioned previously, *Trypanosoma evansi* is transmitted to horses through the bite of blood sucking flies *Tabanus* and *Stomoxys* which act as mechanical vectors across Asia and in North Africa (in addition to *Glossina*), where *T. evansi* is endemic. However, *T. evansi* has relatively recently been introduced into Central America and South America, where it can be transmitted to horses by one of the species of vampire bats, *Desmodus rotundus*, which can serve as both vector and reservoir host (Brun et al., 1998). Vampire bats become infected with *T. evansi* by feeding on the blood of infected horses. Parasites enter the bat's bloodstream through the mucus membranes lining the buccal cavity, and some of the infected bats die due to disease caused by the initial phase of infection (Desquesnes et al., 2013). However, some individuals survive the initial infection with the trypanosomes achieving a chronic infection with high blood parasitemia and some individual bats with a chronic infection have trypanosomes in their saliva. These bats then act as biological vectors and can transmit *T. evansi* to horses via their saliva during blood feeding. Additionally, because infected vampire bats commonly groom each other and/or feed other bats in the colony regurgitated blood, these infected vampire bats can propagate the infection among other individuals in the colony (Desquesnes et al., 2013). As a result, vampire bat colonies can maintain *T. evansi* in the absence of infections in horses, and the infected bats can serve as reservoir hosts for infections in horses! Finally, there are reports of canids becoming infected by eating freshly killed mammals that are infected with *T. evansi* (see Woo, 1977).

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5

Parasites in Relation to Other Organisms

Life Cycles

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Chapter 5

Life Cycles

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Introduction

Life cycles of parasites have evolved into complex sequences of improbable events, with as many as 4 host species being included in the life cycle of certain parasite species (Bolek et al., 2010). Relative to their hosts, parasites in their infective stages are rather small and have limited mobility in the external environment. As a result, one can make the argument that the most dangerous part of any parasite's life cycle is when the parasite is away from its host. Consequentially, adaptive scenarios and evolutionary contingencies are both often invoked to explain the complexity of parasite life cycles and the resulting transmission events (Poulin and Cribb, 2002).

In order for a particular parasite to infect and live in or on an appropriate host, there must be suitable conditions enabling access to the host(s), including: 1) A dependable means of transmission from one host to another, 2) the ability of the parasite to establish itself within that host after reaching it, and 3) specific conditions within that host for the parasite to survive, grow, and reproduce. To accomplish this, parasites have evolved various types of life cycles which enable them to complete the necessary steps (that is, colonize, survive, grow, and mature) among a variety of different but often specific host species. A **parasite life cycle** is defined broadly as including the ontogenetic stages of a specific parasite species, and a set of events, such as growth and reproduction, that must occur before the parasite can survive and reproduce. In the case of parasites, the life cycle also includes all necessary hosts and all transmission events that enable a specific species of parasite to complete its life cycle.

Infection Site

Depending on the species, many parasites occupy a specific infection site and/or location in 1 or more of the hosts infected during their life cycle. Parasites that inhabit the lumen of the intestines, lungs, or other hollow organs of their hosts are said to be **coelozoic**, whereas parasites that live within tissues of their hosts are referred to as **histozoic**. For example, amphibians are commonly infected with 2 distinct genera of myxozoans, a group of parasitic cnidarians (Jirků et al., 2006; 2007; Hartigan et al., 2012). *Cystodiscus serotinum* produces infective spore stages in the gallbladder of amphibians; whereas *Sphaerospora ohlmacheri* produces infective spores in the tubules of the kidneys of frogs and toads. Both species are coelozoic because they infect the lumen of the gallbladder or tubules of the kidneys. However, each species is considered **site specific** in amphibians, such that *C. serotinum* can only develop in the gallbladder and *S. ohlmacheri* can only develop in the tubules of the kidneys (Figure 1). In contrast, many cercaria, which are the larval stages of trematodes, are histozoic and encyst within various tissues of their second intermediate hosts. For example, tadpoles (larvae) of many amphibian species serve as second intermediate hosts for various trematode species (Rhoden and Bolek, 2015). The metacercariae of some trematode species only encyst in specific tissues and organs whereas metacercariae of other species are infection site generalists and can be found in various tissues and organs of tadpoles (Figure 2); thus, some species of trematodes have metacercariae that are generalists and some that are specialists. Studies indicate that cercariae of echinostomes actively seek and enter tadpoles via the cloaca, then migrate to the kidneys where they encyst (Thiemann and Wassersug, 2000; Taylor et al., 2004). In contrast, species of *Telorchis* will penetrate any surface on the body of a tadpole (Schell, 1962). Notably, tadpoles have a greater chance of becoming infected with species of *Telorchis* by mechanically sucking in infective stages of flukes from the water column, whereas cercariae of echinostomatid flukes can only infect tadpoles when they enter through the cloaca (Rhoden and Bolek, 2012).

To a parasite, a host represents multiple microenvironments, and only certain environments meet the parasite's very specific needs. Clearly, not all hosts will be equal, and some

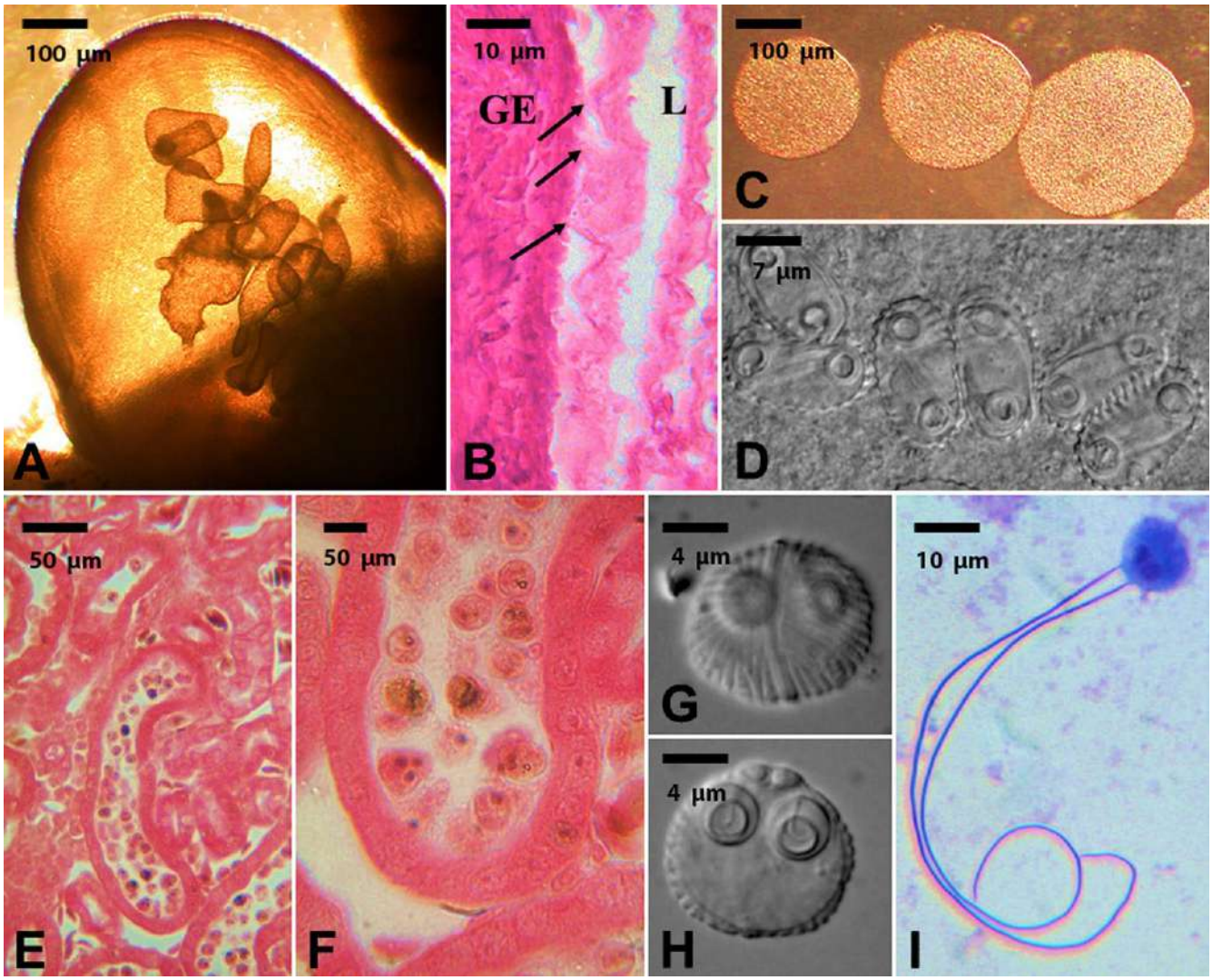

Figure 1. Example of coelozoic parasites with restricted site specificity; showing detailed development of the next infective stage in the life cycle. A–D) Developmental stages of *Cystodiscus serotinum* in the gallbladder of a green frog *Rana clamitans*. A) Gallbladder showing developing plasmodia stages. Scale bar = 100 µm. B) Histological section showing the distribution of plasmodia in the lumen (L) of the gallbladder and their intimate association (arrows) with the epithelial cells of the gallbladder (GE). Scale bar = 10 µm. C) Removed plasmodia from the gallbladder. Scale bar = 100 µm. D) Infective spore stages within the plasmodia. Scale bar = 7 µm. E–F) Developmental stages of *Sphaerospora ohlmacheri* in the kidneys of a Blanchard's cricket frog *Acris blanchardi*. E) Histological section of the kidney showing renal tubule occluded by plasmodia of *Sphaerospora ohlmacheri*. Scale bar = 50 µm. F) Close up of renal tubule occluded with developing spores of *Sphaerospora ohlmacheri*. Scale bar = 50 µm. G–I) Detailed morphology of infective spores of *Sphaerospora ohlmacheri*. Note the detailed surface structures on the spores and the everted extruded polar filaments (I) indicating the spore stages are infective to the next host in the life cycle. Scale bars = 4 and 10 µm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

parasites that infect different host species can behave differently. As a result, the site of infection for some parasites can be influenced by their host species. For example, a recent study on the frog tongue fluke *Halipegus occidualis* showed that these flukes commonly infect 3 species of frogs (Stigge and Bolek, 2016). Anurans (frogs) become infected with *H. occidualis* when they ingest a dragonfly paratenic host that contains encysted metacercariae. However, when green frogs *Lithobates clamitans* and leopard frogs *L. pipiens* ingest an infected dragonfly paratenic host, the worms migrate from the stomach and attach to the lingual vein under the tongue, where they mate and lay eggs. In contrast, when dragonfly paratenic hosts are ingested by bullfrogs *L. catesbeianus*, the worms never attach to the lingual veins under the tongue, but instead reside in the frog's stomach where they mate and lay eggs. It is unclear why *H. occidualis* behaves so differently

in bullfrogs than in green frogs and leopard frogs; nonetheless, the study clearly indicates that different species of host-parasite combinations matter in understanding parasite life cycles and host-parasite interactions.

Host Specificity

To begin, note that host specificity is covered briefly in the introduction to this book. While there is some debate about whether this is the proper framework to consider life cycles, this contention will be left aside for now, since it is referenced extensively in the literature as it has served as the basis for numerous robust studies in the concept of ecology of parasites.

As observed with infection site, parasites can vary in their host-range (also known as specificity) at 1 or more stages in their life cycles. Although most species of parasites are known to develop only in a restricted range of hosts, different parasites exhibit varying degrees of host specificity. For example, some species of cestodes, such as the pork tapeworm *Taenia solium* (Cyclophyllidea: Taeniidae) are only known to mature to egg producing adults in humans *Homo sapiens* definitive hosts and are considered host-specific at the definitive host level. In contrast, and at the other extreme, species of *Trichinella* (class Nemata: family Trichinellidae) can mature in almost any species of mammal. Another example of a parasite with a wide host-range is the coccidian protozoan *Toxoplasma gondii*. This parasite uses cats (order Carnivora: family Felidae) as the definitive host (any cat species will do) but it can use almost any vertebrate as the intermediate host.

These examples exhibit the variety of host-range shown by parasites across 3 phyla of phylogenetically unrelated parasites. A parasite that is specific for a single host species is said to be **oioxenous**, a parasite that infects closely-related hosts is considered **stenoxenous**, whereas a parasite that infects unrelated hosts is considered **euryxenous**. Finally, some parasites exhibit **stadium specificity** where hosts are only susceptible to infection by a particular parasite at a specific developmental stage. Some protozoans such as gregarines (apicomplexans) that infect holometabolous insects and some species of nematodes and acanthocephalans that occur in amphibian definitive hosts, can only infect either the larva or adult stage of their host (Nickol and Heard, 1973; Clopton et al., 1992; Rhoden and Bolek, 2011; Childress et al., 2017). For example, tadpole pinworms, *Gyrinicola batrachiensis* are constrained to the large intestine of tadpole stages of anurans (Adamson, 1981). One explanation for this dramatic difference in host specificity between tadpoles and frogs is differences in their diets and digestive tracts. In general, pinworms feed on the bacteria found in the hindgut of animals that consume plants as a significant portion of their diet. As tadpoles metamorphose to the adult anuran stages, their feeding and correlated digestive tract changes dramatically from a predominantly herbivorous diet to a strictly carnivorous diet, and all *G. batrachiensis* are lost from their intestines (Adamson, 1981). As a result, separate and distinct parasite niches corresponding to distinct life cycle stages of free-living animals can affect parasite host range and measured specificity.

To understand the nature of host range, some parasitologists contend that experimental cross infections should be conducted to determine whether host-parasite associations may be established by true host-parasite incompatibility (Janovy et al., 2007). However, potential host species may simply not be infected with a particular parasite species because they never encounter the infective stage of the parasite in nature due to various environmental factors. With most systems involving parasites of vertebrates, logistical burdens make studying cross infection very difficult, especially when the species are not routinely reared in captivity. There are a number of studies on protozoa, trematodes, nematodes, and annelids testing host compatibility in insect, amphibian, and reptile host-parasite systems (Bolek and Janovy, 2007a; 2007b; 2008; Janovy et al., 2007; Bolek et al., 2009; 2010; Langford and Janovy, 2009; 2013; Childress et al., 2017; Andrews et al., 2015; Stigge and Bolek, 2016). In general, what these studies suggest is that host specificity has a strong ecological component, such that many potential and competent hosts never come in contact with the infective stages of a particular parasite species in nature, undoubtedly affecting host-parasite patterns of associations. Additionally, these studies indicate that it is difficult to predict the range of compatible hosts a particular parasite can infect. For example, Langford and Janovy (2013) tested the host specificity of 7 species of lungworms which infect snakes and anuran definitive hosts. Their field studies and experimental infections indicated that both species of snake lungworms were generalist snake parasites, and in nature and the laboratory they could infect up to 5 species of snakes. However, their laboratory experiments also suggested that lizards can be infected under some environmental conditions. In contrast, lungworms from anurans were found not to infect salamanders or reptiles in nature or in the laboratory. Additionally, amphibian lungworm species ranged from being strictly host specific, infecting only 1 species of frog or toad, to relative generalists, able to infect multiple species of distantly related frog and toad species. Overall, these studies indicate that for many parasite species, host specificity or host-range in nature appears to be limited by both ecological and physiological factors, which vary among parasite species and their hosts.

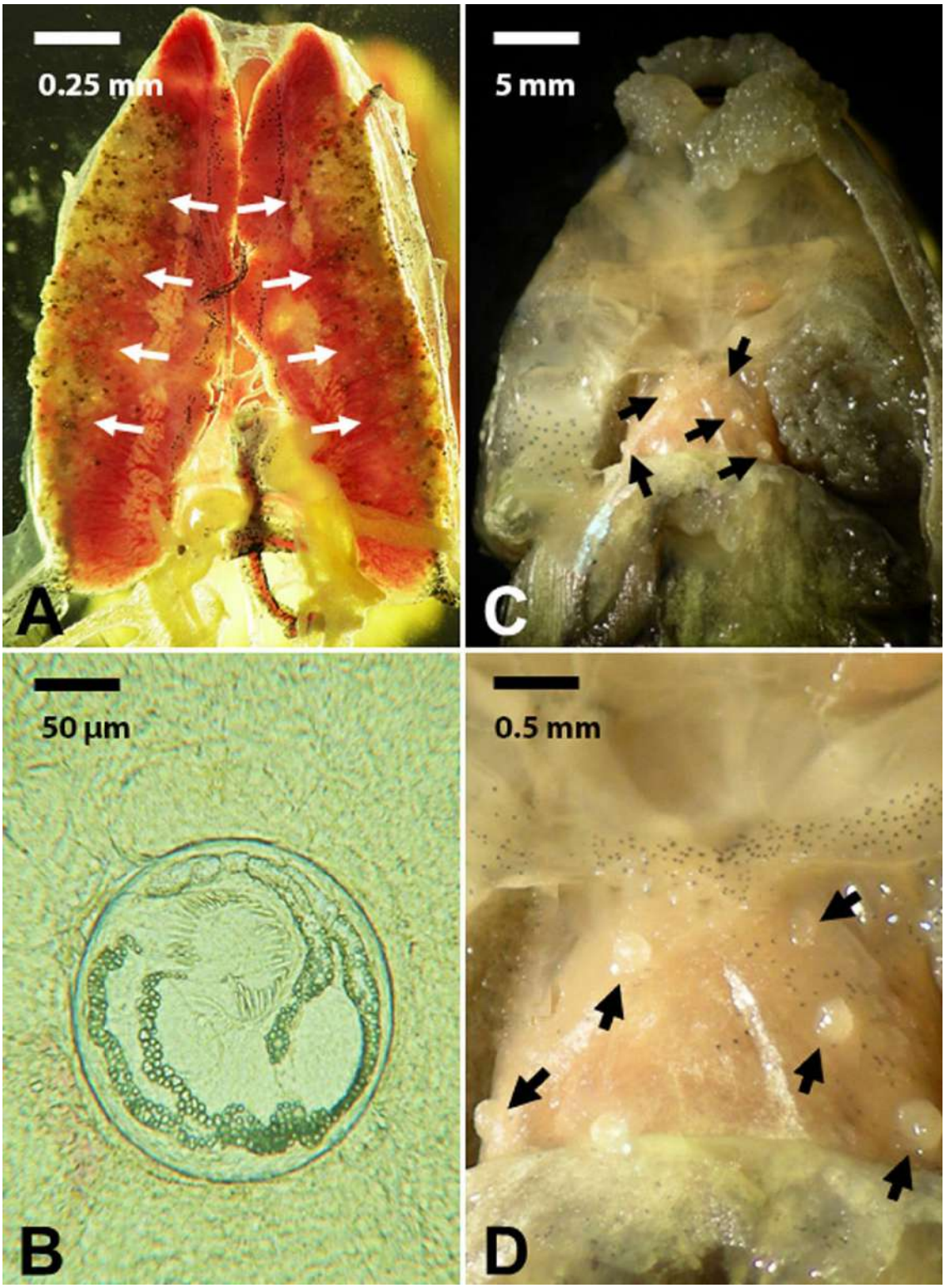

Figure 2. Example of histozoic parasites with variable site specificity in a bullfrog tadpole. A) Removed kidneys from a bullfrog tadpole showing hundreds of echinostomatid metacercarial stages encysted on the lateral sides of each kidney (arrows). Scale bar = 0.25 mm. B) A single echinostomatid metacercaria encysted in kidney tissue of bullfrog. Scale bar = 50 µm. C) Ventral body region of a bullfrog tadpole with the musculature removed showing encysted metacercarial stages of *Telorchis* sp. (arrows). Scale bar = 5 mm. D) Higher magnification of the heart showing the distribution of encysted metacercariae of *Telorchis* sp. (arrows) on the heart. Scale bar = 0.5 mm. Source: M. Bolek.

Parasite Development and Types of Parasite Life Cycles

Parasite development can be categorized as **monoxenous** where the parasite lives and develops within a single host during its life cycle, or **heteroxenous** where a parasite lives and develops within more than 1 host during its life cycle. Additionally, life cycles can be categorized as **simple** or **direct** where a parasite only infects a single host in its life cycle, or as **complex** or **indirect life cycles**, where a parasite uses 2 or more hosts in its life cycle. However, some parasites with direct or indirect life cycles also go through complex reproductive events within their hosts where they alternate sexual and asexual generations in 1 or multiple hosts. As a result, distinct sets of terms are used to differentiate between parasite reproductive events within their hosts and life cycle complexity. For example, many coccidian species in the genus *Eimeria* have direct or simple life cycles and infect their definitive vertebrate host when the host ingests the infective oocyst stages. However, once inside the intestinal epithelial cells of its host, the coccidian goes through a complex set of multiple asexual multiplication events, followed by the production of male and female gametes and eventually sexual reproduction (Figure 3). Parasites that have alternations of sexual and asexual generations in their life cycle are commonly referred to as **heterogenetic parasites**. In contrast to *Eimeria*, all acanthocephalan species (phylum Acanthocephala) have indirect or complex life cycles, including a definitive, intermediate, and commonly an additional paratenic (transport) host. However, except for sexual reproduction in the definitive host, no other complex asexual multiplication or alternations of generations occurs in the intermediate or paratenic hosts in the life cycle (Figure 4). Parasites that have no alternation of sexual and asexual generations in their life cycles, are sometimes referred to as **monogenetic parasites**. As a result of the enormous diversity of parasite species, different combinations of direct or indirect and heterogenetic or monogenetic development can occur in different groups of parasites during their life cycles.

In addition to the examples above, there are other life cycle variations, particularly in parasite species that must exit their host into the external environment and develop into free-living adults and/or to find mates and reproduce in the external environment. For example, life cycles of some species of flies which cause **myiasis** (a term for an infestation of tissues, wounds, or body cavities of living animal by fly maggots) fall into this category (Zumpt, 1965). Many species of flies causing myiasis are **obligate** parasites and their maggots must develop within their hosts to complete the life cycle. For example, flies in the subgenus *Bufolucilia* commonly

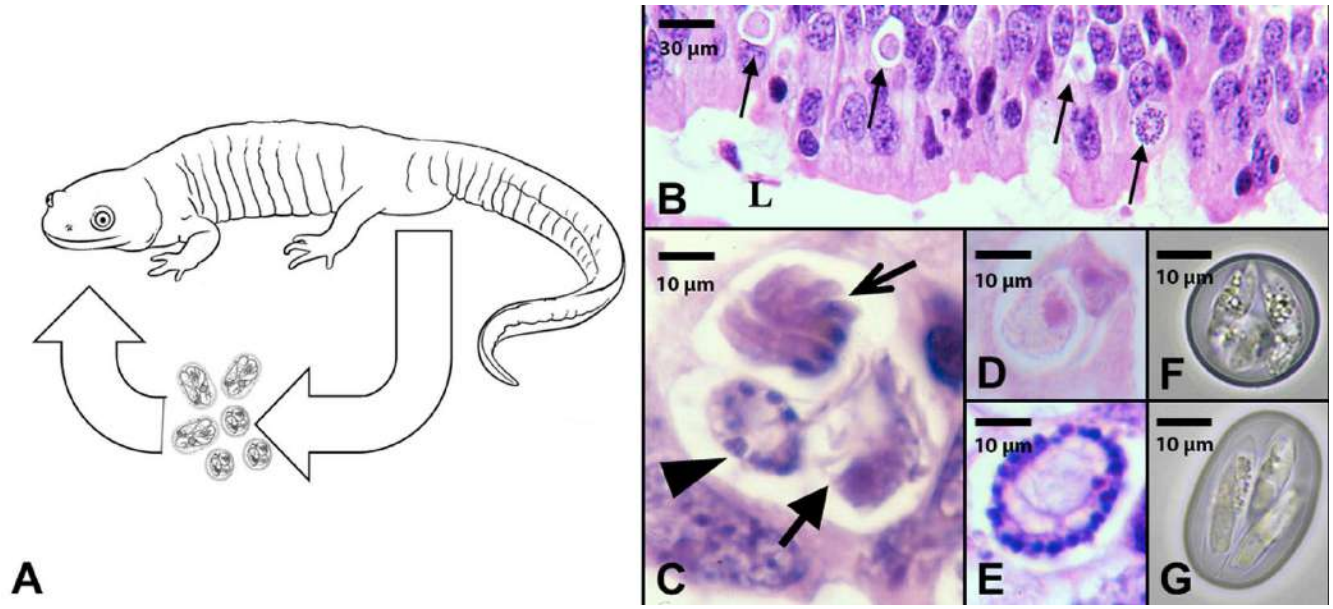

Figure 3. An example of a direct, monoxenous, but heterogenetic life cycle of salamander *Eimeria* spp. A) A tiger salamander *Ambystoma tigrinum* showing the routes of transmission of *Eimeria* species. Salamanders defecate infective stages (oocysts) into the external environment and become infected when they accidently ingest oocysts. B) Histological section of the small intestine of a tiger salamander showing different developmental stages of *Eimeria* species (arrows) in the epithelial cells of the small intestine. Scale bar = 30 µm. C) Higher magnification of an epithelial cell showing asexual multiplication (thin arrow) and development of microgametes (sperm; middle arrow) and macrogametes (ova; thick arrow). Scale bar = 10 µm. D–E) Epithelial cells showing developing oocysts (zygotes) after fertilization. Scale bar = 10 µm. F–G) Fully developed and infective oocysts recovered from the feces of *Eimeria urodela* and *E. ambystomae*. Scale bar = 10 µm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

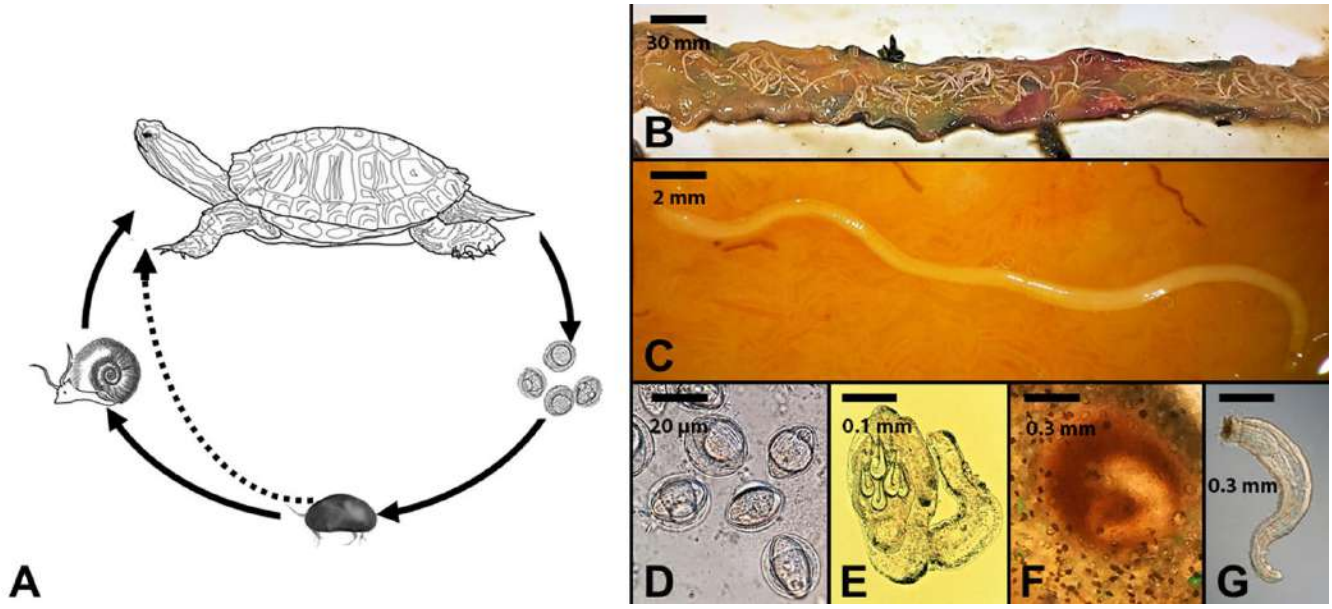

Figure 4. An example of an indirect, heteroxenous, but monogenetic life cycle of the turtle acanthocephalan *Neoechinorhynchus emydis*. A) Turtle definitive hosts release eggs into the external environment where they are ingested by ostracod intermediate hosts. Once the parasite develops to the next infective stages, the infected ostracod can be ingested by a snail paratenic host where no development of the parasite occurs or a turtle definitive host where sexual reproduction occurs. Additionally, turtles can become infected when they ingest snail paratenic hosts. B) The small intestine of a turtle showing hundreds of adult acanthocephalan parasites attached to the intestine. Scale bar = 30 mm. C) Higher magnification of a single adult female worm attached to the intestine mucosa. Scale bar = 2 mm. D) Eggs of an acanthocephalan. Scale bar = 20 µm. E) Developing larval stage recovered from the body cavity of an ostracod intermediate host. Scale bar = 0.1 mm. F) Encysted juvenile acanthocephalan in a snail paratenic host. Scale bar = 0.3 mm. G) Infected juvenile acanthocephalan removed from a snail paratenic host. Scale bar = 0.3 mm. Note the dramatic morphological changes among the different stages in the life cycle. Source: M. Bolek. License: CC BY-NC-SA 4.0.

infect amphibian hosts throughout Europe and North America (Bolek and Coggins, 2002; Bolek and Janovy, 2004; Tantawi and Whitworth, 2014; Arias-Robledo et al., 2019). Female flies locate amphibian hosts visually and deposit eggs on the back and flanks of their unsuspecting frog or toad victims (Figure 5). The larvae hatch, migrate through the skin, and eventually disappear into the frog's tissues. Within 2 to 3 days of infection, an open wound appears and displays the posterior spiracles of the maggots, which allows the maggots to breathe (Figure 5). Within these wounds, maggots develop to mature third instar larvae within 5 to 7 days of hatching, migrate out of the amphibian host, burrow into the soil, turn into pupae, metamorphose into adult flies, mate, and start the process all over again.

Other variations on parasite life cycles include the alternation of free-living and parasitic generations known as **heterogonic reproduction**. For example, lung nematodes in the genus *Rhabdias* alternate between parasitic and free-living generations. Parasitic individuals within the lungs of their amphibian hosts are **protandrous hermaphrodites**, a term for individuals that are functional males before becoming females. The spermatozoa are used to fertilize the eggs, and the eggs are then transported from the host's lungs into the gastrointestinal tract, and defecated into the soil (Runey et al., 1978). The released eggs hatch and begin a free-living generation resulting in adult free-living males and females which undergo sexual reproduction in the external environment (Langford and Janovy, 2009). Next, the free-living female nematode's progeny hatch within her body, where they feed on her internal organs, killing their mother in the process, and exiting her body as infective stages, a process known as **matricidal endotoky**. Finally, the infective juveniles enter the anuran host body cavity orally and/or via skin penetration and eventually migrate to the lungs to begin egg production to continue the life cycle (Baker, 1979).

The Role of Parasite Life Cycles in Transmission

Arguably, some of the most complex parasite life cycles belong to the digenetic trematodes, also known as flukes. During their life cycle, trematodes undergo sexual reproduction in the definitive host, followed by asexual reproduction in the first intermediate host in a process known as **polyembryony**, the formation of more than 1 embryo from a single fertilized ovum. Hundreds to thousands of free-living stages

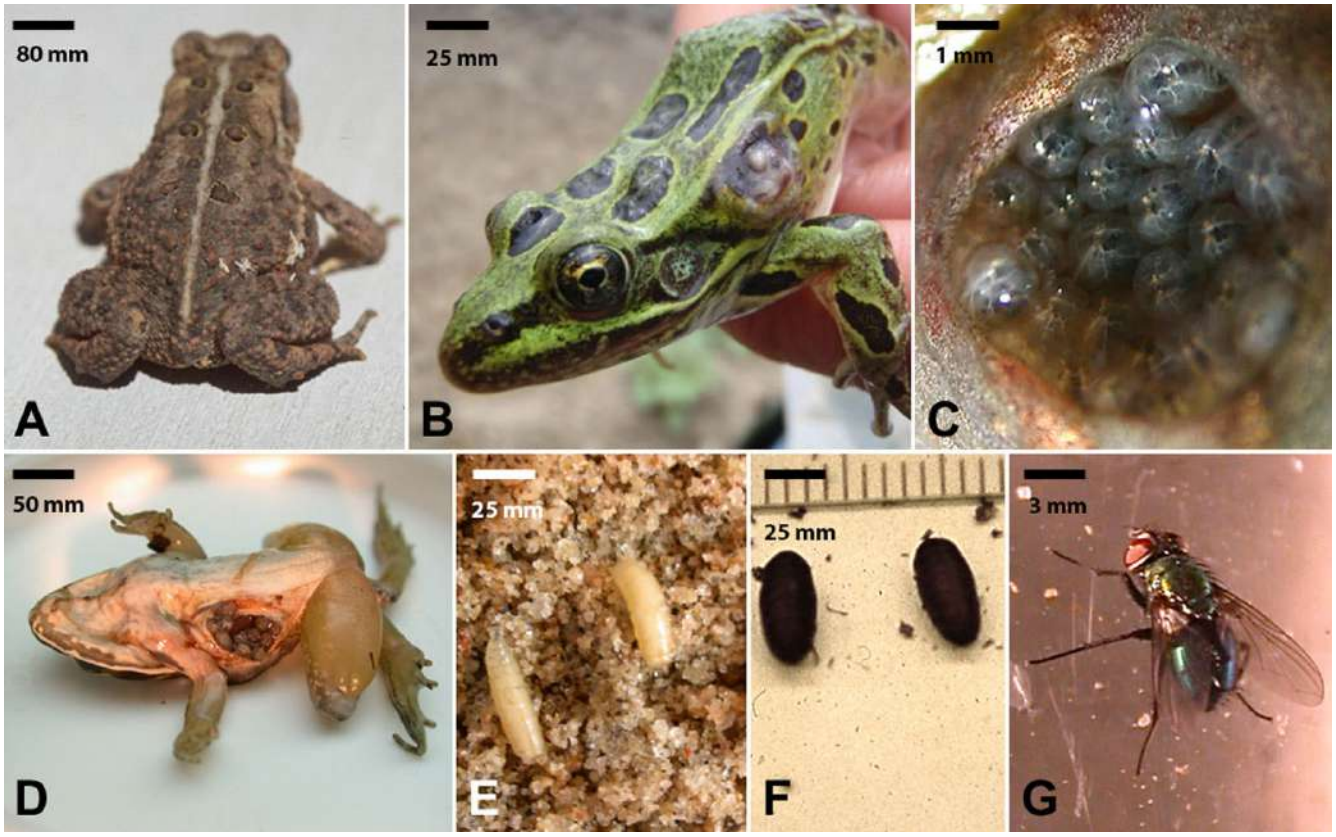

Figure 5. An example of a direct life cycle parasite where the parasites must exit the host and develop into a free-living adult and reproduce. A) Eggs of *Bufolucilia silvarum* glued to the back of an American toad *Bufo americanus*. Scale bar = 80 mm. B) Opened wound on the left lateral side of a northern leopard frog *Rana pipiens*. Note visible third instar maggots of *Bufolucila silvarum* in the wound. Scale bar = 25 mm. C) Third instar maggots of *Bufolucila silvarum* congregating and feeding as a group in an infected wood frog *Rana sylvatica*. Scale bar = 1 mm. D) Third instar maggots of *Bufolucila elongata* in a single wound on the right ventral side of a wood frog *Rana sylvatica*. Scale bar = 50 mm. E) Third instar maggots of *Bufolucila silvarum* searching for a place to pupate after leaving the host. Scale bar = 25 mm. F) Fully formed pupae of *Bufolucila silvarum*. Scale bar = 25 mm. G) An adult male green toad fly *Bufolucila silvarum*. Scale bar = 3 mm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

are then released from the first intermediate host, some of which infect a second intermediate host, which is then ingested by the definitive host (Figure 6).

A typical digenetic trematode life cycle offers a good example of the complexity of the transmission challenges faced by parasites during their life cycles (Lafferty and Kuris, 2002). First, eggs released into the external environment by adult worms in the definitive host hatch into a short-lived miracidium stage, which then must find a suitable first intermediate host, usually a snail. Second, and after asexual reproduction within the snail first intermediate host, the free-living but short lived cercariae emerge from the snail and must locate a suitable second intermediate host where they encyst as metacercariae stages. Third, the metacercaria stage must be ingested along with the second intermediate host by an appropriate definitive host for the life cycle to be completed.

It is hypothesized that parasites with complex life cycles have evolved by either adding or subtracting hosts based on trophic interactions of potential hosts (Poulin and Cribb, 2002). In trophically transmitted parasites with more than 1 host, or in parasites that are transmitted by vectors that take a blood meal from a vertebrate host, there are 2 hypotheses that support the addition of a host. One hypothesis proposes that the original host was preyed upon by other potential hosts higher up in the trophic food chain, and all other hosts have been added over time to the parasite life cycle (Smith-Trail, 1980; Poulin, 2007; Parker et al., 2003). Another hypothesis suggests the opposite. In this case, the original host was a top predator in the food web, and all other hosts with lower positions in the food web than the original host have been added secondarily to the parasite life cycle (Smith-Trail, 1980; Gibson and Bray, 1994; Lafferty, 1999; Parker et al.,

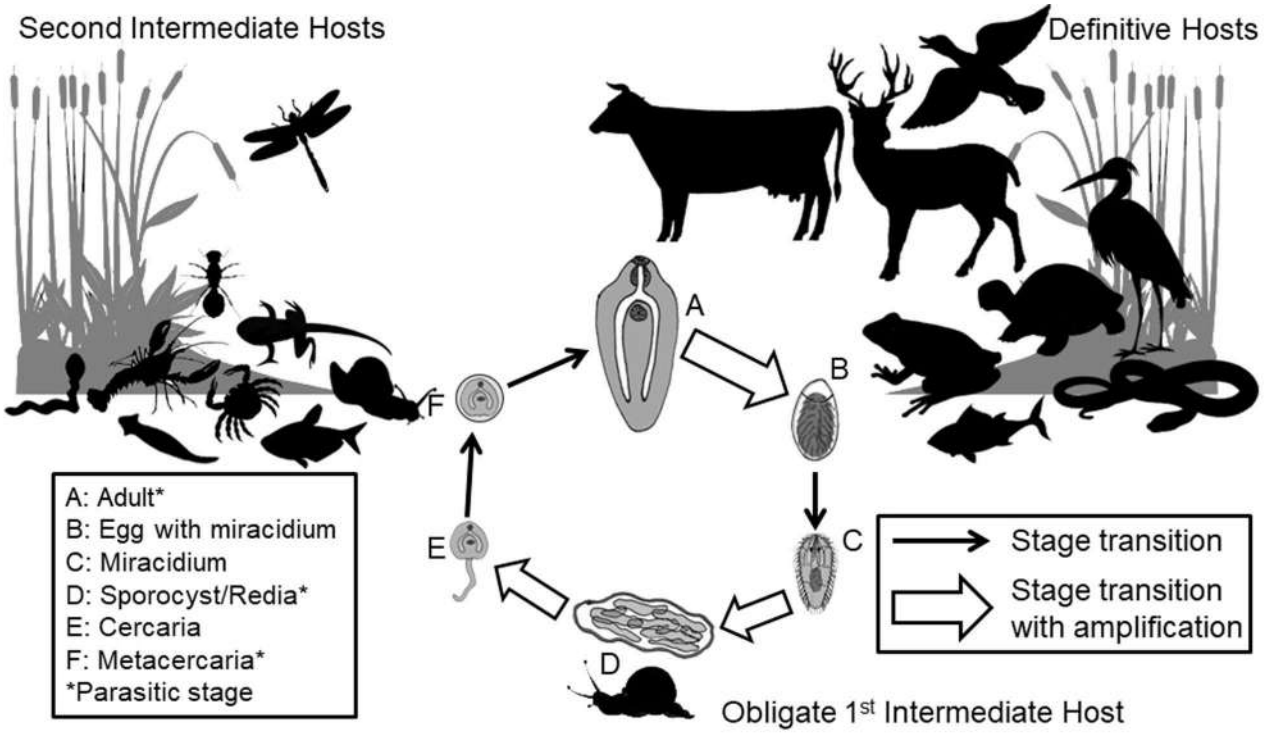

Figure 6. A representative diagram of a typical complex life cycle of a digenetic trematode. Note that most digenetic trematodes are host specific at the snail first intermediate host in the life cycle and much less host specific at the second intermediate and definitive host level. Also note the trematode developmental stages in the life cycle (A–F); including adult worms (A) producing eggs (B) through sexual reproduction in the definitive host (C), asexual reproduction (D) and production of free living cercariae (E) in the obligate snail first intermediate host. Source: M. Bolek. License: CC BY-NC-SA 4.0.

2003). Finally, hosts can also be lost if the life cycle no longer requires a particular host for completion (Poulin and Cribb, 2002; Parker et al., 2003).

A number of studies on parasite life cycles indicate that some species of parasites can survive in the alimentary canal of the predators of their definitive hosts. For example, **post-cyclic transmission** has been reported in a number of acanthocephalan and nematode species (Bolek, 1997; Nickol, 2003). In these cases, when a predator ingests a definitive host, instead of dying or being lost, the parasites simply reattach themselves to the intestine of the predator and resume growth or reproduction. Importantly, the predator may be the same as or a different species than the original definitive host of the parasite. Additionally, the direct life cycle of aspidobothrean trematodes, which parasitize molluscs, commonly promotes their survival and they reproduce in the intestines of turtles and fish that in turn consume infected clams as part of their diet. The aspidobothrean trematodes are considered a basal sister group to the digenetic trematodes which also infect molluscs as first intermediate hosts, but have complex life cycles (Zamparo and Brooks, 2003). As a result, one can imagine the evolution of complex life cycles by the addition of hosts to a direct life cycle.

However, understanding the specific steps of how and why these life cycles have evolved is difficult to decipher due to the lack of a fossil record for most parasites, complex host-parasite associations, and the lack of empirical data on host use for most parasite species in nature (Stigge and Bolek, 2015). For example, it is currently unclear if these processes occur gradually or require less evolutionary time (Stigge and Bolek, 2015). As a result of these difficulties, understanding how life cycles operate in nature and what hosts are used by those parasites can provide empirical data for future hypotheses testing on parasite life cycle evolution.

Parasite Adaptations, and Life Cycle Variation and Plasticity

Reproduction is certainly the most important task that individuals of any species of parasite must accomplish during their lifespan within a definitive host. However, in order for any parasite to reproduce within its host, it must be able to **infect** that host. In combination, these 2 principles (**infection** and **reproduction**) dictate that parasite life cycles have been selected for their ability to increase the probability that individual propagules will infect their hosts and achieve reproductive output (consisting of more propagules).

Understanding parasite life cycles is fundamental for many types of parasitological inquiries because life cycles inform understanding of life history strategies, host-parasite interactions, community and population ecology, life cycle evolution, and the epidemiology of diseases. Yet, the propensity for biologists to portray life cycles as a fixed, invariable unit is a monumental error, as actual real-world life cycles are not captured fully in so-called iron wheel diagrams such as those depicted in textbooks or health agency websites (Bolek et al., 2016). Indeed, understanding life cycle plasticity and variability is crucial to understanding how parasites evolve and function in hosts and the external environment. Despite the importance of this area of investigation, few biologists focus on life cycles of parasites as the center of their research. Furthermore, most parasitologists who have studied life cycles only do so until the life cycle could be completed. Once elucidated, most investigators do not continue to search for alternative hosts to complete the life cycle in nature. It is therefore unsurprising that published life cycles tend to be accepted as absolute truth and their validity is rarely questioned (Krull, 1952; Bolek and Janovy, 2008; Bolek et al., 2009; 2010).

Two examples are given in the following that provide realistic snapshots of how some parasites live in nature, while also highlighting specific life cycle adaptations that may increase both transmission probabilities and reproduction. In addition, these examples demonstrate how unrealistic paradigmatic life cycle diagrams are in deciphering transmission strategies of parasites in nature (Bolek et al., 2016).

The first example considers the life cycles of 2 closely related but host specific species of polystomatid flatworms (phylum Platyhelminthes: family Polystomatidae): *Polystoma nearcticum* and *Pseudodiplorchis americanus* (see Tinsley, 1990). *Polystoma nearcticum* infects the urinary bladders of 2 closely related treefrogs, *Hyla chrysoscelis* and *H. versicolor*, which reside in forests and grassland habitats throughout the eastern United States (Tinsley, 1990; Bolek and Coggins, 1998; Du Preez et al., 2007; Muzzall and Kuczynski, 2017). Interestingly, the life cycle of *Po. nearcticum* is synchronized with the reproductive biology of its treefrog definitive hosts (Figure 7). During the spring, when treefrogs enter permanent ponds to breed, adult forms of *Po. nearcticum* that live in the frog's urinary bladder begin laying unembryonated eggs concurrently with the oviposition activities of their treefrog definitive hosts. The eggs of *Po. nearcticum* are released into the pond in the frog's urine, and over a period of 10 days the eggs develop and hatch into short-lived motile larvae. Once hatched, the larvae of *Po. nearcticum* must find and infect their tadpole hosts within 20 hours of hatching. Interestingly, because tadpoles do not possess a urinary bladder, larvae of the worms enter the gill chamber of their tadpole hosts, where they mature in weeks and begin releasing eggs into the pond. The second generation of eggs produced by the branchial (gill) generation of *Po. nearcticum* develop and hatch coinciding with the metamorphoses of their tadpole hosts. When tadpoles transform into froglets they develop a urinary bladder and the larvae from the second generation of eggs of *Po. nearcticum* enter the froglet's cloaca and migrate into the urinary bladder (Figure 7). Once inside the urinary bladder of their treefrog definitive hosts, *Po. nearcticum* reaches sexual maturity and begins producing eggs when its treefrog hosts return to their breeding ponds the following spring.

In contrast to *Polystoma nearcticum*, *Pseudodiplorchis americanus* infects the urinary bladder of Couch's spadefoot toads, *Scaphiopus couchii*, an amphibian species that lives in deserts and arid habitats throughout the southwestern United States (Tinsley, 1990). Unlike the treefrog hosts of *Po. nearcticum*, Couch's spadefoot toads only enter temporary desert pools to mate and deposit eggs for approximately 21 hours per year (Tinsley, 1990). Since spadefoot toad tadpoles must complete metamorphosis in rapidly drying desert pools, they have one of the shortest developmental periods of any anuran species ranging from 7 to 20 days (Dodd, 2013). However, even with rapid metamorphosis, spadefoot toad tadpole mortality is often quite high in these desert pools, making tadpoles unreliable hosts for *Ps. americanus*. As a result, the transmission of *Ps. americanus* is confined to 1 to 3 nights each summer when the desert-adapted toads spawn.

To overcome this temporal problem, selection has favored a dramatic modification in the life cycle of *Pseudodiplorchis americanus*. Instead of producing eggs that must develop for weeks in the external environment and infect tadpoles, the larvae of *Ps. americanus* complete their development inside the uterus of worms in the urinary bladder of spadefoot toads. Once spadefoot toads enter desert pools to spawn, the larvae hatch within seconds of being released with the toad's urine into freshwater (Figure 7). When the larvae encounter a spadefoot toad in the water, they crawl up the chest of the amphibian and invade the nostrils. The larvae then migrate via the buccal cavity into the lungs where development occurs. Within a few weeks, the juvenile worms then migrate from the lungs by the intestine and cloaca into the urinary bladder. In the bladder, juvenile worms mature and then mate, accumulating new larvae in their uteri that will infect spadefoot toads the following year. Remarkably, the larvae of *Ps. americanus* appear to have specific adaptations for infecting adult spadefoot toads. For example, they are 2 to 4 times the size of larvae of any other species of polystomatid flatworms. Additionally, these giant larvae can swim for twice as long as larvae of *Polystoma nearcticum*, allowing them 2 days to

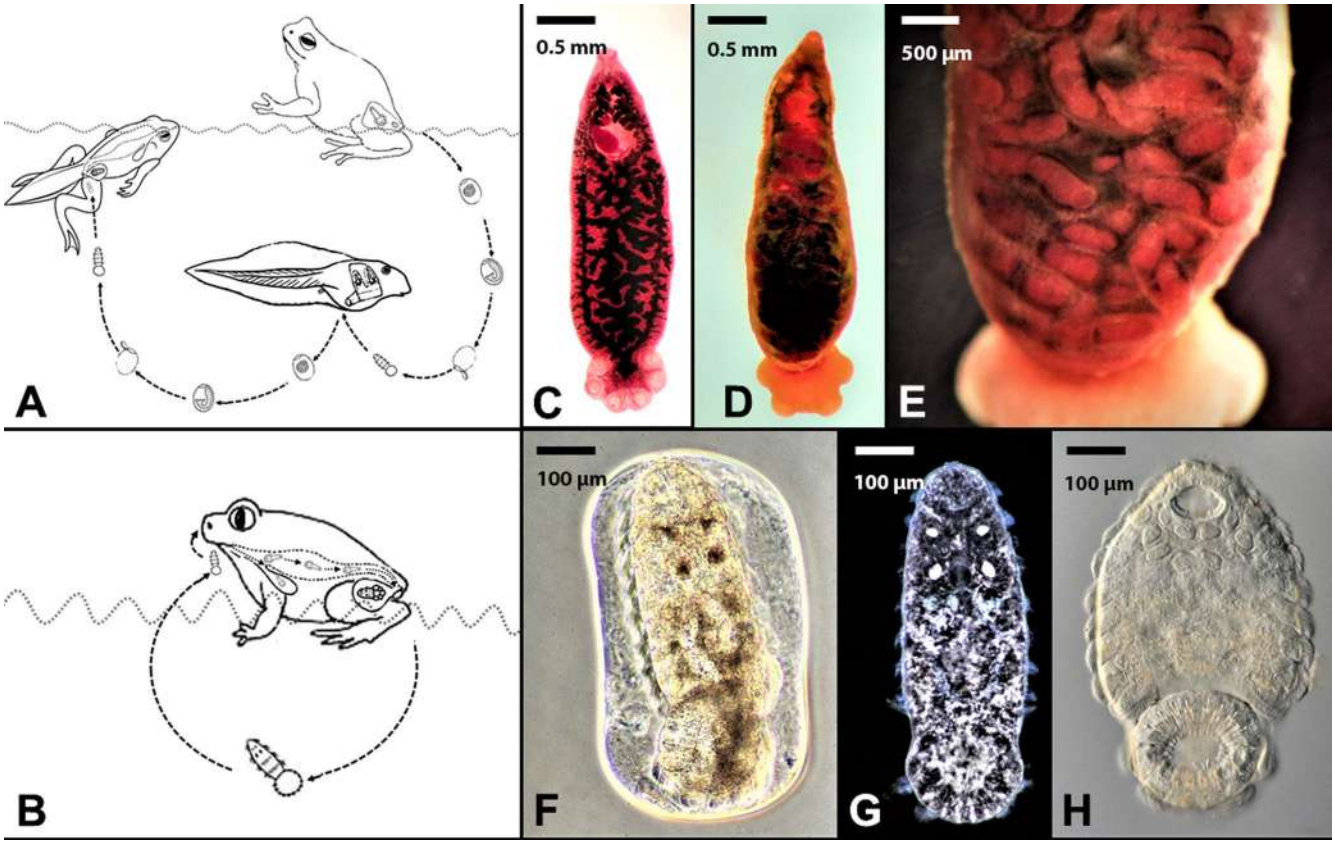

Figure 7. Example of life cycle variation for 2 closely related and host specific polystomatid trematodes. A) Transmission strategies of *Polystoma nearcticum* in the eastern gray treefrog *Hyla versicolor*. Note the egg being released by the urinary bladder generation of worms when their treefrog hosts enter ponds to breed followed by eggs being released from the branchial generation of worms on the gills of tadpoles. In all cases the eggs must develop in the external environment and the larval stage must find and infect metamorphosing froglets by entering their cloaca. B) Transmission strategy of *Pseudodiplorchis americanus* in Couch's spadefoot toad, *Scaphiopus couchii*. Larval stages are released directly from the bladder of spadefoot toads when they enter breeding pools. C) Adult *Po. nearcticum* recovered from the urinary bladder of a Cope's gray treefrog *H. chrysoscelis*. Scale bar = 0.5 mm. D) Adult *Ps. americanus* recovered from the urinary bladder of a Couch's spadefoot toad *S. couchii*. Scale bar = 0.5 mm. E) Higher magnification of *Ps. americanus* showing fully developed larvae in the uterus. Scale bar = 500 µm. F–H) Egg and hatched larvae of *Ps. americanus*. Note the 4 eyespots in (F) and (G) and the ciliated cells containing hundreds of cilia used for swimming in (H). Scale bar = 100 µm. Source: M. Bolek. License: CC BY-NC-SA 4.0.

encounter a spadefoot toad in water. Finally, the larvae of *Ps. americanus* can survive drying for up to an hour, which is likely an adaptation that allows the larvae of *Ps. americanus* to leave the water and crawl up the chest of their spadefoot hosts and enter the nasal passages (Tinsley and Earle, 1983).

The second example demonstrates how a generalist parasite, the tadpole pinworm, *Gyrinicola batrachiensis*, has a modified life cycle that appears to increase its reproductive success in different species of hosts. *Gyrinicola batrachiensis* infects the large intestine of tadpoles and has been reported from 18 species of frogs and toads (Pierce et al., 2018). Adult anurans are resistant to infections and (as noted above) tadpoles lose their pinworm infections when they metamorphose into adults, which in turn gives *G. batrachiensis* limited time for reproduction in its tadpole hosts. To make matters more complex, not all tadpole hosts are equal in terms of pinworm development and reproduction. For example, tadpoles of some anuran species metamorphose in just a few weeks **(short developmental period)** giving limited time for pinworm reproduction, while tadpoles of other anuran species take months to years **(long developmental period)** to metamorphose, giving pinworms more time for reproduction. However, pinworms cannot choose what species of tadpoles they will infect because all tadpoles become infected with *G. batrachiensis* when they accidentally ingest a pinworm egg on the pond bottom.

Investigation has shown that *Gyrinicola batrachiensis* exhibits 2 different but related lifestyles that appear to solve the problem for both short- and long-lived larval anurans. To overcome these constraints, *G. batrachiensis* has evolved 2 different reproductive strategies. The first strategy involves asexual reproduction, by **parthenogenesis**, when unmated female pinworms produce thick-shelled environmentally resistant eggs that are passed in tadpole feces to infect other tadpoles in the pond. The second strategy involves sexual reproduction by female and male pinworms, which results in female *G. batrachiensis* that produce 2 types of eggs: thick-shelled and thin-shelled. The thick-shelled eggs are released into the external environment to infect other tadpoles, which are similar to eggs produced by parthenogenic females. In contrast, thin-shelled eggs never leave the tadpole's intestine and they are **autoinfective**, hatching quickly in the tadpole's gut thus rapidly increasing the number of pinworms in a single tadpole.

Production of thin-shelled autoinfective eggs varies according to the amphibian species and its tadpole developmental time (Figure 8). In tadpoles with short developmental periods that provide limited opportunities for pinworm recruitment and reproduction, pinworms can reproduce **parthenogenetically** (Adamson, 1981). Parthenogenetic pinworms are **monodelphic** and produce thick-shelled environmentally-resistant eggs. While parthenogenetic pinworms do not benefit from sexual recombination, reproduction via parthenogenesis increases the probability that the nematode offspring will infect another tadpole before their current host metamorphoses. Alternatively, in tadpoles with long developmental periods that allow *Gyrinicola batrachiensis* more time for development and reproduction, nematodes reproduce sexually (Adamson, 1981; Rhoden and Bolek, 2011; Childress et al., 2017; Pierce et al., 2018).

Female nematodes in tadpoles with long developmental periods are **didelphic**, producing thick-shelled environmentally resistant eggs in 1 uterine branch and thin-shelled autoinfective eggs in the second branch of the uterus. As a result of the autoinfective reproductive strategy, pinworms in long-developing tadpoles increase their numbers quickly and in the long run, a female worm can produce numerous reproductively active progeny inside a single tadpole host. So, although *Gyrinicola batrachiensis* might not always end up in their ideal host, that is, a long developing tadpole, they always try to make the most of their lot in life!

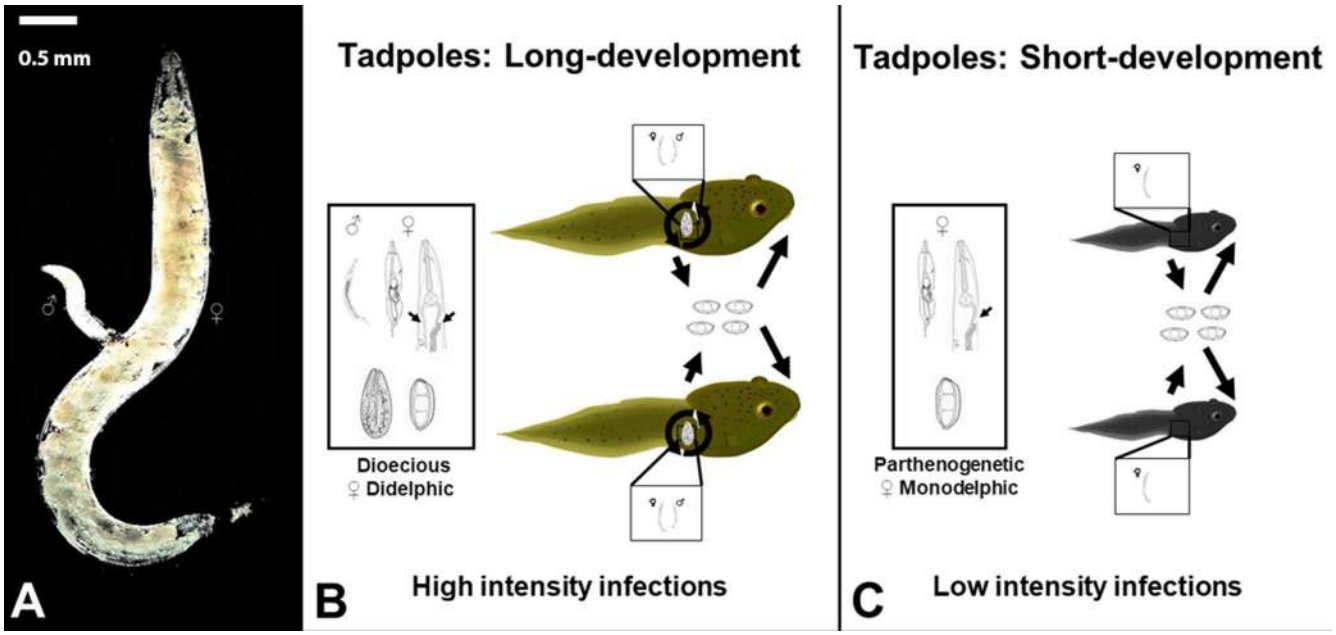

Figure 8. Example of plasticity in a direct life cycle of a generalist parasite *Gyrinicola batrachiensis*. A) A male (♂) in the process of mating with a female *G. batrachiensis* (♀). Scale bar = 0.5 mm. B) Dioecious reproductive strategy of *G. batrachiensis* in tadpoles with long developmental periods. Female worms are didelphic and produce thick-shelled and thin-shelled autoinfective eggs. As a result, tadpoles with long developmental periods have high intensities of *G. batrachiensis*. C) Parthenogenetic reproductive strategy of *G. batrachiensis* in tadpoles with short developmental periods. Female worms are monodelphic and only produce thick-shelled eggs. As a result, tadpoles with short developmental periods usually have much lower intensities of *G. batrachiensis*. Source: M. Bolek. License: CC BY-NC-SA 4.0.

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6

Parasites in Relation to Other Organisms

Behavioral Parasitology

Megan Wise de Valdez

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Chapter 6

Behavioral Parasitology

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Introduction

As pointed out in the previous sections, parasites have an intimate relationship with their hosts and can affect many aspects of their host's biology. By definition, parasites live at the expense of their host, causing some type of physical or physiological damage, but they can also affect host behaviors. Throughout this section, the who, what, when, where, why, and how of parasite manipulation of host behaviors will be investigated.

To categorize before moving on to examples, direct versus indirect effects on host behaviors should be described. Parasites can influence host behaviors **directly** through the physical presence of the parasite within a host or they may **indirectly** influence host behavior when potential hosts exhibit behaviors in order to avoid becoming infected with the parasite. Some examples of these parasite-avoidance behaviors are swatting, moving to a different habitat, feeding/foraging at specific times of day, and grooming (see Moore, 2002 for full review). Although these indirect effects on host behavior are interesting and certainly worthy of study, the direct effects of parasites on host behaviors are most salient. **Parasite-induced behavioral alteration/modification** refers to a behavioral change in a host that is caused by the presence of a parasite; there is no underlying assumption that the behavioral change is advantageous to either the host or the parasite. Note that the word *modification* and *alteration* can be used interchangeably. **Parasite *manipulation* of behavior** implies that the parasite is actively changing a host behavior in order to benefit itself. In the rest of this chapter, the basic principles of parasite-induced behavioral modifications will be established by exhibiting case-studies from the scientific literature to help answer 3 basic questions:

Question 1) **Why** are there parasite-induced behavioral modifications of hosts?
Question 2) **Which** host behaviors or traits are likely to be altered?
Question 3) **When** are host behaviors altered?

At the end of the chapter, there is a set of more advanced questions for those students who may want to delve deeper into the complexity of this aspect of host-parasite relationships.

Learning Objectives

1) Apply the scientific method to address questions about parasite-induced modification of host behaviors.
2) Analyze examples in the scientific literature to learn how scientists have experimentally addressed questions about parasite manipulation of host behaviors.
3) Be able to provide some classic examples of parasite-induced modification of host behaviors.
4) Understand the evolutionary principles of parasite manipulation of host behaviors.
5) Understand the types of host behaviors likely to be altered in relation to the parasites' life cycles.
6) Think critically about host-parasite relationships yet to be investigated from a behavioral standpoint.

Why Are There Parasite-Induced Behavioral Modifications of Hosts?

There is no simple answer to this question, but there are 3 primary hypotheses: 1) The altered behavior is a side-effect of infection, 2) the host benefits from the altered behavior (host-adaptation), and 3) the parasite benefits from the altered behavior (parasite-adaptation) (Poulin, 2010; Moore, 2013). Each will be discussed in turn.

Behavioral Changes as Side-Effects of Infection

Behavioral alterations as side-effects of infection appears to be the simplest answer because an infected host is expected to act sick, especially if the behavioral changes appear to be of no obvious advantage to either host or parasite. However, unless the hypothesis is tested it should not be used as the default explanation. Wise de Valdez (2007) conducted a study to determine whether parasite-induced behavioral changes were a side effect of infection or if they were advantageous to the parasite. The host-parasite system used was mermithid-mosquito system. Mermithids are nematode worms that can use aquatic mosquito larvae for development where they then emerge to a free-living state. During their development

Box 1. Notes on the Scientific Method

No matter in what stage someone is in their scientific career, all employ the scientific method, or at least parts of it, when embarking on new areas of study. In fact, most scientists have internalized the process as they use it on a daily basis. It is helpful to periodically revisit the formal nature of the scientific method. Thus, because for many students, this will be the first time considering parasite manipulation of host behaviors, you may approach it as new scientists using the scientific method. It all starts with an observation followed by a question (or many). Then use your previous scientific knowledge, or read a bit more, to come up with an educated answer to that question: The hypothesis. All good hypotheses must be testable. Now, the hypothesis may or may not be the right answer to the question; it is only a best guess based on previous understanding of a system. Therefore, to determine if the hypothesis is correct, the hypothesis must be tested, and data must be gathered through observational or experimental studies. Through data analysis, it can then be confirmed whether the hypothesis may be supported or rejected.

they grow and eventually take over much of the space inside the mosquito larvae after which they exit the mosquito larvae, killing it. Wise de Valdez (2006) found that mermithid nematodes made their mosquito larvae hosts less active and it is tempting to hypothesize that the change in activity levels is simply a side effect of the worm filling up the entire space of the mosquito larvae. An alternative hypothesis would be that the behavioral changes benefit the parasite by making the mosquito less likely to be eaten by a predator and thus survive long enough to emerge to its free-living stage. To test these hypotheses, Wise de Valdez (2007) experimentally infected *Aedes aegypti* mosquito larvae with mermithid nematodes and confirmed that their activity levels were lower than those without an infection. Predation experiments were then conducted using the predatory mosquito larva *Toxorhynchites rutilus* and it was found that the predator consumed both infected and uninfected larvae at equal rates. Therefore, the experiment supported the hypothesis that the behaviors are a side effect of infection, and the reduction in activity levels did not appear to benefit the parasite because they were eaten just as often as uninfected mosquitoes.

However, a singular set of experiments supporting a hypothesis does not necessarily make the hypothesis definitive. The important thing is that data were gathered and allowed the investigators to begin to make more educated assumptions about a system. Future scientists could use this study to develop new hypotheses that might lead to other conclusions after testing. This is what is so great about science, new hypotheses can always be tested! When the host's behaviors don't necessarily fit the classic sick behavior or when entirely new and unexpected behaviors are observed, other explanations may be sought.

Host Adaptation: The Host Benefits from Altered Behaviors

Another hypothesis to consider how to answer the why of behavioral alterations is that the host could benefit in some way from a change in behavior. The altered behavior would then be considered a host adaptation. An **adaptation** is a character that increases the fitness of an organism and **fitness** is the ability of an organism to survive long enough to successfully reproduce. Adaptations arise through **natural selection**; individuals that exhibit a particular trait survive and reproduce more than individuals that do not exhibit that trait. The parasite-induced behavioral changes of an infected host would be a host adaptation if they help to reduce or rid the host of parasites and thereby increase host survival and reproductive capacity (its fitness).

Unusual foraging habits that are a form of self-medication have been observed as a behavioral change that benefits the host. For instance, chimpanzees will eat medicinal plants that are not part of its normal diet (Moore, 2013, citing Huffman, 1997). Caterpillars infected with a parasitoid fly will switch plant food source and increase its survival (Moore, 2013, citing Karban and English-Loeb, 1997). Two other classic behavioral strategies that have evolved in some infected hosts in response to parasitism are known as **behavioral fever** and **behavioral chills** which are characterized by movement of infected hosts to a higher or lower than normal temperature to rid themselves or reduce the impact of a pathogen (see Moore, 2002 for review). Both of these are most likely to occur in organisms that cannot regulate their temperature metabolically. Metabolic fever in endotherms is well documented. It induces a behavioral change that brings the afflicted individuals to a habitat with a particular temperature. Müller and

Schmid-Hempel (1993) found that bumblebees infected with parasitoid fly larvae remained outside the hive where it was colder and when given a choice they spent more time in cold areas than uninfected bumble bees (behavioral chills). By altering their behavior to choose colder temperatures, these infected bumblebees lived longer and had fewer fully-developed parasitoids that the infected bumblebees that were kept at normal temperatures. A study by Watson et al. (1993) showed that house flies infected with a fungal pathogen that spent at least 8 hours in 40 ºC temperature shortly after infection survived longer than those that did not. Interestingly, this behavior did not benefit the house fly if the infection was more advanced (after 5 days post infection). Even more interesting, and evidence that parasite-induced behavioral alterations are complex, was that the flies that did not successfully employ behavioral fever moved to cooler temperatures, a behavior that benefited the fungal parasite; cooler temperatures enhanced the propagation of the parasitic fungus!

Parasite Adaptation: The Parasite Benefits from Altered Behaviors

In the first half of the 20th century, several researchers proposed that parasites may be able to alter the behaviors of their hosts in ways that increase their transmission success (Lefèvre et al., 2009 citing Cram, 1931; Van Dobben, 1952). Later, in the 1970s and 1980s, researchers provided some of the first empirical evidence that intermediate hosts infected with parasites exhibit different behaviors than those that were uninfected. Furthermore, the infected hosts were more likely to be consumed by the next host in their life cycle, thereby increasing transmission success (Hindsbo, 1972; Bethel and Holmes, 1973; 1974; 1977; Moore, 1983). These studies involved acanthocephalan parasites and their crustacean intermediate hosts. Bethel and Holmes (1973; 1977) demonstrated that small aquatic crustaceans, *Hyalella azteca* and *Gammarus lacustrus*, infected with 1 of 2 different species of acanthocephalans, *Polymorphus paradoxus* or *Corynosoma constrictum*, exhibit behaviors that move them to areas where their habitat overlaps with the feeding area of the parasites' definitive host and may make them more conspicuous. Through predation experiments using birds and muskrats, they found that infected crustaceans were more vulnerable to predation by mallard ducks and accidental ingestion by muskrats (both definitive hosts) than uninfected crustaceans. A study by Moore (1983) showed that the juvenile stage of *Plagiorhynchus cylindraceus* induces risky behavior of its isopod pill bug host, thereby causing it to be more conspicuous to its definitive host predator, the European starling (the details of this study will be discussed later in the chapter).

These initial studies kick-started research on parasite manipulate of host behaviors in earnest and since then researchers have found examples across all parasite taxa: protozoan parasites, Plathyhelminthes parasites in the classes Trematoda (flukes) and Cestoda (tapeworms), Acanthocephalans, Nematodes, Nematomorphs, and parasitic arthropods (see reviews by Adamo, 1997; Moore, 2002; Lefèvre et al., 2009; Hughes et al., 2012). Discovering the adaptive nature of these behavioral alterations in a scientifically sound way became a main area of discussion (Poulin, 1995; 2010). Furthermore, the types of questions being asked about parasite-induced behavioral alterations have expanded to include more complex questions (see end of chapter). For the remainder of the chapter the primary focus will be on the hypothesis of parasite manipulation of behaviors as parasite adaptations. The next question is: Which host behaviors or traits are likely to be altered, and when?

Which Host Behaviors or Traits are Likely to Be Altered, and When?

Life Cycles and Transmission Routes

In order to understand the adaptive nature of a parasite-induced behavioral change, the life cycle of the parasite in question must be understood. The parasite life cycle plays a major role in which host is likely to be manipulated and which behaviors are manipulated. Parasites with **complex life cycles** have multiple hosts; 1 or more **intermediate hosts** which are infected with an immature stage of the parasite, and a **definitive host** in which the parasite reaches sexual maturity. Parasites with **simple life cycles** have only 1 host, and **parasitoid life cycles** are unique in that 1 host is always killed by the parasite as it emerges to a free-living stage. Each life cycle has different requirements for how the parasite moves within the environment to reach a reproductive stage. Parasites with complex life cycles require movement from 1 host to the next. This movement can be up the food chain where 1 host lower on the food chain is consumed by the next host in the life cycle that is higher in the food chain (trophic transmission; Figure 1A). Movement can be through a vector, where 1 host (the vector) transmits the parasite to the next host (often via a bite) without being killed (vector-borne transmission; Figure 1B). Additionally, some parasites with complex or simple life cycles might require a host to bring them to a specific habitat where their eggs or larvae might be deposited (Figure 1C). Parasites with simple life cycles (1 host) are interesting because they may live their entire life within the single host or they may have 1 or more free-living stages, spending only part of their life cycle in the host. Some of these single-host parasites may use their host as

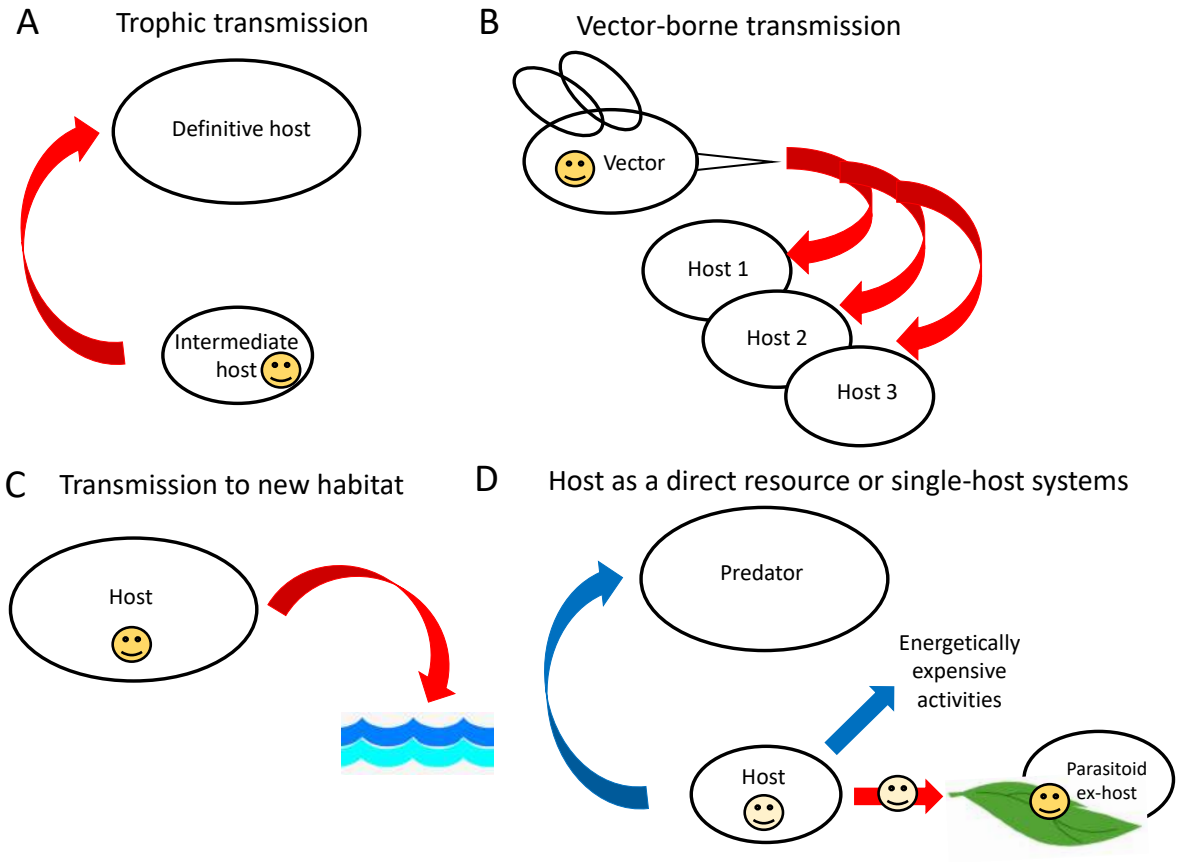


Figure 1. Presented are 4 main scenarios in which behavioral alterations have been seen. The smiley face is the parasite and the arrows indicate the stage in the life cycle where behavioral alterations are likely to occur. Red arrows indicate behaviors that increase the likelihood and blue arrows indicate behaviors that decrease the likelihood. A) Trophic transmission: Trophically-transmitted parasite behaviors of the intermediate host should be altered to increase transmission to the next host. B) Vector-borne transmission: In vector-borne parasite transmission, behaviors of the vector should be altered to increase transmission to multiple hosts or to increase the parasite load delivered. C) Transmission to a new habitat: Parasites that require delivery to a new environment, either themselves or their propagules, should manipulate the host to bring it to the appropriate habitat. D) Hosts as a direct resource: Parasites that use a host as a direct nutritional resource, usually parasitoids, should modify host behaviors to increase nutritional access or to prolong its survival and in some cases to elicit post-emergence protection. Note: These scenarios are not mutually exclusive. Source: Adapted from Poulin, 2010. License: CC BY-NC-SA 4.0.

a direct nutritional resource (Figure 1D), especially parasitoids, that usually consume much of their host in order to develop to their free-living stage. All of these different life cycles and transmission requirements open the door for some interesting ways in which parasite-induced behavioral modifications are manifested.

Trophic Transmission

In complex life cycles where trophic transmission is required, it would be expected that the host likely to be manipulated would be the intermediate host and the altered behaviors should result in an increase in consumption of that intermediate host by the next host in the life cycle (Figure 1A). Even these expectations have their nuances; the behaviors manipulated will be different if the next host is a natural predator or if the next host is not a natural predator of the intermediate host. If the intermediate host is a natural food source of the next host in the life cycle, it would be expected that the parasite would alter its normal predator avoidance behaviors. For example, the intermediate hosts of *Toxoplasma gondii* are rats and the definitive hosts, cats, are a natural predator. Normally, rats find cat urine odor repulsive. This is a natural defense mechanism that elicits an avoidance behavior. However, when infected with *T. gondii* rats are attracted to cat urine and might even seek out the cat which should theoretically increase the rate of predation on infected rats and thereby promote trophic transmission (Berdoy et al., 2000). On the other hand, if the intermediate host is not a regular food source of

the next host in the life cycle, parasites might manipulate behaviors that increase the contact these hosts have with their non-natural predator. For example, the trematode parasite *Dicrocoelium dendriticum* uses an ant as its intermediate host. In order for the life cycle to be completed, the ant harboring the juvenile trematode must be consumed by a grazing herbivore (usually a sheep or cow) which does not intentionally consume ants. The parasite manipulates the behavior of the ant in order to increase contact with the grazing definitive host. Ants infected with *D. dendriticum* act normally during the day but when the temperature drops, they climb to the top of blades of grass and clamp down with their mandibles. The ants are unable to release until the temperature rises again, thus positioning themselves to be eaten by grazing definitive hosts (Anokhin, 1966). Another extraordinary example that is quite evolutionarily complex is a nematode that not only causes the posterior end of an ant to turn red, but also manipulates the ant to hang out near a cluster of red berries. Yanoviak and fellow researchers (2008) conducted predation experiments and found that this manipulation of the phenotype and climbing near berries increased predation by the definitive host, frugivorous (fruit-eating) birds, that do not normally consume ants.

Included above are brief descriptions of just a few of the many studies that support the hypothesis that infected intermediate hosts behave differently than uninfected hosts and that these behavioral changes may be adaptive by increasing trophic transmission to the next host. However, many studies reported in the scientific literature (see review in Moore, 2002) have not provided experimental evidence that definitively supports that hypothesis. The reason these studies are less frequent in the literature is that they are simply hard to do. Pick any life cycle illustrated in this book and imagine what it would take to study the primary questions of parasite-induced behavioral changes. Not only would it first need to be established that the behaviors of infected and uninfected hosts differ, but then the next host in the life cycle would need to be included to determine if they became infected more often due to this behavior. Sometimes that next host in the life cycle is an animal that simply can't be used in experiments (think humans, large carnivores) or may be uncooperative in experimental arenas. Despite this difficulty there are studies out there. Following is a detailed description of one of the seminal works that provides experimental evidence of parasite manipulation of hosts in a trophically transmitted parasite system.

Moore (1983) investigated the acanthocephalan parasite *Plagiorhynchus cylindraceus* and the behavioral manipulation of its intermediate host *Armadillidium vulgare* (common pillbug). The life cycle of *P. cylindraceus* requires that the intermediate host, the pillbug, be eaten by the definitive host, the European starling (*Sturnus vulgarus*; Figure 2).

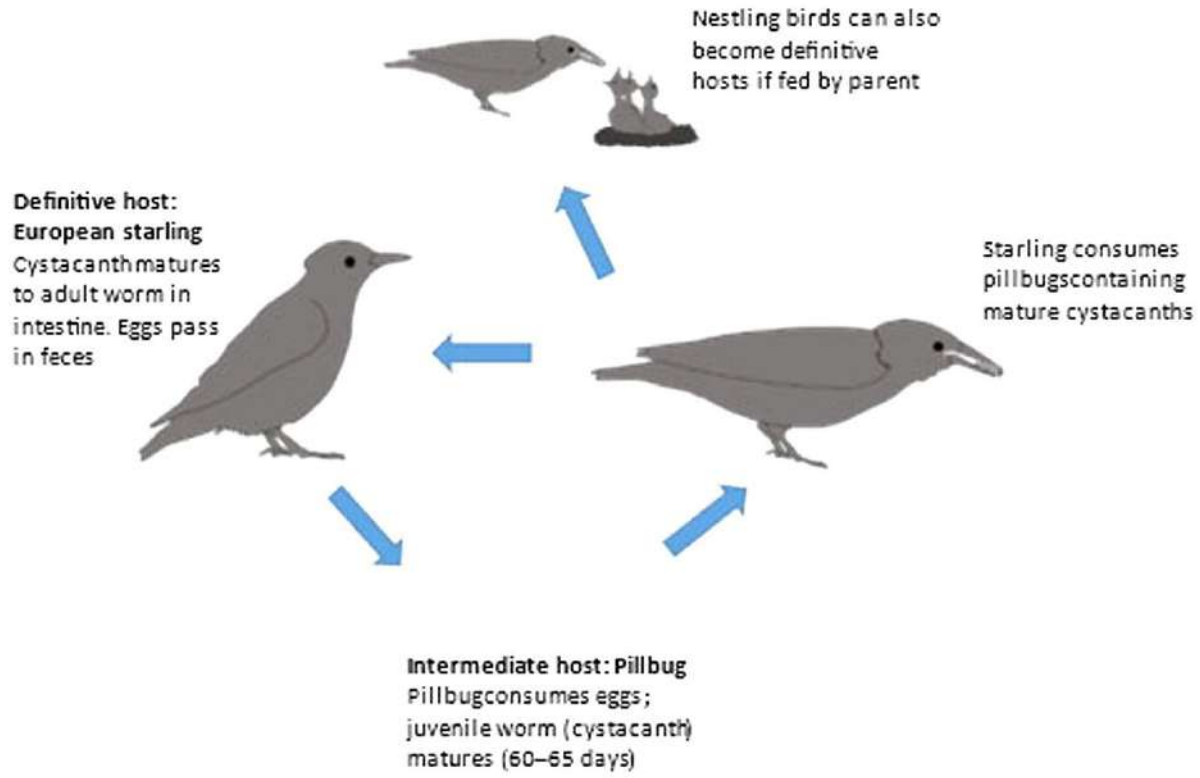


Figure 2. Life cycle of the acanthocephalan parasite *Plagiorhynchus cylindraceus*. Source: M. Wise de Valdez. License: CC BY-NC-SA 4.0.

Box 2: Stop and Think

Before reading further, take a look at the life cycle (Figure 2) and think about what you already know about pillbugs and birds. Where do they live? How do they behave? What behaviors might be targeted by the parasite that might help it reach the starling? By doing this you are starting to formulate one or more hypotheses. How might these hypotheses be tested?

Moore conducted both laboratory and field experiments to investigate this host-parasite system. For this example, it is interesting to consider how 2 primary questions were answered: 1) Do infected pillbugs behave differently from uninfected pillbugs, and 2) Are infected pillbugs more likely to be eaten by starlings?

In order to answer the first question, "Do infected pillbugs behave differently from uninfected pillbugs?" Moore experimentally infected pillbugs and sham-infected others (Figure 3A). **Sham infection** is when the researcher treats the control animals similarly during the infection experiments but does not include the actual parasite. In behavioral experiments it is

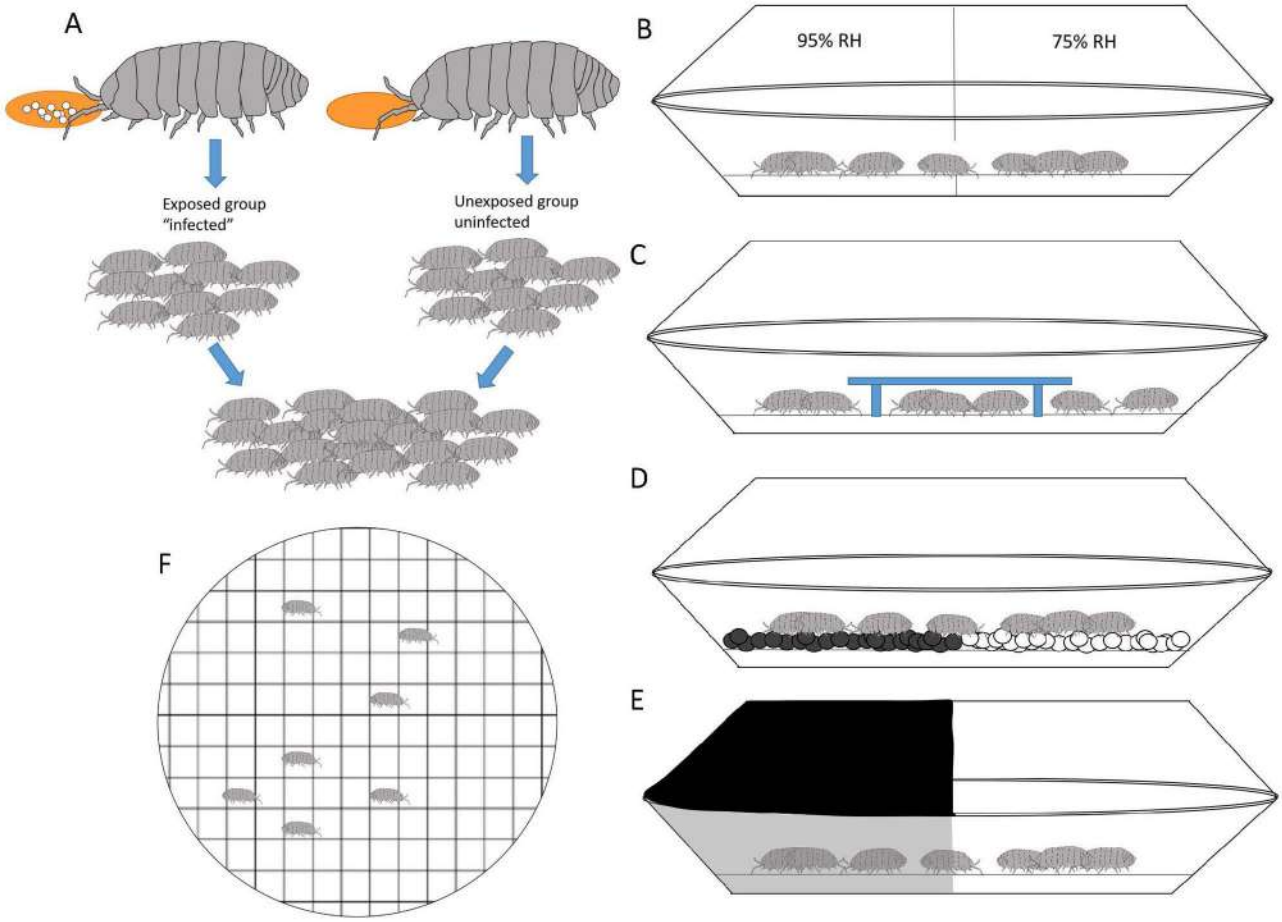


Figure 3. Experimental design to test behavioral differences between uninfected pillbugs and those infected with *Plagiorhynchus cylindraceus.* A) Experimental infections: Pillbugs were fed carrots with (exposed) or without (unexposed) *P. cylandraceus* eggs. Pillbugs were maintained for 3 months to ensure the cysticanth had reached the stage when it could be infectious to birds. Prior to placement in the arenas (B–F), an equal number of exposed and unexposed pillbugs were combined into a group, then each was uniquely marked. At the end of each trial all pillbugs were dissected to look for cysticanths. Each behavior trial was thus **blind** (the observer did not know infectious status during behavioral observation). All arenas consisted of 2 pie plates, one on top of the other. A wire mesh bottom was placed as a platform for pillbugs. Different aspects were manipulated to test the behavioral response. B) Humidity choice: High relative humidity (RH) or low RH. C) Shelter seeking: Under a shelter or exposed. D) Substrate preference: White or black. E) Phototaxis: Light or dark. F) Locomotion: Distance moved and resting behaviors. Source: Adapted from Moore, 1983.

important to institute multiple controls in order to ensure that behavioral differences observed are the result of the parasitic infection and not a difference in treatment of the organisms. Another important thing to note is that the pillbugs in the intentionally-infected group may not always become infected. Exposure to parasite eggs does not ensure that the infection will take. For this reason, the 2 groups are referred to as exposed and unexposed (Figure 3A).

Because pillbugs are normally found in areas of high moisture and under leaf litter, bark, or rocks, and because these habitats also provide protection from potential visual predators, Moore chose to look at behaviors associated with habitat preference (humidity, shelter, substrate, and light) and overall activity level of the pillbugs. Moore set up several arenas to test habitat preference of infected and uninfected pillbugs (Figure 3B-E) and one to determine activity level (distance moved and time resting; Figure 3F).

Before adding the pillbugs to the arenas, 5 exposed and 5 unexposed pillbugs were mixed together and were then marked with a unique identifier. By mixing them before the study, it enabled Moore to conduct **blind assays** in which she did not know which pillbugs were exposed and which were unexposed. In this way she controlled for observational bias. The trials consisted of placing the 10 pillbugs in the arena, allowing them to acclimate, and then recording the location of each pillbug every minute for 30 minutes. At the end of each trial, the pillbugs were dissected to determine infection status. Moore did this for each of the different arenas: humidity choice (95% relative humidity:75% relative humidity; Figure 3B), shelter seeking (under a shelter:exposed; Figure 3C), substrate choice (white:black; Figure 3D), and phototaxis (movement to or away from light; Figure 3E).

Moore found that infected pillbugs spent significantly more time in less humid and unsheltered areas and spent

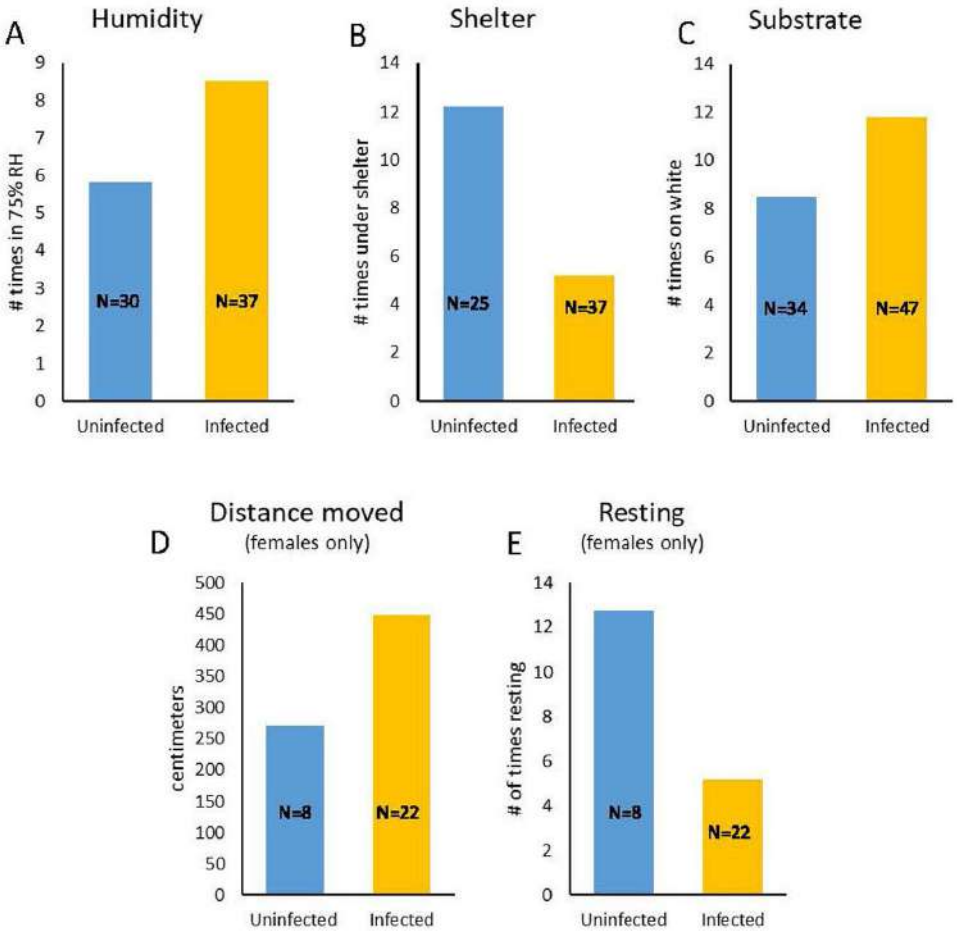


Figure 4. Infected pillbugs were found more frequently in less humid areas, in unsheltered areas, and on white substrate than uninfected pillbugs. There was no difference in phototaxis between infected and uninfected groups, both were negatively phototactic. Infected females were more active than uninfected females; infected females moved a greater distance in a set time period and rested less than did uninfected females. Males did not show differences between infection groups. Error bars not shown (but see Moore, 1983). Source: Adapted from Moore, 1983. License: CC BY-NC-SA 4.0.

more time on white substrate than uninfected pillbugs (Figure 4A-C), however there was no difference in preference for darkness (all preferred dark). Activity levels differed between infected and uninfected female pill bugs; infected females traveled further and rested less than uninfected females (Figure 4 D-E).

The second aspect of Moore's study was to establish whether starlings fed preferentially on infected pillbugs. Moore used experimental data from outdoor cage studies as well as observational data from the field. In the outdoor cage trials Moore used 5 individual wild-caught adult starlings and provided each individual bird with 10 infected and 10 uninfected pillbugs (pillbugs were unmarked; Figure 5). Pillbugs being presented to the birds were on a pan where they were offered a choice of black/humid or white/less-humid substrate. After the bird had eaten 10 pillbugs, the uneaten pillbugs were dissected in order to determine which pillbugs the starling had eaten (infected or uninfected). Moore found that 71% of the infected pillbugs were eaten and only 44% of the uninfected pillbugs were eaten (Figure 5), indicating that behavioral differences in the pillbugs led to an increase in predation rates on infected pillbugs.

In the field, Moore used the infection rate of nestling starlings to establish if parents were foraging randomly or preferentially on infected pillbugs. Note that nestlings can become infected by being fed infected pillbugs by their parents (Figure 2). Moore collected data from wild starlings to determine how often pillbugs were fed to nestlings and the natural infection rate of pillbugs in the field area. With these data, she calculated the probability of nestlings receiving infected pillbugs from their parents if the parents chose pill bugs randomly in the field arena. She then compared this probability to the actual infection rate of nestlings in the field. She found that more nestlings were infected than would be expected if parents were choosing pillbugs randomly. Which means that adult starlings were feeding their nestlings infected pillbugs more often than they were feeding them uninfected pillbugs because the adults are more likely to capture infected pillbugs due to the risky behavior exhibited by the infected pillbugs. These field observations corresponded with what she saw in the lab predation experiments. In conclusion, Moore provided experimental and field-based evidence that the behavioral manipulation of pillbugs by *Plagiorrhynchs cylindraceus* is a parasite adaptation to increase the chance of being consumed by the next host in the life cycle.

Vector-Borne Transmission

Not all parasites that have a complex life cycle involve trophic transmission. Parasites that use a vector to transmit parasites to multiple hosts are also exhibiting a complex life cycle (Figure 1B), but in this case, 1 host transmits the parasite to the other without being consumed. A vector-parasite life cycle often involves an arthropod that is capable of blood-feeding (think mosquitoes, ticks, kissing bugs, sand

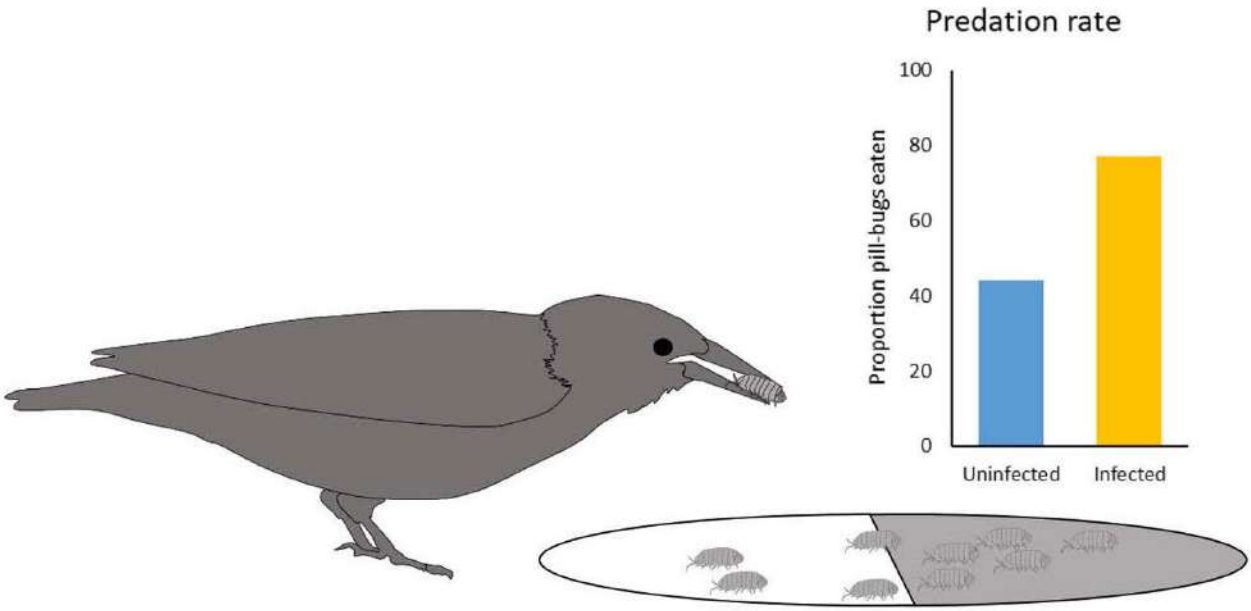


Figure 5. Field-cage predation experiment. Starlings were offered an equal number of infected and uninfected pillbugs in a pan that allowed pillbugs to choose their habitat. The habitat provided was either white dry sand or dark humid sand. Over 5 trials, 71% of infected pillbugs were eaten and only 44% of uninfected pillbugs were eaten. Source: Adapted from Moore, 1983. License: CC BY-NC-SA 4.0.

flies) on a vertebrate host. Through the act of blood-feeding, parasites are transmitted to the other host in the life cycle, often a vertebrate.

Box 3: Stop and Think

Before reading further, think about times when you have been bitten by a mosquito. You hear and see them and you likely swat or slap them or you just give up and go inside. What if that mosquito was infected with a parasite that could be transmitted to you? What mosquito behaviors might the parasite manipulate to ensure transmission to you? What behaviors might be manipulated to ensure it was also transmitted to the other people hanging around outside with you? How would you formulate these questions into hypotheses that you could test?

Behaviors that are likely to be altered in this type of host-parasite relationship are those that increase the transmission rate or the parasite load delivered upon transmission. The vector behaviors that are most often targeted are the feeding behaviors, although host-seeking/finding behaviors have also been shown to be altered by parasites (see reviews: Molyneux and Jefferies, 1986; Hurd, 2003; Lefèvre et al., 2006). One of the first accounts of modified feeding behavior of a vector was by Bacot and Martin, 1914 (referenced in Moore, 2013), where they observed that fleas carrying the plague bacteria (*Yersinia pestis*) were less successful at feeding due to blockage of their feeding apparatus by *Y. pestis* and that the blockage led to plague transmission. *Plasmodium*, the parasite that causes malaria, is vectored by mosquitoes and multiple studies have shown that *Plasmodium* can alter mosquito host-seeking and blood-feeding behavior in ways that can potentially increase transmission rates (Cator et al., 2012).

Another well-studied vector-borne parasite that has been studied in light of its behavioral manipulations is the protozoan parasite *Leishmania* (Killick-Kendrick et al., 1977; Beach et al., 1985, citing Chung et al. 1951; Rogers et al., 2002). *Leishmania* are single-celled parasites that are transmitted to humans or other mammals by the bite of a sand fly and in humans can cause various debilitating pathologies and symptoms (see Chapter 12 for detailed descriptions). The most common form is cutaneous leishmaniasis which is characterized by painful open sores on the skin that have difficulty healing. Rogers and Bates (2007) investigated whether 2 species of *Leishmania* that cause cutaneous leishmaniasis, *L. mexicana* and *L. infantum*, manipulate the behavior of their sand fly hosts (*Lutzomyia longipalpis*) in ways that increase transmission efficiency in a mouse model (use of humans in experimental infections is reasonably restricted). An elegant multi-dimensional study provides evidence that *Leishmania* can manipulate host behavior to increase transmission and infectivity, described below.

In order to interpret when and how behavioral alterations are likely to occur in the sand fly-*Leishmania* system, the life cycle of *Leishmania* must be understood (see Chapter 12 for more on *Leishmania*). In short, the life cycle of *Leishmania* involves an infected sand fly biting an uninfected mammalian host and injecting the motile promastigote stage. The promastigotes invade white blood cells and develop into amastigotes. An uninfected sand fly becomes infected when it bites an infected mammal and ingests blood containing the amastigote stage. In the sand fly, the amastigote stage transforms into the promastigote stage over the course of 7–10 days (extrinsic incubation period). Thus, it is important to remember that the promastigote stage is the stage that is infective to the mammal and that the amastigote stage is infective to the sand fly.

Some of the previous work on this system must be understood before delving into the study by Rogers and Bates (2007). Several research teams established that *Leishmania* damage the stomodeal valve and physically block the gut with a matrix made by a gel they secrete (Schlein et al., 1992; Stierhof et al., 1999; Rogers et al., 2004). This blockage interferes with sand fly feeding and limits the amount of blood it can take in. As a result, they take longer to feed and probe the skin more often (Rogers et al., 2002). A different group studying the rodent malaria-mosquito model of *Plasmodium yoelli* and *Anopheles stephensi* found that feeding persistence increased in infected mosquitoes but only after *Plasmodium* had reached the stage in which it was infective to humans (Anderson et al., 1999).

Box 4: Stop and Think

How is it advantageous to the parasite to alter vector behavior only during certain times? Think about what a vector has to go through when it needs to feed? What are the risks?

Hypotheses	Assay	Experiment	Result	Interpretation
1) *Leishmania* manipulate sand flies to persist with blood feeding only after they become infective to the mammalian host	Biting persistence assay (Figure 7)	Compared biting persistence of uninfected sand flies with those infected with either *L. mexicana* or *L. infantum* after interruption over the course of 11 days as the *Leishmania* developed from amastigote to the infective promastigote	Infected sand flies persisted longer than uninfected (Fig.ure8A) and the persistence increased as the infection matured	**Hypothesis supported** Infection with either species of *Leishmania* leads to a change in biting persistence and that the change is stage-specific, occurring to a higher degree when the parasite is infective to the mammalian host
2) *Leishmania*-infected sand flies feed on multiple hosts	Second host choice assay. Similar to Figure 7 but with 2 mice	Compared probability of a host-switch after repeated disturbance of uninfected sand flies and those infected with either *L. mexicana* or *L. infantum*	Infected sand flies were more likely to feed on multiple hosts whereas uninfected flies often gave up on feeding after repeated interruption (Figure 8B)	**Hypothesis supported** Infection with *Leishmania* increases the number of hosts on which sand flies feed and thus can be considered a mechanism for increased transmission success
3) *Leishmania*-infected sand flies that have been behaviorally manipulated will deliver a higher parasite inoculum per host than non-manipulated infected sand flies	Biting persistence assay (Figure 7) and uninterrupted feeding assay using only infected sand flies	Compared the lesion thickness of mice bitten by sand flies exhibiting feeding persistence and lesion thickness of mice bitten by non-persistent feeders after the biting persistence assay and after an assay where they were allowed to feed uninterrupted	Sand flies that were more persistent delivered a greater inoculum of *Leishmania* (as measured by lesion thickness) than sand flies that were less persistent (Figure 8C) when interrupted, but when uninterrupted there was no difference (Figure 8D)	**Hypothesis supported** The behavior manipulation by *Leishmania* to cause greater biting persistence can lead to a greater parasite load delivered than if the behavior did not occur. This indicates that the behavioral manipulation is indeed an adaptation to increase transmission

Table 1. Summary of experimental design to establish that *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. Source: Adapted from Rogers and Bates (2007), 2019. License: CC BY-NC-SA 4.0.

Armed with that background information, it can be understood how Rogers and Bates (2007) developed their hypotheses: 1) *Leishmania* manipulate sand flies to persist in blood-feeding only after they become infective to mammals (when the parasite reaches the promastigote stage), 2) *Leishmania*-infected sand flies feed on multiple hosts, and 3) *Leishmania*-infected sand flies that have been behaviorally manipulated will deliver a higher parasite inoculum per host than non-manipulated infected sand flies.

In order to answer these questions, Rogers and Bates used a biting persistence assay in which individual sand flies were allowed to land and attempt feeding on an anesthetized mouse for 1 minute, after which they were disturbed by brushing the leg or antennae every 10 seconds until they stopped trying to feed (Figure 6).

The time it took for the sand fly to stop attempting to feed was considered their feeding persistence. The biting assay was modified to address each of the hypotheses. In order to test the first hypothesis, Rogers and Bates experimentally infected sand flies by feeding them rabbit blood with *Leishmania* amastigotes (they used 2 species of *Leishmania*; *L. mexicana* and *L. infantum*), or rabbit blood alone (the uninfected group; Figure 7).

They then used the biting persistence assay as described; testing both infected and uninfected sand flies. Recall that the first hypothesis also stated that the parasite should alter the behavior only when it becomes infective to the next host. Therefore, they conducted this assay daily over the course of the infection: Four days post-infection (non-infective stages) through 11 days post-infection (highly-infectious stages). They found that sand flies infected with either *L. mexicana* or *L. infantum* exhibited greater feeding persistence than uninfected sand flies and that this occurred later in infection when the parasite could be effectively transmitted to a mammalian host (Figure 8A).

The second hypothesis, which stated that infected sand flies are more likely to feed on multiple hosts, required modifying the biting persistence assay to include a second mouse. The sand fly was allowed to locate and begin feeding on a mouse for 1 minute and then disturbed every 10 seconds until the sand fly switched to the other mouse or stopped

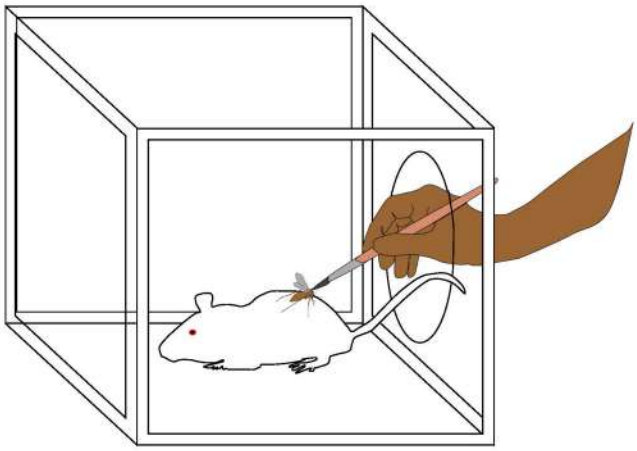


Figure 6. Biting persistence assay. One sand fly at a time was placed in a cage with a single anesthetized mouse. The sand fly was allowed to feed for 1 minute, after which it was disturbed every 10 seconds by brushing its hind legs until the sand fly stopped trying to feed. The total time it took to stop attempting to feed was known as its feeding persistence. After the trial sand flies that were experimentally infected with *Leishmania* were dissected and parasite load determined. Source: M. Wise de Valdez, 2019. License: CC BY-NC-SA 4.0.

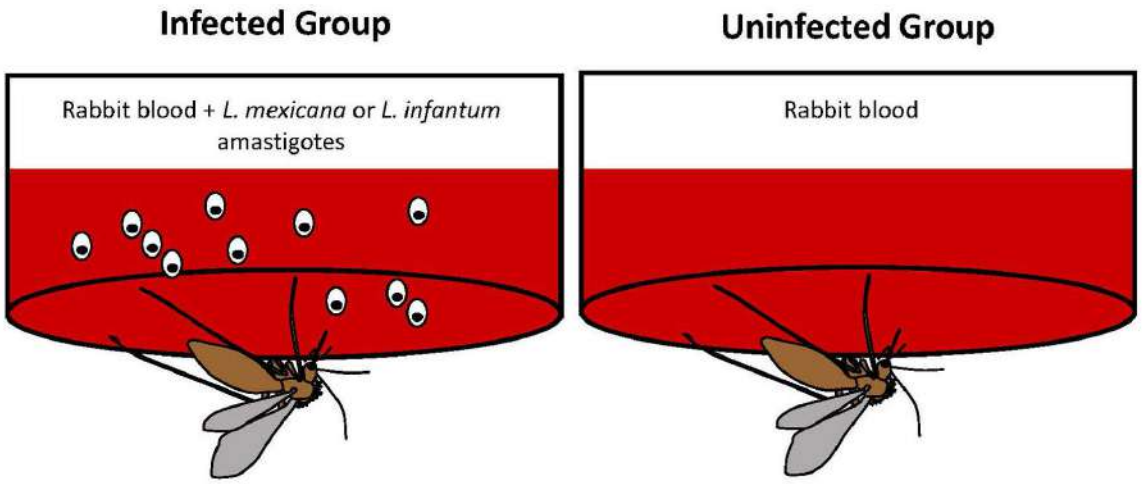


Figure 7. Experimental infection of sand flies was carried out using an artificial membrane system. Each feeder held fresh rabbit blood with either *Leishmania* amastigotes (*L. mexicana* or *L. infantum*) or rabbit blood alone. Source: M. Wise de Valdez. License: CC BY-NC-SA 4.0.

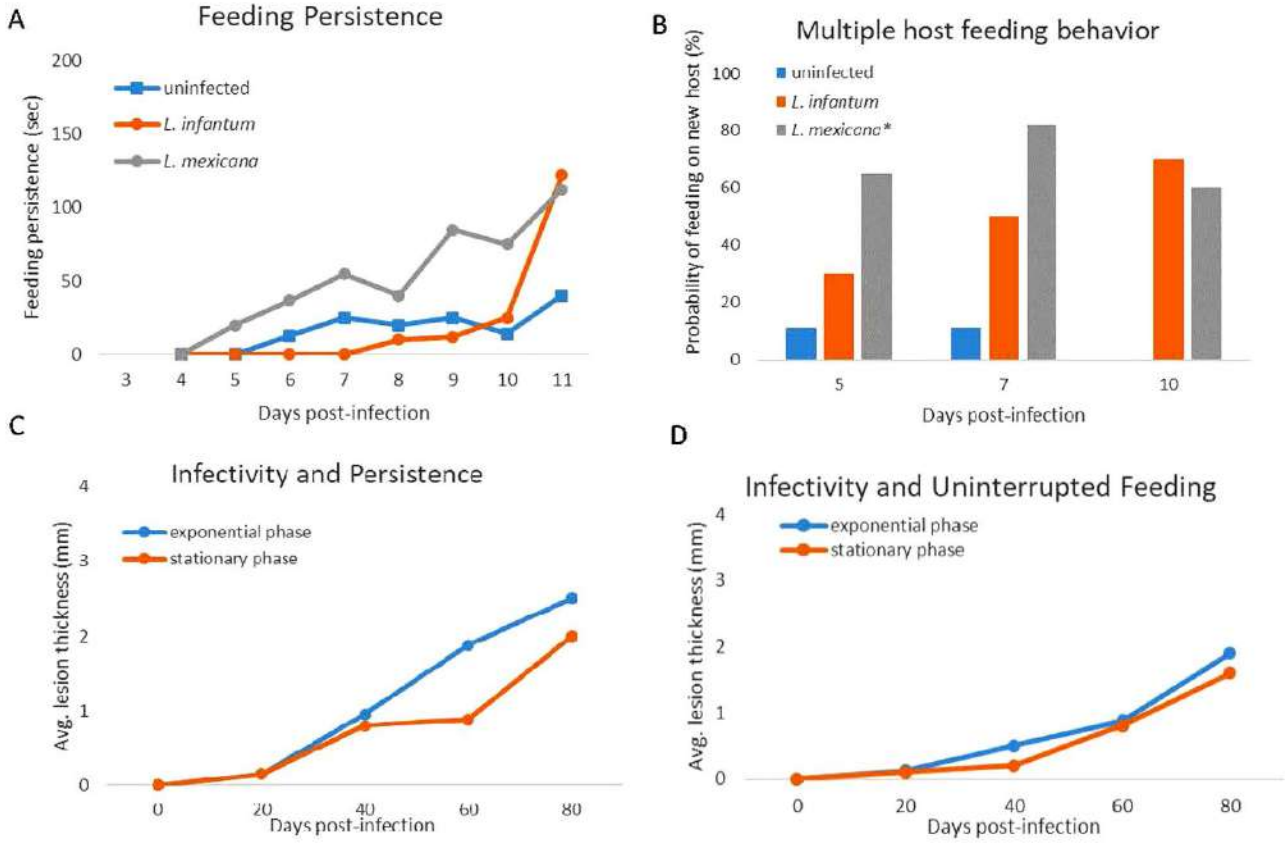


Figure 8. Results from the biting persistence assays. A) Feeding persistence of infected and uninfected sand flies. Infected sand flies exhibited greater feeding persistent than uninfected sand flies. For each day post infection, 16 infected and 16 uninfected sand flies were assayed to establish an average feeding persistence. Error bars not shown. B) Proportion of sand flies assayed that switched to a novel host after repeated interruption. On days 5, 7, and 10 post-infection 12 sand flies from each group were assayed. Error bars not shown. C) Average lesion thickness of mice bitten by persistent sand flies (blue) or non-persistent sand flies (orange). Error bars not shown. Persistent sand flies produced greater lesions and thus delivered a greater inoculum of parasite than non-persistent sand flies. D) Average lesion thickness of mice after being bitten by uninterrupted sand flies. Error bars not shown. There was no difference in lesion thickness between the exponential and the stationary stage infected sand flies when they were allowed to feed without interruption. Source: Adapted from Rogers and Bates, 2007. License: CC BY-NC-SA 4.0.

attempting to feed. The researchers observed sand flies on days 5, 7, and 10 post-infection. They found that sand flies infected with *L. mexicana* or *L. infantum* were more likely to switch to a new host than uninfected sand flies (Figure 8B).

The third hypothesis required a more elaborate set-up. The hypothesis was: An increase in biting persistence leads to a greater parasite load delivered to the mammalian host. In order to test this, they had to be able to compare a group of infected sand flies that exhibited increased feeding persistence and infected sand flies that did not. Rogers and Bates were able to isolate different phenotypes of *L. mexicana*: One that elicited an early increase in biting persistence (7 days post-infection; exponential phase) and another that did not elicit an increase until closer to day 10 (stationary phase). They experimentally infected sand flies with either the exponential phase or the stationary phase. On day 7 post-infection, they conducted the biting persistence assay and followed the development of the resulting lesions on the mice. They used the thickness of the lesions as a proxy for the inoculum size (the number of parasites injected by the sand fly). They found that the average lesion thickness was greater in mice bitten by more persistent sand flies than less persistent sand flies (Figure 8C). In a parallel experiment to confirm that the biting persistence was the primary mechanisms for an increased inoculum, the authors allowed sand flies of both infection types to feed without interruption. They found that the average lesion thickness on mice did not differ between the 2 groups (Figure 8D). This is further evidence that the modified behavior of increased feeding persistence was the mechanism for an increase in transmission efficacy.

Rogers and Bates's primary conclusions were that, 1) Timing of parasite development is linked to feeding persistence, 2) parasites do not increase risky feeding behavior until the stage that is infective, and 3) that this behavioral manipulation strategy enhances *Leishmania* transmission by increasing transmission to multiple hosts and increasing parasite load during biting. Thus, this set of experiments provided evidence for adaptive parasite manipulation of the vector behavior and the fact that it occurs in more than 1 species lends strength to this conclusion.

Transmission to a New Habitat

Some life cycles require that the parasites be delivered to a new habitat where they emerge themselves or where their propagules (eggs or juveniles) are released (Figure 1C). Delivery to a new habitat can be as simple as the parasite taking advantage of where its host is already going, or it may require the manipulation of a behavior to take a host where it wouldn't normally go. Mermithid nematodes (*Gastromermis*) in adult mayflies (*Baetis bicaudatus*) do both. *Gastromermis* nematodes that infect mayflies use the female mayfly's natural oviposition behavior of laying eggs in streams to reach a water source where they then emerge to mate (Vance, 1996a). However, when the nematodes find themselves in a male mayfly they are a bit stuck because males do not display oviposition behavior. Vance (1996a) showed that mermithids feminize male mayflies which causes them to exhibit oviposition behavior, thus delivering the worms to water where they can emerge. This type of study provides unique evidence that the behavioral manipulation is adaptive because the parasite does not manipulate behavior of all hosts, only those that do not exhibit the behavior necessary for it to complete its life cycle. This selectivity within the same system regarding which hosts are manipulated and which are not is indicative of a phenotype that is a direct result of natural selection. This host-parasite system is also unique because it exhibits host sex-specific manipulation.

Sometimes it is not about the adult stage emerging in a habitat where it can mate, it is also about delivering the immature stages to habitats where they can get to the next host. *Plagiorchis elegans* manipulates its snail intermediate host (*Stagnicola elodes*) to rise to the water surface to release the cercarial stage (Lowenberger and Rau, 1994) and several parasitic fungi alter the behavior of their insect host to find perching areas to better release their fungal spores (Poulin, 2010, citing Andersen et al., 2009; Maitlan, 1994).

One of the most well-known examples of parasite behavioral manipulation is horsehair worms (phylum Nematomorpha) that cause their terrestrial insect host to jump into water. Thomas et al. (2002) carried out field observations and experiments in the field and lab to evaluate this behavior. This study bears highlighting since it 1) includes non-manipulative field observations of multiple host species being manipulated by 2 different species of nematomorphs, and 2) the authors use a y-tube olfactometer which is a tool in studying preference and/or choice (Figure 9). Behavioral biologists across many fields use some form of the y-tube olfactometer regularly.

The field observations made by Thomas et al. (2002) involved recording the number of insects coming from a nearby forested area (with known natural habitats for nematomorphs), moving across a concrete pathway towards a swimming pool, and jumping into a pool. They also recorded how many of those insects were infected. They conducted these observations every night over 2 consecutive summers. They recorded 9 different species that jumped into the swimming pool and all were infected (Figure 10C). The most common species recorded were *Nemobius sylvestris* (Figure 10A), with 70 individuals that committed suicide, and *Meconema thalassinum* (Figure 10B), with 30 individuals taking a dip.

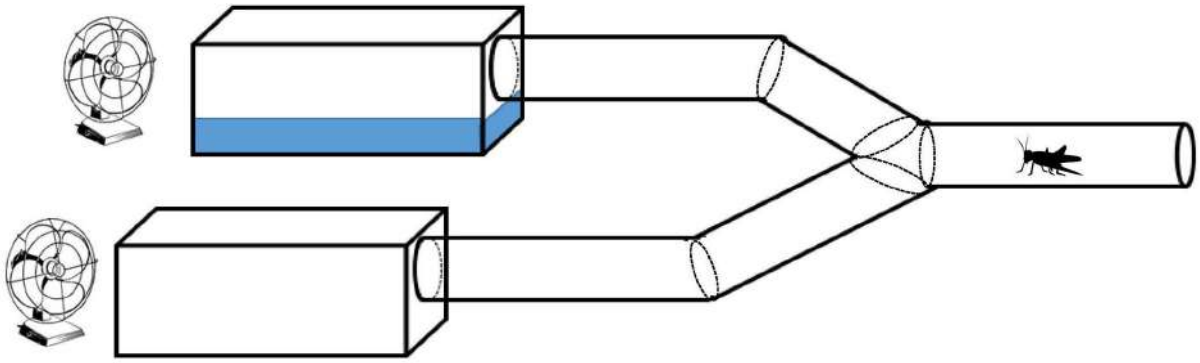
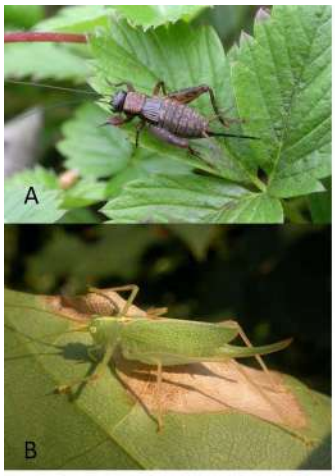


Figure 9. Y-tube olfactometer. A hypothetical design of the y-maze choice assay conducted by Thomas et al. (2002) to assess whether water served as an attractive stimulus. At one end of each arm was a trough, 1 with water and 1 without. A fan was placed at the end of each arm to gently send the "odor" down each arm. Crickets were tested one at a time by placing them at the end of the tube. After 15 minutes their location was recorded. Source: Adapted from Thomas et al. (2002), 2019. License: CC BY-NC-SA 4.0.

In the field-based experiment, Thomas and others used field-caught crickets. They collected 33 *Nemobius sylvestris* crickets from the forested area (presumed uninfected) and 38 from the concrete area around the pool (presumed infected). They then placed the 4 crickets, 2 from the forest and 2 from the pool, under a cup on the concrete near the pool. They studied the crickets' behavior for 15 minutes, recording which individuals jumped into the pool. After the trial, they dissected all crickets to establish their infection status. They found that significantly more infected crickets entered the water than did uninfected crickets (Figure 10D). When they analyzed which of the 33 forest-collected crickets were infected, they found that 15% were infected, while 95% of the poolside-caught crickets were infected. This significant difference between the infection prevalence of poolside versus forest-caught crickets indicates that water-seeking behavior is more common in infected crickets.

The goal of the laboratory experiment was to determine if the presence of water was an attractive stimulus for infected crickets. They used the y-tube olfactometer (Figure 9) to allow crickets (uninfected and infected) to choose an arm with water at the end, or one without water. Again, they used field-caught crickets (forest-caught and poolside-caught). They found that infection status did not affect the arm that the crickets chose. However, of the crickets that chose the arm with water, all infected crickets jumped into the water and only 1 of the 12 uninfected crickets jumped into the water. These data clearly show that nematomorphs manipulate water-seeking behavior but the mechanism by which they alter the behavior is not via an increase in water detection.



C

Cricket host species	Nematomorph species	# of times observed
Nemobius sylvestris (A)	*Paragordius tricuspidatus*	70
Meconema thalassinum (B)	*Spinochordodes tellinii*	30
Pholidoptera griseoptera	*S. tellinii*	11
Uromenus rugosicolis	*S. tellinii*	5
Ephippiger cunii	*S. tellinii*	1
Barbitstes serricauda	*S. tellinii*	1
Leptophyes punctatissima	*S. Tellinii*	1
Antaxius pedestris	*S. Tellinii*	1
Yersinella raymondi	*S. Tellinii*	7

D

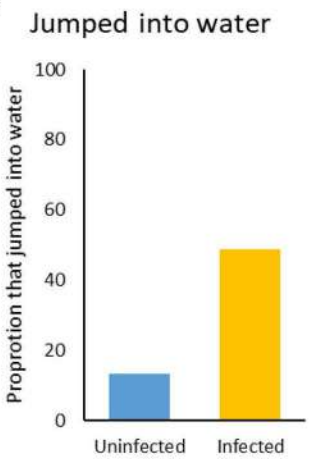


Figure 10. A) The European bush cricket *Nemobius sylvestris*. B) The oak-bush cricket *Meconema thalassinum*. C) Results of the field observations: species of crickets that jumped into water, the species of nematomorph they harbored, and the number of times they observed individuals of each species jumping into the water over the course of 2 summers. D) Results of the field experiment: Proportion of infected and uninfected crickets that jumped into the water. Source: Adapted from Thomas et al. (2002). License: CC BY-NC-SA 4.0.

Box 5: Stop and Think

What might be some other mechanisms for how the nematomorph manipulates the behavior? How would you test this?

Hosts as a Direct Resource or Single-Host Systems

Parasite-host relationships in which the parasite has only a single host for the duration of its life cycle or which relies on the host for its own development offer a unique set of hypotheses on adaptive manipulation of host behaviors (Fritz, 1982). First, it would be expected that these parasites alter host behaviors in ways that decrease the host's risk of predation. The parasite requires the host to stay alive long enough for the parasite to reach maturity and by altering behaviors that reduce predation on the host, the parasite thereby increases its own chance of survival. Second, it would be expected that these parasites would alter host behaviors in ways that ensure that sufficient nutritional reserves are available to the parasite. Parasites that develop to maturity in a host and then emerge usually require vast nutritional resources directly from the host. There are 2 sets of behaviors that might be targeted. Parasites might reduce energetically expensive behaviors in order to reserve nutritional stores or they might increase host foraging behavior to keep up with the nutritional needs of both the parasite and host. There are relatively few studies that experimentally investigate whether these behavioral changes occur (Moore, 2002) and fewer still that provide evidence for adaptation (but see Benton and Pritchard, 1990; Vance, 1996a; 1996b; Vance and Peckarsky, 1997; Wise de Valdez, 2007; Barquin et al., 2015; Soghigian et al., 2017).

One theme that emerges from the literature, however, is that there appears to be a trade-off between reducing predation risk and ensuring that enough nutrition is obtained. Revisiting the mermithid-nematode system helps to explain this point. Mermithid nematodes infect juvenile mayflies (in their aquatic stage) and there they undergo partial development. The mayfly larvae have to stay alive long enough to emerge into flying adults in order for the mermithid to complete its development. Therefore, it might be expected that the mermithids in the larval mayflies would reduce risky behaviors so as not to become fish food. However, they in fact increase their risky behaviors and are preyed upon more often than uninfected mayflies (Benton and Pritchard, 1990; Vance, 1996a; 1996b; Vance and Peckarsky, 1997). The researchers propose that there is a trade-off between maintaining nutritional reserves and predator avoidance. They suggest that the developing mermithid induces a nutritional deficit and therefore increasing feeding behaviors (and thus risky behaviors) may make up for that deficit. Note however, that the study has not continued past the point of establishing that a behavioral difference between infected and uninfected larval mayflies exists.

In 2 larval mosquito-parasite systems researchers have been able to extend the study to answer whether behavioral changes were adaptations or not. The research by Wise de Valdez (2007) described earlier concluded that the reduction of activity levels of mosquito larvae infected with mermithid nematodes was likely not a parasite adaptation because predation rates did not decrease. Soghigian et al. (2017) on the other hand investigated a protozoan gregarine parasite that uses mosquito larvae as its only host. They looked at larval behavior of *Aedes triseriatus* infected with *Ascogregarina* and found that they were less active and these behavioral changes *did* lead to reduced predation rates by the predatory larval mosquito *Toxorhynchites rutilus*. This difference in results is likely due to the evolutionary relationship in these 2 systems. The latter system is common in nature where they are exposed to natural selection pressures and which presumably has a longer evolutionary relationship. The former system however used laboratory-reared colonies of the mosquito, the parasite, and the predator. Laboratory environments can shift the selection pressures these organisms face. Therefore, it is important to acknowledge and consider the source of the test organisms when interpreting the results.

Box 6. Stop and Think

Addressed earlier was how nematomorphs manipulate their hosts to jump into water so that they can emerge. This host-parasite system is also one in which the parasite uses the nutritional stores of the insect in order to complete development and requires that the cricket host stays alive for more than a month. What other types of cricket behaviors might the nematomorph alter while it is developing?

In the cricket-nematomorph parasite system, Barquin et al. (2015) used information from several studies on the impact of insect parasitoids on the calling behavior of infected crickets compared to uninfected crickets (Cade, 1984; Zuk et al., 1993;

Orozco and Bertram, 2004; Kolluru et al., 2002) to hypothesize that calling behaviors of crickets should be manipulated by nematomorphs because calling is both energetically costly and attracts the attention of auditory predators. Although this study addresses only whether behavioral alterations occur and not whether they are adaptive, this study is highlighted because it exemplifies how hosts are handled in a laboratory setting and how some behaviors need to be assessed through means other than visual observation. Next, one of the experiments conducted by Barquin et al. (2015) is summarized.

Box 7. Stop and Think

What might be the next set of experiments someone would want to develop in order to test these remaining questions? Reading papers that have unanswered questions and then coming up with ideas for how someone could answer them is what budding scientists should be doing. So students should find those biological systems that have unanswered questions, or have questions yet to be asked, and find a way to answer them! (Hint: Students should talk to professors and ask if they can do research in their lab.)

Barquin and colleagues (2015) exposed *Acheta domesticus* crickets to *Paragordius varius* nematomorph larvae 2–3 days after wing development (30 exposed, 30 sham-exposed). Crickets were marked with waterproof paint to give them each a unique identity (Figure 11B). Crickets were housed in an insectary with a 12–12 light/dark cycle to keep the circadian rhythms. Individual cricket chirping frequency was recorded for 12 hours (dusk to dawn) on day 5 post-exposure and every 6 days thereafter using individual cages, microphones, and a computer program set up to record sound (Figure 11A).

The computer program allowed them to measure how much time they spent chirping and the intensity of the chirping events (Figure 11C). Note that the same cricket was followed throughout the course of its infection, for this reason it was imperative that each cricket had a unique identifier that would not wear off over the course of a month. After the trials infection status was determined by placing the cricket in a bowl of water and checking for worm emergence (Figure 11D). Note that exposure does not necessarily result in infection, therefore there were fewer infected crickets than uninfected crickets when data were analyzed (Figure 12).

This section would be incomplete without mentioning that some insect parasitoids manipulate the behaviors of their hosts in ways that protect them even after they have emerged. One species of parasitic wasp manipulates its orb-weaving spider host to spin it a specialized protective pouch just before it emerges. The wasp larva is then deposited in this pouch which serves to protect it while it pupates (Poulin, 2010, citing Eberhard, 2000). Another species of parasitic wasp, which uses a caterpillar host, somehow has manipulated the caterpillar to stick around even after it emerges in order to protect it from other predators (Poulin, 2010, citing Brodeur and Vet, 1994; Grosman et al., 2008).

Box 8. What Did All These Studies Have in Common?

- Started with questions and developed hypotheses that could be tested.
- The life cycle of the parasite had to be well understood.
- Needed source of infected individual: experimental infections.
- Hosts were always dissected afterwards to establish infection status.
- All studies required uninfected controls so that behaviors could be compared.
- Required both definitive and intermediate hosts as well as the appropriate habitats in the experimental design.
- Experiments were repeated: scientists used multiple organisms and multiple trials of each assay performed.
- None of them had all the answers.

A Quick Note: How Do Parasites Do It?

The mechanisms by which parasites manipulate host behaviors are elusive but more often than not they can be categorized into direct or indirect mechanisms: A direct mechanism is something produced by the parasite and an indirect mechanism might be physical interference with a biochemical pathway. Often the manipulation passes through neurological

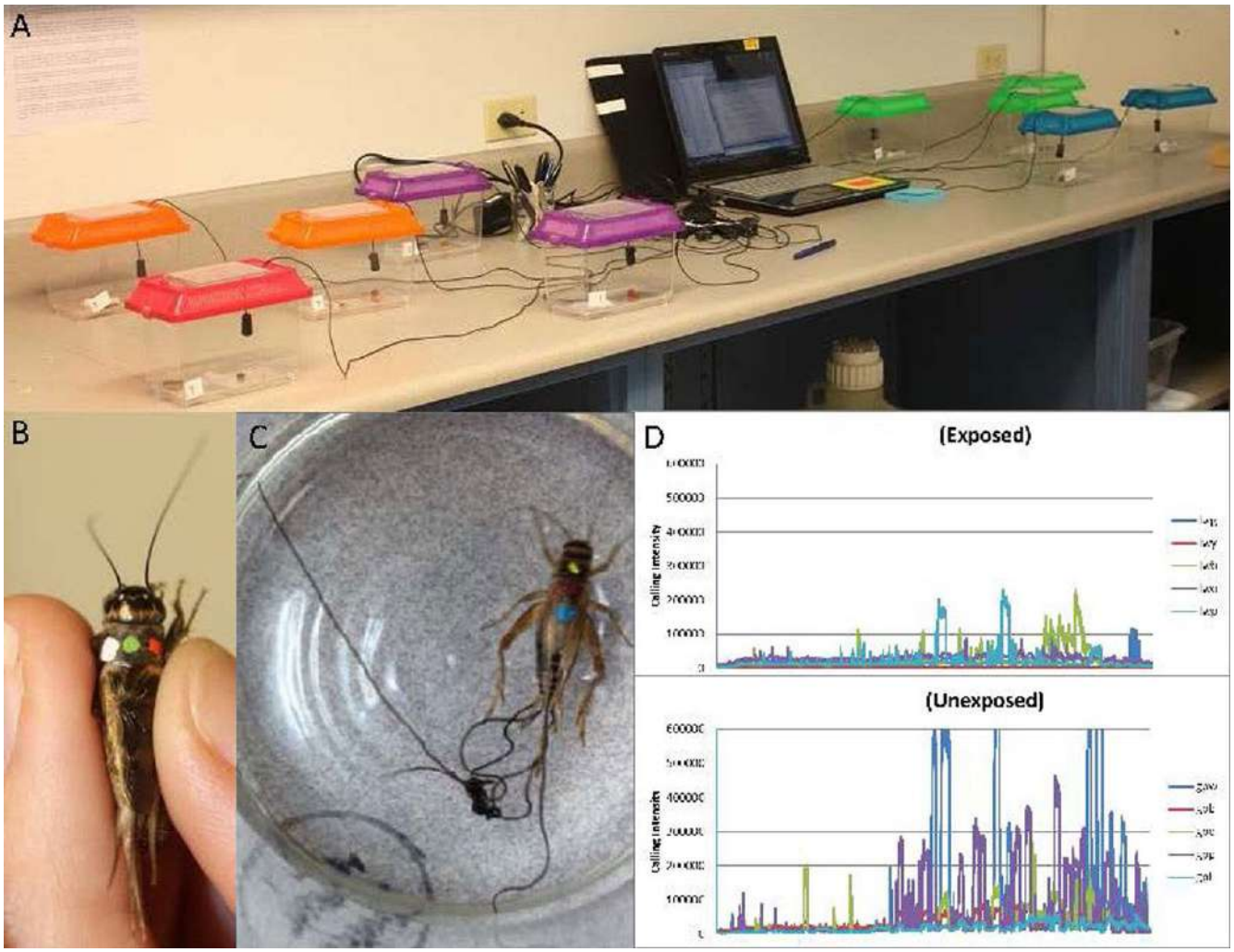


Figure 11. Experimental design used to study the effect of nematomorphs on calling behavior of crickets. A) Set up: Each cage held a microphone attached to a computer that ran a program to record frequency and intensity of calling over 12 hours. A single cricket was housed in the cage with a source of water and food. B) Example of a unique identifier. C) Sample output from a 12-hour recording period. Each different colored line was an individual cricket. Notice that on the sample day when this was recorded (6 days post-infection) the uninfected called more often and with greater frequency than exposed crickets (they did not yet know their infection status). D) Example of how the researchers checked the infection status, the nematomorph is emerging from the posterior end of the cricket. Source of images: M. Wise de Valdez, 2019. License: CC BY-NC-SA 4.0.

routes; some parasites secrete peptides that influence neural function, others can either directly or indirectly alter concentrations of hormones or neurotransmitters of their hosts (Poulin, 2010). A more recent area of study, proteomics, involves seeing which proteins may be manipulated by parasites and the downstream effect of those proteins on behavior (Lefèvre et al., 2009). It has also been suggested that perhaps parasites may alter the expression of host genes in a way that results in a behavioral change but this has yet to be studied (Poulin, 2010). For a more thorough discussion and concrete examples of research on how parasites manipulate behavior check out reviews by Thomas et al. (2005; 2010); Lefèvre et al. (2009); Poulin (2010); and Adamo (2012).

Summary

Review: **Learning objectives 1, 2, and 5:** Apply the scientific method to address questions about parasite manipulation of host behaviors. Analyze examples in the scientific literature to learn how scientists have experimentally addressed questions about parasite manipulation of host behaviors. Understand the types of host behaviors likely to be altered in relation to the parasites' life cycles. The details of 4 experimental studies were described where the researchers first asked questions, formulated hypotheses, tested them, gathered and analyzed data, and interpreted the results to either support or reject their hypotheses. Each study highlighted a specific mode of transmission and the behavioral

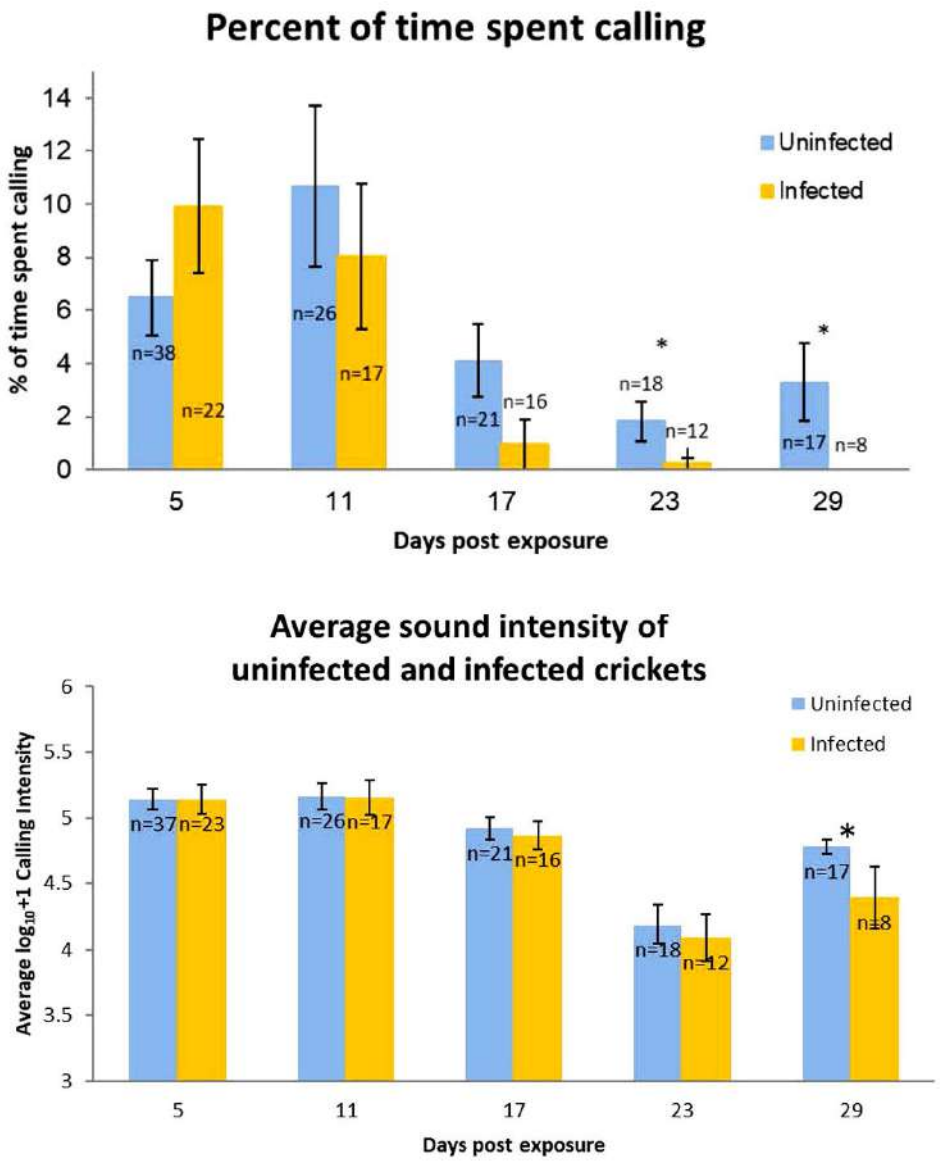


Figure 12. Time spent calling and calling intensity of male *Acheta domesticus* crickets infected with *Paragordius varius*. Source: Adapted from Barquin et al., 2015. License: CC BY-NC-SA 4.0.

manipulations we expected to see based on those transmission modes: Trophic transmission, vector-borne transmission, transmission to a new habitat, and remaining in a host for development. **Learning objective 3**: Be able to provide some classic examples of parasite manipulation of host behaviors. There are 3 primary groups of parasites that always seem to be cited in the literature for behavioral parasitology: Nematomorphs, mermithid nematodes, and acanthocephalans (with a few trematodes and protozoans thrown in). **Learning objective 4:** Understand the evolutionary principles of parasite manipulation of host behaviors. An adaptation is any character that increases the fitness of an individual. In order for parasite-induced behavioral changes to be an adaptation they must increase the fitness of the parasite by increasing its survival so it can reproduce, increase its reproductive/transmission output, or increase its chance to make it to the next host or habitat in order to complete its life cycle. **Learning objective 6:** Think critically about host-parasite relationships yet to be investigated from a behavioral standpoint. Throughout the section, call out boxes urged you to stop and think. These were meant to be a pause in the reading so that you could assess whether what was being conveyed could be applied to a new scenario.

Advanced Questions

Indeed, the questions addressed throughout this section are only a few of the questions one can ask about this interesting relationship between parasites and their hosts. See also the following papers to investigate a few more relevant questions. In Poulin (2010), Moore (2002; 2013), Libersat et al. (2018), Poulin and Maure (2015), Lefèvre et al. (2009), Thomas et al. (2010), Hughes et al. (2012), numerous questions are asked, such as:

- Are some taxonomic groups of parasites more likely manipulate host behavior than others?
- Why do some parasites alter behaviors and others do not?
- How effective is host manipulation?
- What behavioral changes might occur in hosts with more than 1 species of parasite?
- What other parasite-induced behavioral alterations that may benefit the host?
- How do hosts alter their behavior in order to compensate for their eventual sexual demise?
- What role might parasites that manipulate host behavior play on the ecology of the habitat in which they are found?
- What are the evolutionary mechanisms by which parasites evolve behavioral manipulation?
- What research is being conducted to determine the physical mechanism of parasite-induced altered behavior?

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7

Parascript Approaches

Biostatistics for Parasitologists:

A Painless Introduction

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Chapter 7

Biostatistics for Parasitologists: A Painless Introduction

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Introduction

Students of veterinary or human epidemiology, evolutionary biologists, and ecologists alike, are often asked how heavily a particular host species (or population, or herd, etc.) is infected by parasites. Further questions arise in comparisons regarding which one is more infected, or which one is more subjected to more pathogenic pressure than the others. After carefully reading this chapter, you won't be able to answer such questions–simply because such questions make no sense.

The occurrence of parasites within the host population, just like the harm exerted by them, is a complex pattern that cannot be described by a single statistical measure. Different indices capture different aspects of infection. Statistical indices have to be chosen that have clear (easy to understand) and distinct (non-overlapping) biological interpretations, and appropriate statistical tests must be chosen that are not based on assumptions that are not fulfilled in host-parasite systems. Unfortunately, some of the most widespread indices have vague if any biological interpretation, or they merely statistically predict each other, causing a redundancy of information.

Further, when applying appropriate indices to describe infection, it is a common situation that one index is higher in the host population A, the other index of infection is higher in population B, and so on. Even if all indices appear to be higher in one population than the other, we can never exclude the possibility that further meaningful indices can be proposed. A definite answer like "sample A is more infected than B" arises only in some rare and self-evident–and frankly not really interesting scientifically—cases when parasites are totally absent from the latter.

The aim of the present chapter is to advise readers how to choose appropriate statistical indices, and then, to choose the appropriate statistical tests to handle them. Finally, we offer a free statistical toolset to carry out the recommended statistical procedures in a relatively painless manner. The text below is based closely on a review paper by the authors of this chapter (Reiczigel et al., 2019a).

Taking Samples

Constrained by time, and financial and ethical limitations, investigators usually cannot collect and analyze every individual of a **host-parasite system**. Rather they take a random sample from the whole, with the hope that the sample will represent the unknown totality with reasonable accuracy. Of course, the larger the sample, the better accuracy we get. When taking a sample of a host-parasite system, typically, host individuals serve as ordinary units of sampling. First, a sample of host individuals is collected to represent the host population and, second, their bodies are searched for parasites. It is usually presumed that all parasites harbored by a particular host individual are found and identified, which may not be true.

Thus, we collect groups of parasite individuals inhabiting the same host individual, so-called **parasite infrapopulations** (Bush et al., 1997). Statistically speaking, **random sampling** of hosts implies **cluster sampling** of parasites. The size of these infrapopulations is most often expressed as the number of parasite individuals, thus we limit the discussion here to this particular situation.

Frequency Distribution of Host Individuals across Infection Classes

For sake of simplicity, first we focus our interest on the occurrence of a single species of parasite within a sample of

hosts. After collecting a sample, all **conspecific** parasite individuals need to be identified and counted from each host. Then host individuals are characterized by the number of parasites they harbor, then they can be grouped into so-called **infection classes**, such as the group of non-infected hosts, the next group of hosts each harboring 1 parasite, the next group of those harboring 2 parasites, etc. Alternatively, wider categories are often applied, such as 0, 1–10, 11–20, etc. It is a common practice to replace the number of host individuals by the proportion (%) or probability (0–1 scale) that host individuals belong to a particular infection class. Such frequency distributions are visualized as **histograms**, and often used to characterize host-parasite systems.

Host-parasite **frequency distributions** do not approximate a normal distribution (a symmetric bell curve) nor a uniform distribution. Rather the distribution of parasites always exhibits an **aggregated** (also known as **right-skewed**, or **positively-skewed**) **distribution**: The majority of hosts harbor 0, or just a very few, parasites, a few hosts harbor more, and only a very few hosts harbor many more of them (see Figure 1; Crofton, 1971). The experienced frequency distributions, as visualized by histograms, can be approximated by mathematical models. In the case of natural infections by macroparasites, the so-called **negative binomial distribution model** often provides a good approximation.

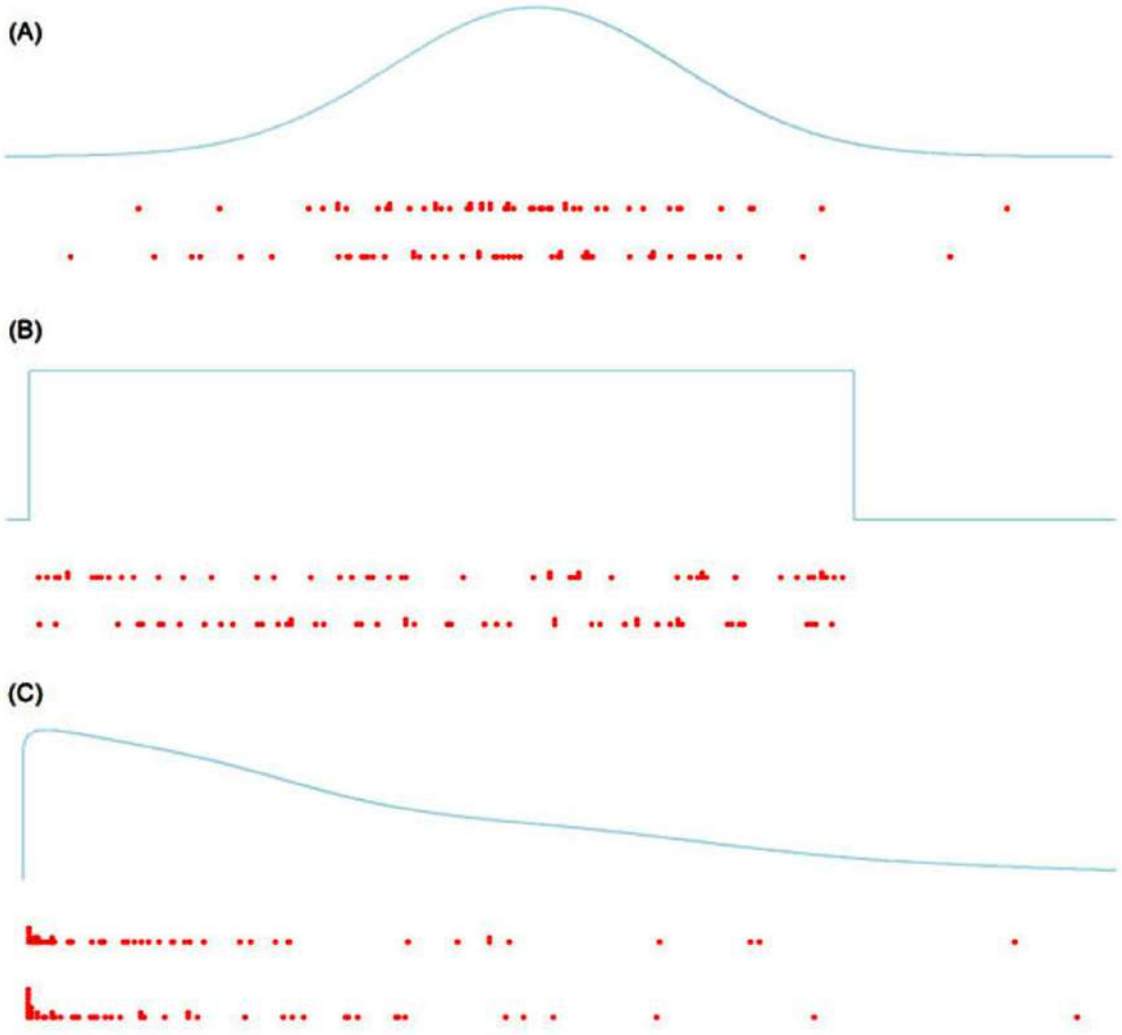

Figure 1. Density function (light blue) and dot plots of samples (n = 50) taken from different distributions. A) **Normal distribution**, where the mean is the most frequent value and the exceedingly smaller or greater values are exceedingly rare. B) **Uniform distribution**, where all values in a certain interval are equally likely. C) **Aggregated (or right-skewed) distribution**, where low values are frequent but high values are rare. Hosts grouped into parasite infection classes typically exhibit this type of distribution. Source: J. Reiczigel, M. Marozzi, F. Ibolya, and L. Rózsa. License: CC BY-NC-SA 4.0.

Sample Size

Providing information on **sample size** is essential partly because it affects the accuracy of the sample estimates, and partly because low sample sizes tend to bias some of the estimated indices of infestation/infection (Reiczigel and Rózsa, 2017). Since hosts usually act as natural sampling units, authors typically express sample size as the number of host individuals. However, in certain cases (see below), the number of parasites collected/examined may remain totally unknown–a shortcoming that should be carefully avoided.

Prevalence

Prevalence (also called extensity in the early literature) is the proportion of infected individuals, traditionally expressed as a percentage (0–100% range) or as a probability (the probability that a randomly chosen individual is infected, 0–1 range). **Sample prevalence** is an estimate of the unknown true population prevalence and, thus, its 95% confidence interval (CI) must be calculated to express its precision or uncertainty: The wider the CI, the lower the precision of the estimate (or the higher the uncertainty).

There are several methods that can be used to calculate a CI for a proportion. It is traditional to apply the Clopper and Pearson's (1934) method. Alternatively, Sterne's (1954) method and Blaker's (2000) method provide narrower, and thus more informative, interval estimates (see Reiczigel, 2003 for a comparison of their efficacy).

In epidemiology, the proportion of host individuals developing new infections within a specified period is called **incidence** or **cumulative incidence**. If calculated for a year (or month, week, etc.) it is called **incidence rate** or **incidence density**. The incidence expresses the risk of developing new infection in a certain time period. From a statistical point of view, incidence is handled similarly to prevalence, often modeled by the Poisson distribution.

Naturally, studies based on methods that can only differentiate the infected versus uninfected status of examined hosts (like serological methods) will report only sample size and prevalence (sample prevalence and its CI) to quantify results.

Mean Intensity

Intensity is the number of parasites found in an infected host. **Sample mean intensity** is the mean number of these values calculated for a sample, with all the 0 values of uninfected hosts excluded. Given the typical aggregated nature of parasite distributions, this value does not characterize a typical (say, characteristic, or usual) level of infection, rather it is highly dependent on the presence or absence of 1 or a very few highly infected host individuals. However, provided that sample size and prevalence are already known, mean intensity exactly defines the total number of parasites found in the sample. It is advisable to provide its 95% CI enabling readers to extrapolate it as an estimation of true **population mean intensity**. This CI is calculated by means of the bias-corrected and accelerated (BCa) bootstrap method of Efron and Tibshirani (1993).

Do not apply the scheme 'mean ± SD,' because it is meaningful only for symmetrical distributions, but not for the aggregated ones so characteristic to parasites. Thus, nonsense expressions like 'mean intensity = 10 ± 20' (erroneously suggesting that mean intensity can have negative values) are also avoided.

Before the era of computer-intensive methods, investigators often log-transformed raw values in order to normalize the data set. Then they calculated the mean of these transformed data, and statistically compared these means by parametric tests (like Student's t test or ANOVA) applied on the log-scale, and finally back-transformed the mean and obtained the 'geometric mean.' However, log-transformed parasite distributions very poorly approximate the normal distribution model, and the resulting index, the 'geometric mean' of intensity is hard to interpret biologically. Given that computer-intensive methods like bootstrap have opened new avenues of statistical analyses, using geometric means should now be abandoned.

Median Intensity

Median intensity, unlike mean intensity, is not strongly affected by the values of the very few highly infected host individuals, thus it is more suitable to provide information about a typical (characteristic, usual) level of infection. Thus, while mean intensity (combining host sample size and prevalence) defines the number of parasites collected, median intensity informs about a characteristic state of infected hosts (of course, with the uninfected hosts excluded).

A 95% CI of median intensity is useful to express the accuracy of estimating population median intensity. For this purpose, the method introduced by Arnold et al. (2008) is followed. Due to the discreteness of data, it is often impossible to construct exact 95% confidence limits, thus, the shortest interval that reaches at least the desired confidence level is reported instead.

The most common method for the comparison of 2 medians is the non-parametric Wilcoxon-Mann-Whitney U-test (WMW). However, it should be noted that, without imposing some rather restrictive assumptions on the population distributions, WMW does not compare medians (there are examples where the sample medians are exactly equal and WMW detects a significant difference between the samples). One such assumption is that the frequency distributions to be

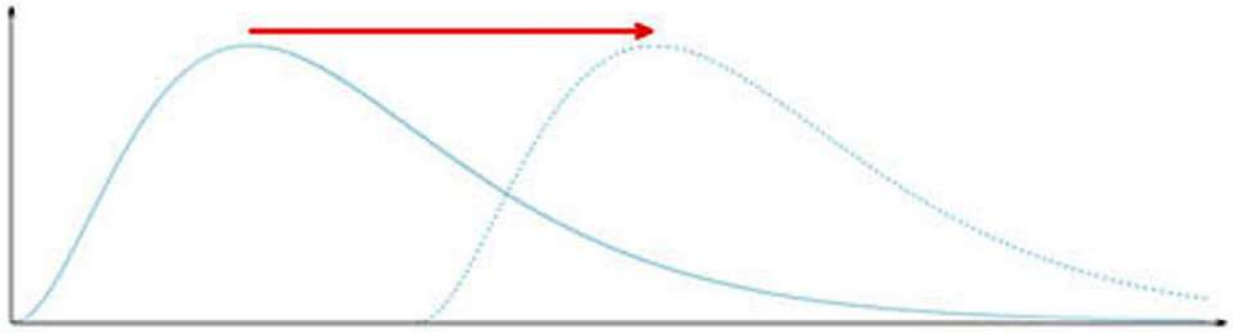

Figure 2. The classical assumption of the Wilcoxon-Mann-Whitney U-test is that the distributions to be compared have same shapes (and therefore same variances) but may be shifted along the horizontal axis (above). Unfortunately, real host-parasite systems do not fulfill this assumption, thus results of the WMW test are difficult to interpret. Source: J. Reiczigel, M. Marozzi, F. Ibolya, and L. Rózsa. License: CC BY-NC-SA 4.0.

compared have the same shape, the only difference between them is a shift along the horizontal axis (see Figure 2). There are other assumptions, but all of them are similarly restrictive, and most parasite distributions do not seem to fulfill them. If none of these assumptions hold, the result of the WMW test can be misleading (Divine et al., 2018). If the test detects a significant difference, the most one can say is that the distributions (rather than the means or medians) differ. Therefore, if differences between medians are of interest, the best choice is Mood's Median Test (Sen, 1998).

Stochastic Equality of Intensities or Abundances

The bootstrap test for **stochastic equality of distributions** (Reiczigel et al., 2005a) is a variant of the WMW test. It compares pairs of values taken from the 2 samples and tests whether the probability of getting higher values from one sample than from the other is same (50%–50%) or different. If using this method, the question regards only *how often* a value taken from one sample is higher than that from the other sample, but not *how much higher*. Therefore, if this test shows that infections in one sample tend to exceed those in the other, it does not necessarily mean that the latter sample hosts fewer parasites.

Abundance

Abundance is defined and treated similarly to intensity, but the 0 values of non-infected host individuals are not excluded. Due to the inclusion of the infection class 0 (non-infected hosts), the frequency distribution of abundance classes is more aggregated and, thus, their analysis is less accurate than that of the intensity classes, resulting in wider CIs and weaker statistical tests (greater *p*-values). Therefore, it is preferable to calculate intensity rather than abundance, and to avoid confusion, it is best to not provide both indices. Presuming that sample size (*N* hosts) and prevalence are provided, readers already have all the information about the non-infected hosts, thus, the further inclusion of these calculations in quantitative descriptions is redundant. The relationship between mean abundance, mean intensity, and prevalence can be described by a simple formula, enabling calculation of any 1 of them when knowing the other 2 of them:

mean abundance = prevalence * mean intensity

Median abundance is a less informative measure, in particular, because, by definition, it equals 0 whenever prevalence is less than 50%, irrespective of the actual prevalence and the intensity values of infected hosts.

Overall, abundance measures (mean and median, their CIs) combine information on prevalence and intensity. Apply them only if such a combined index is definitely needed.

Crowding

Crowding is the size of the infrapopulation to which an individual parasite belongs (Reiczigel et al., 2005b). Although this equals intensity, intensity is defined as a host character, while crowding is a character of the parasite individual. Therefore, mean intensity refers to the intensity values averaged over host individuals, but mean crowding is obtained by averaging the crowding (= intensity) values over the parasite individuals. Say, mean intensity for 3 individuals infected by 1, 2, and 6 parasites is (1 + 2 + 6) / 3 = 3, while mean crowding for the parasites in the same sample is (1 + 2 + 2 + 6 + 6 + 6 + 6 + 6 + 6) / 9 = 4.56. Note that, due to the aggregated shape of distributions, an 'average' individual lives in a host that is more 'crowded' by conspecific parasites than the mean number of parasites per hosts (here: 4.56 > 3). Mean crowding is a rarely used index; however, it is a potentially meaningful measure of infection when speaking about

Box 1. Money Flows Like Parasites

Since counting money is much closer to our everyday experience than counting parasites, here is a surprising parallelism between them.

Most people possess little if any money, while a very few people are extremely rich. Thus, money, just like parasites, exhibits an aggregated distribution across human (analogous to host) individuals. The value of average richness is affected differently by different individual changes. It is very sensitive to the presence or absence of a single very rich person, but much less sensitive to the presence or absence of a single poor person. Similarly, **mean intensity** (or **mean abundance**) of infection is sensitive to the presence or absence of one or few highly infected individuals. Therefore, mean values do not reliably characterize the wealth of "average people;" likewise, neither the infection of a "typical" host individual.

There are similar causes responsible for the rise of aggregated distributions both in monetary and epidemiological systems. First, money (just like parasites) tends to move from one person to another in groups, such as sums of money, similar to multiple infections by more than one propagule at the same time. Second, some people are inherently good at earning and accumulating money, while others consistently spend all the money they happen to have–just like individual differences between susceptible and resistant individual hosts. Finally, money can multiply itself if hosted by a careful person; this is termed interest on capital. Similarly, most parasites can multiply themselves within the body of a susceptible host.

For such reasons, money behaves very much like parasites, at least from a statistical point of view.

density-dependent parasite characters (such as body size, fecundity, or sex ratio) in relation to the putative social environment of parasites.

Due to the usual sampling, that is, sampling the hosts, there are dependencies between the crowding values of parasite individuals: All of the conspecific parasites infecting the same host have identical values and, therefore, all of these values change simultaneously whenever a parasite is added or removed. This makes crowding values notoriously hard to handle statistically. As random sampling from the parasite population is practically infeasible, statistical methods assuming independence of the sample values—practically all classical methods, that is—cannot be validly used for the analysis of crowding.

A CI (confidence interval) for **mean crowding** can be created by the BCa bootstrap method as demonstrated by Efron and Tibshirani (1993). A 95% CI is useful to characterize the accuracy of sample mean crowding as an estimate of the true population value. Statistical comparisons of mean crowding across 2 (or more) different samples are also based on CIs. First, 97.5% CIs are generated for both samples. If these intervals overlap, the difference between the 2 samples is non-significant at the prescribed level of 0.05, that is, $p > 0.05$ (Reiczigel et al., 2005b). Unfortunately, the power of this testing method is rather low. Therefore, Neuhäuser et al. (2010) proposed applying Lepage's (1971) location-scale test as a more suitable alternative.

From a purely mathematical point of view, diversity and crowding are closely related notions; one can be transformed into the other (Lang et al., 2017).

Levels of Aggregation

While all natural, and most experimental parasite infections exhibit an aggregated frequency distribution across host individuals, the level of aggregation may differ considerably from sample to sample. The most frequent indices to quantify these levels are, 1) The variance-to-mean ratio of abundance, 2) the exponent k of the negative binomial model fitted to the data (presuming acceptable fit of the model), and 3) Poulin's (1993) 'index of discrepancy,' which includes a modified version of the so-called Gini-coefficient (a well-known index in the literature of economics).

Although these indices aim to quantify the same feature (level of aggregation) of frequency distributions,

unfortunately, their values do not exactly predict each other, thus, they cannot be transformed into each other and the are not interchangeable.

Just like in the case of mean crowding, these indices can be compared across samples by testing the potential overlap between their 97.5% CIs.

Parasite Sex Ratio

Samples of dioecious parasites can be characterized by their **sex ratios**. Note that the term *sex ratio* is quite misleading. Mathematically speaking, a 'ratio' should be expressed as the frequency of 1 sex divided by the frequency of the other sex. However, the index males/females would be unfavorable to apply; for example, it cannot be calculated for samples without females (since one cannot divide a number by 0). Instead, it is traditional to apply the proportion of males among adult dioecious parasites as a measure of sex ratio. Thus, the index called *sex ratio* actually means ***male-proportion***. As it is a proportion, the recommended statistical tests are identical to those of prevalence.

Parasite Species Richness

Species richness is a simple and frequently used index to quantify diversity. Unfortunately, small samples tend to underestimate the true parasite species richness in populations of animals. General advice about the required sample size cannot be given because it depends on many other factors such as the levels of aggregation, interactions between parasite species, etc. There are several methods that have been designed to extrapolate sample values to the true parasite species richness harbored by the whole host population, so as to correct for this sample size bias. Walther and Morand (1998) compared the reliability of several methods using real parasitological datasets and found that the first-order jackknife (Heltshe and Forrester, 1983) and the Chao2 estimators performed best (Chao, 1987; Chao and Chiu, 2016). This latter method estimates the number of unobserved parasite species from the number of rare species (those occurring only in 1 or 2 hosts in the sample). Thus, the estimation fails in the absence of rare species in the sample, but it performs well if the number of rare species is < 50% of all parasite species in the dataset. It is also advised that a large sample of hosts is needed to obtain a reliable estimate, a sample size of at least a few hundred host individuals is recommended, but of course this depends on the estimated size of the population under study.

Interactions Between Parasite Species

Two parasite species coexisting in the same host population may exhibit a positive or negative interaction, making their co-occurrence in a particular host individual more or less likely than expected by chance. The simplest method to analyze such interactions is to summarize the presence or absence of the 2 species on each host in a 2 × 2 contingency table and apply Fisher's Exact Test to analyze it. The sensitivity of this method, unfortunately, may be rather poor because the difference between hosting 0 or 1 parasite individuals is often negligible. Therefore, computing the Spearman Rank Correlation coefficient to explore potential interactions between abundance values of the 2 parasite species is recommended as it provides a more robust or sensitive estimate.

Quantitative Parasitology on the Web (QPweb)

Misuse of biostatistics and misinterpretation of statistical results are very common in the parasitological literature. Therefore, we have published a brief overview of the suitable biostatistical tools together with some new methods proposed by us (Rózsa et al., 2000) to address these important issues. The Rózsa et al. (2000) paper was accompanied by freely distributed software called Quantitative Parasitology (QP) to make the recommended statistical procedures easily accessible. Subsequent software versions QP1.0, QP2.0, and QP3.0 followed with increasing numbers of new functions. These were made available as downloadable software that ran on Windows PCs. Each was capable of handling only 1 type of parasite per host sample, thus, multispecies infections or sex ratios could not be analyzed. Finally, we introduced Quantitative Parasitology on the Web (QPweb) in 2013, which is an R-based interactive web service capable of communicating with computers via an internet browser, independently of the operating system used. Contrary to former versions, this one is already capable of representing different types of parasites (different species, different sexes, and so on) co-occurring in the same host sample, opening new possibilities for analyzing parasite communities.

Parallel to the introduction of subsequent software versions, we also published new biostatistical procedures potentially useful in characterizing the infection level of a sample or comparing infection indices across samples of hosts (Reiczigel, 2003; Reiczigel et al., 2005a; 2005b; 2008). All these new procedures were incorporated into the newer software versions. The latest version of QPweb (v1.0.15, as of 2020, and still in 2024) is freely available on the web (Reiczigel et al., 2019b; available at https://www2.univet.hu/qpweb/qp10/index.php) to carry out most of the procedures mentioned above, including a simple users' guide to help work through potential technical difficulties (Figure 3).

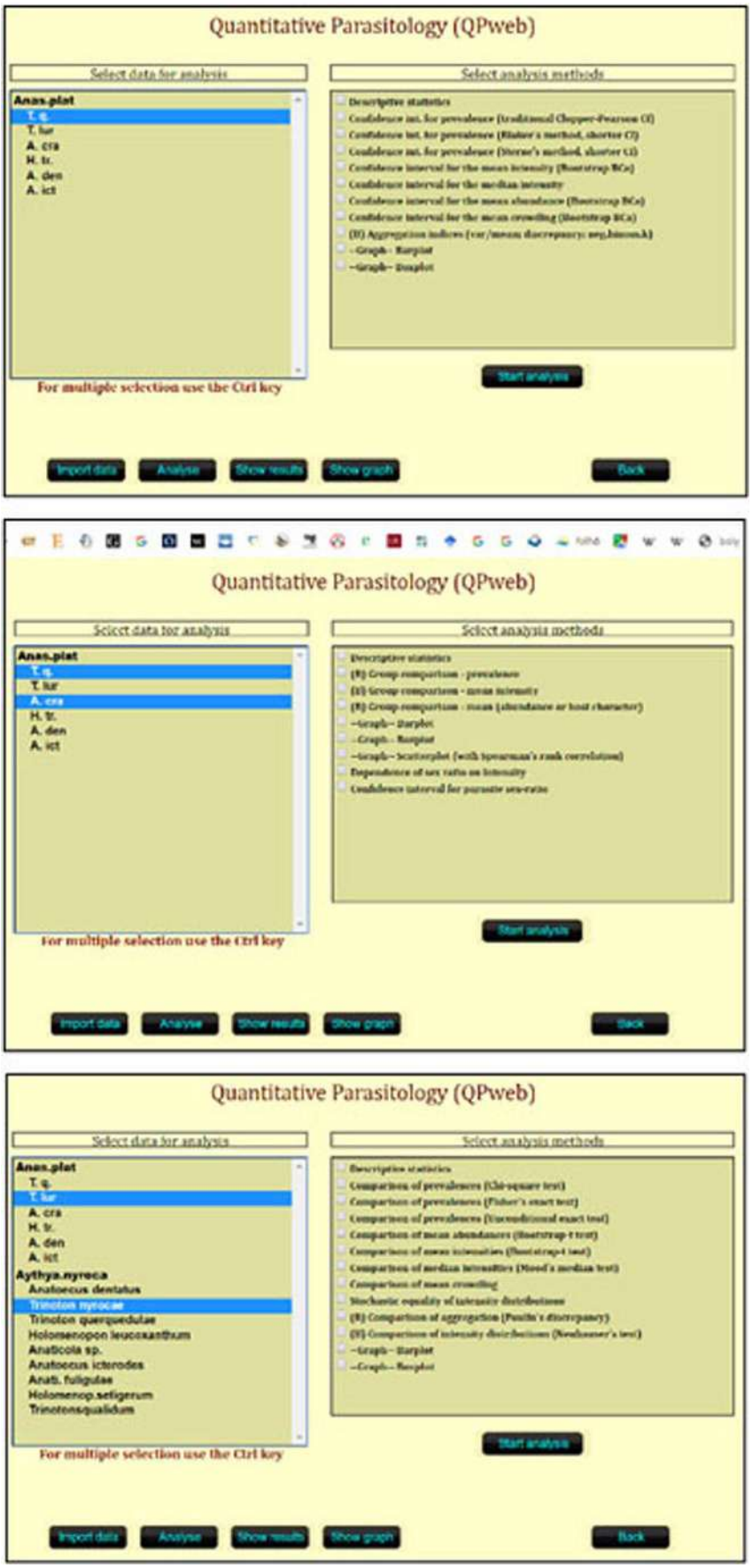

Figure 3. Analysis tools offered by QPweb when choosing different combinations of samples. Top: One species of parasite in 1 sample of host. Middle: Two species of parasites in 1 sample of host. Bottom: Two species of parasites in 2 samples of hosts. Source: J. Reiczigel, M. Marozzi, F. Ibolya, and L. Rózsa. License: CC BY-NC-SA 4.0.

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8

Parascript Approaches

Distributional Ecology of Parasites

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Chapter 8

Distributional Ecology of Parasites

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Introduction

Organisms in general experience an array of factors in shaping their geographic distributions. These factors are studied in the field that is coming to be called **distributional ecology** and range from spatial to environmental and historical to current; as such, the complexity of the situation is quite impressive. The field of distributional ecology is simultaneously pretty old (Grinnell, 1914; 1917a; 1917b) and quite new and novel (Soberón and Nakamura, 2009; Peterson et al., 2011)—distributional ecology centers around the question of why populations of a species are where they are, and why they are not where they are not. These ideas became popular with the development of large-scale and openly accessible data resources (Peterson et al., 2016), and of sophisticated computational algorithms for relating known occurrences of species to raster (that is, grid-based) GIS datasets to discover dimensions ostensibly of the fundamental **ecological niche** (Escobar and Craft, 2016). This old-and-new field has now seen intensive research attention from across the fields of ecology, biogeography, and systematics, and even fields as far afield as public health, invasion biology, and agricultural planning (for example, Mainali et al., 2015; Reddy and Nyári, 2015; Samy et al., 2016; Ramírez-Gil et al., 2019).

Parasites, of course, present several additional levels and dimensions of complexity for distributional ecology. The distributions of many free-living organisms (for example, plants, birds, fish) were hypothesized originally by Grinnell (1917b) to be shaped primarily by **abiotic** factors (for example, temperature, soil pH, precipitation; the important point is that these factors are unaffected by the presence of the species in question). However, parasites often have additional constraints. In particular, Hutchinson (1957) outlined a more complex and comprehensive niche theory that included both abiotic and **biotic** dimensions—these latter biotic dimensions may or may not be important in shaping geographic-scale distributions of species (Anderson, 2017). As a consequence, Peterson et al. (2011) proposed the **Eltonian Noise Hypothesis**, the proposition that biotic interactions do not frequently constrain *geographic-scale* distributions of species (Peterson et al., 2011). This hypothesis—to the extent that it holds true—allows researchers to focus on ecological niches in terms of abiotic factors solely (Peterson et al., 2011). Of course, parasite distributions may be much more complicated in that biotic interactions are at times absolute: Some parasites may be incapable of surviving without specific host species being present. In sum, careful thinking about the distributional ecology of parasites will involve more complexity than is required for free-living organisms (Peterson, 2008; 2014; Escobar and Craft, 2016).

This chapter will provide a review of conceptual bases for distributional ecology. However, distributional ecology is a broad area of inquiry, such that a full and exhaustive review of the field would be too lengthy. As such, in this chapter, the focus is on what is presently perhaps the most popular methodology—that of **correlative ecological niche modeling**—in the parasitology literature over the past couple of decades. Still, without a doubt, other approaches and ideas should also be brought to bear on these questions, as insights based on multiple, complementary sets of analyses from distinct perspectives will generally be more robust and more likely to prove true in the long run.

Conceptual Framework

Early thinking about parasite distributional ecology was laid out by Pavlovsky (1966), who posited that foci ('nidi') of pathogen **transmission** are driven by interactions among various components of ecosystems. However, a genuinely synthetic understanding is still lacking (it is also lacking more generally for free-living, non-parasitic organisms, by the way!). That is to say that, yes, several concepts are well-known: The fundamental ecological niche, which represents an upscaling of organismal environmental physiology, and relates the persistence or fitness of a population or set of populations to a particular set of environmental conditions (Peterson et al., 2011). The fundamental ecological niche can be modified by **biotic interactions** to yield the realized ecological niche (Hutchinson, 1957); most treatments have assumed that these interactions are *negative* (for example, competition, parasitism, predation), but *positive* interactions can also exist. These various niches translate into the **geographic distribution** of the population or species, but in non-specific and non-linear ways, thanks to the complexities of the relationships between geographic and environmental spaces, which has been termed the **Hutchinsonian Duality** (Colwell and Rangel, 2009).

Early contributions in distributional ecology included the concept of an ecological niche that is defined in terms of physical characteristics of the environment (Grinnell, 1917a; 1917b), which has been termed the **Grinnellian niche**, and is roughly equivalent to a fundamental ecological niche defined only in abiotic (non-interactive) dimensions. Later came the idea of the niche being defined in multidimensional spaces and the contrasting ideas of fundamental and realized niches (Hutchinson, 1957). Perhaps least famous but most important is the idea of the existing niche as the subset of the fundamental niche that is manifested on regions that have been accessible to the species (known in previous literature as potential niche; Pulliam, 2000). Although different terminologies do exist (Sillero, 2011), the focus here is on what is probably the most comprehensive theoretical framework in distributional ecology as regards ecological niches of species (Soberón and Peterson, 2005; Soberón and Nakamura, 2009; Peterson et al., 2011).

Grinnell (1917b) developed his niche ideas in terms of tolerances with respect to physical characteristics of the environment, so these environmental dimensions are now called **Grinnellian environmental variables** (Tingley et al., 2009). In modern terminology, those physical characteristics are termed **non-interactive variables**, as they are independent of the presence of the species in question: The presence or absence or high or low abundance of the species in question does not affect Grinnellian variables, such as annual mean temperature (Peterson et al., 2011). Hutchinson (1957) introduced the idea of biotic interactions as a modifying factor in distributional ecology—these biotic factors (for example, presence of prey or a host, absence of a predator, absence of a pathogen) are now known as **interactive variables** (Peterson et al., 2011), and are those that are affected by the presence of the species in question, as direct feedbacks exist between abundance of the species of interest and these variables—for example, prey density.

The environments manifested across the suite of geographic sites that are within the species' fundamental ecological niche are referred to as the **existing niche**, which is the set of conditions that the species has explored and tested, and where the species could potentially establish populations. Given the challenges of understanding where a species could potentially maintain populations, compared to where it actually is present, Soberón and Peterson (2005) emphasized the idea that geographic distributions are limited not just by niche considerations, but also by dispersal ability and access, such that they proposed the so-called **BAM framework**. According to the **BAM** framework, the *occupied geographic distribution* of a species represents the 3-way intersection of the areas suitable with respect to interactive variables (**B** for **biotic**), areas suitable with respect to non-interactive variables (**A** for **abiotic**), and areas accessible to the species over relevant periods of time (**M** for **mobility**).

Species, however, are distributed simultaneously in 2 linked spaces: The **BAM** diagram is cast in *geographic dimensions*, whereas niches are manifested in *environmental dimensions*. This dual-space nature of distributions of species is referred to as the **Hutchinsonian Duality** (Colwell and Rangel, 2009), which is the complex and non-linear set of connections between geographic and environmental spaces, and the idea that the species must maintain a non-null distribution in both spaces continuously and simultaneously. This concept leads to the discussion of distributions of species in environmental dimensions as different sorts of niches and distributions of those same species in geographic dimensions as geographic distributional areas. The **fundamental niche** represents that set of environmental conditions (in non-interactive dimensions) within which the species can maintain populations without immigrational subsidy. The intersection of the fundamental niche with the set of environments represented across **M** (the area accessible to the species over relevant time periods) is termed the **existing niche** (equivalent to the putative potential niche of Pulliam, 2000), and the reduction of the existing niche by the set of environments that are suitable for the species in interactive (biotic) dimensions is the **realized niche** (Peterson et al., 2011). These ideas are presented diagrammatically in Figure 1, as is the idea that the biotic influences themselves reflect **BAM**-type interactions of each interacting species.

In sum, the above is a brief, text-based summary of major concepts in distributional ecology. In effect, in hand, is a **taxonomy** of distributional areas and ecological niches, such that one can be explicit and clear in discussing and describing distributional phenomena. It is not enough to say, "I am developing a niche model" or "I am developing a distribution model" (see title of Godsoe, 2010: "I can't define the niche but I know it when I see it ..."), because the question then has to be asked as to *which niche* or *which distribution* is the object of modeling. Rather, if distributional ecology is to be a rigorous area of inquiry, explicit terminology becomes crucial; the above description is an attempt to provide such a framework for such a terminology (see Table 1 for detailed definitions of each of these concepts).

Relevant Questions in Distributional Ecology

Hutchinson's Duality indicates that the field of distributional ecology can (and indeed must) explore both geographic and environmental dimensions of distributions of species. That is, on one side, questions are feasibly addressed that have to do with geographic distributions. For example,

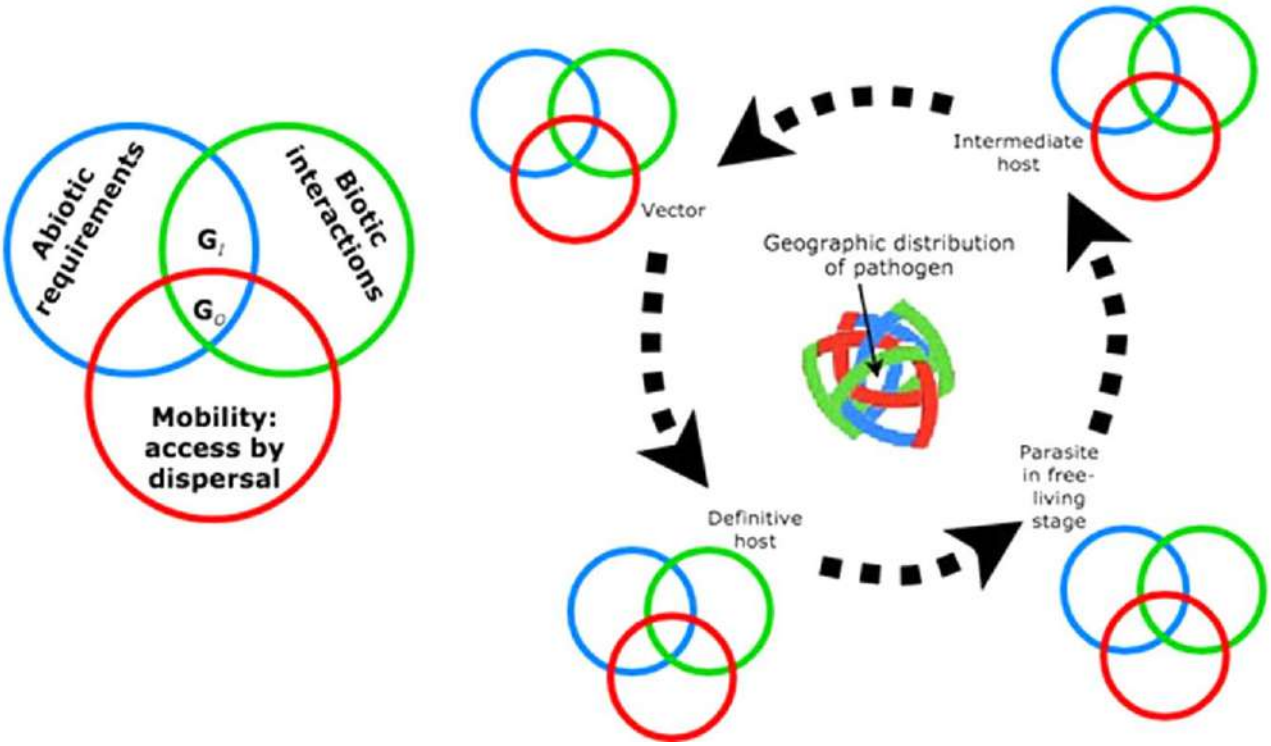

Figure 1. Summary of basic principles of distributional ecology, adapted to parasite biology. Specifically, at the left is the **BAM** diagram, a heuristic useful for conceiving of a species' geographic distribution as the geographic area that (1) fits the species' abiotic requirements (**blue** circle), (2) includes all necessary biotic conditions (**green** circle), and (3) is accessible to the species via dispersal (**red** circle). At the right is a hypothetical parasite life cycle, in which a parasite passes through a free-living stage, and subsequently infects an intermediate host, and is passed by a vector to a definitive host. Each of these steps in the cycle involves a set of interactions with abiotic and biotic environments, and access to a restricted set of areas (that is, a **BAM** intersection for each species in the parasite cycle), such that the 4-way interaction shown in the center of the life cycle would be a hypothesis of the possible geographic distribution of the parasite. Source: A. T. Peterson, 2019. License: CC BY-NC-SA 4.0.

Table 1. Summary of concepts and ideas relevant to species' geographic and environmental distributions. Note that the operator η(**X**) indicates the set of environments associated with some area **X** in geographic space.

Concept	Notation	Relationship	Concept	Notation
Fundamental niche	$\mathbf{N}_F$	$\eta(\mathbf{A}) \subseteq \mathbf{N}_F$	Abiotically (non-interactive) suitable area	**A**
Existing niche	$\mathbf{N}_F$*	$\mathbf{N}_F \cap \eta(\mathbf{M})$		
Realized niche	$\mathbf{N}_R$	$\mathbf{N}_F$*$\cap \eta(\mathbf{B})$		
		$\mathbf{A} \cap \mathbf{B} \cap \mathbf{M}$	Occupied distributional area	$\mathbf{G}_O$
		$\mathbf{A} \cap \mathbf{B}$	Potential distributional area	$\mathbf{G}_P$
		$\mathbf{G}_P$ - $\mathbf{G}_O$	Invadable distributional area	$\mathbf{G}_I$
			Biotically (interactive) suitable area	**B**
			Accessible area	**M**
			Presence sites for the species	$\mathbf{G}_+$

what is the full geographic distribution of a parasite and what host species likely remain to be discovered and documented? If closely related species tend to share the same fundamental ecological niche (Peterson et al., 1999; Peterson, 2011), then these techniques can also be used to make predictions regarding the location of undescribed species (Raxworthy et al., 2003; Peterson and Navarro-Sigüenza, 2009). Similarly, if fundamental ecological niches remain stable across time and if one has raster data layers that describe environmental conditions both at present and in the future or past, one can assess or anticipate future or past potential distributional patterns of the species.

On the environmental side, one can feasibly explore the suites of conditions associated with the distribution of a species, interpreting those conditions as manifestations of the species' realized ecological niche. For parasites in particular, questions of realized versus existing niches emerge, as the degree to which a parasite's range is a function of its own requirements versus those of its host(s) is a critical question in distributional ecology (Maher et al., 2010). Ideally, a deep and detailed understanding of the various niches of a species (that is, realized, existing, fundamental) should permit a predictive understanding of its distribution in time and space, and in relation to other species, including parasites, vectors, hosts, and other competitor parasites. Of particular interest is the opportunity to estimate the fundamental niche, as a fundamental niche represents an evolved characteristic of a species and should be able to be transferred to diverse sets of environmental conditions to hypothesize distributional potential.

Methodology and Study Design

Ecological niche modeling requires 2 major data inputs, and a number of decisions regarding strategy and parameter values (see Figure 2 for a diagrammatic summary, and book-length methodological summaries: Franklin, 2010; Peterson et al., 2011; Peterson, 2014; Guisan et al., 2017). The first data input is that of **species occurrence data**—that is, geographic coordinate pairs that correspond to locations where the species is known to have occurred. Of course, these data need to be explored, and erroneous or inconsistent records need to be detected and removed (Chapman, 2005; Cobos et al., 2018); frequently, geographic coordinates and associated uncertainty measures and documentary metadata need to be added to the data records (Chapman and Wieczorek, 2006). Finally, the occurrence data must be inspected for areas of overly intense sampling, duplicate records, or imprecise records, to avoid introducing biases.

The other major data input is that of **environmental data**, in the form of raster GIS data layers. Most niche-modeling algorithms require that these data layers have the same grid system (that is, spatial resolution, origin, and orientation),

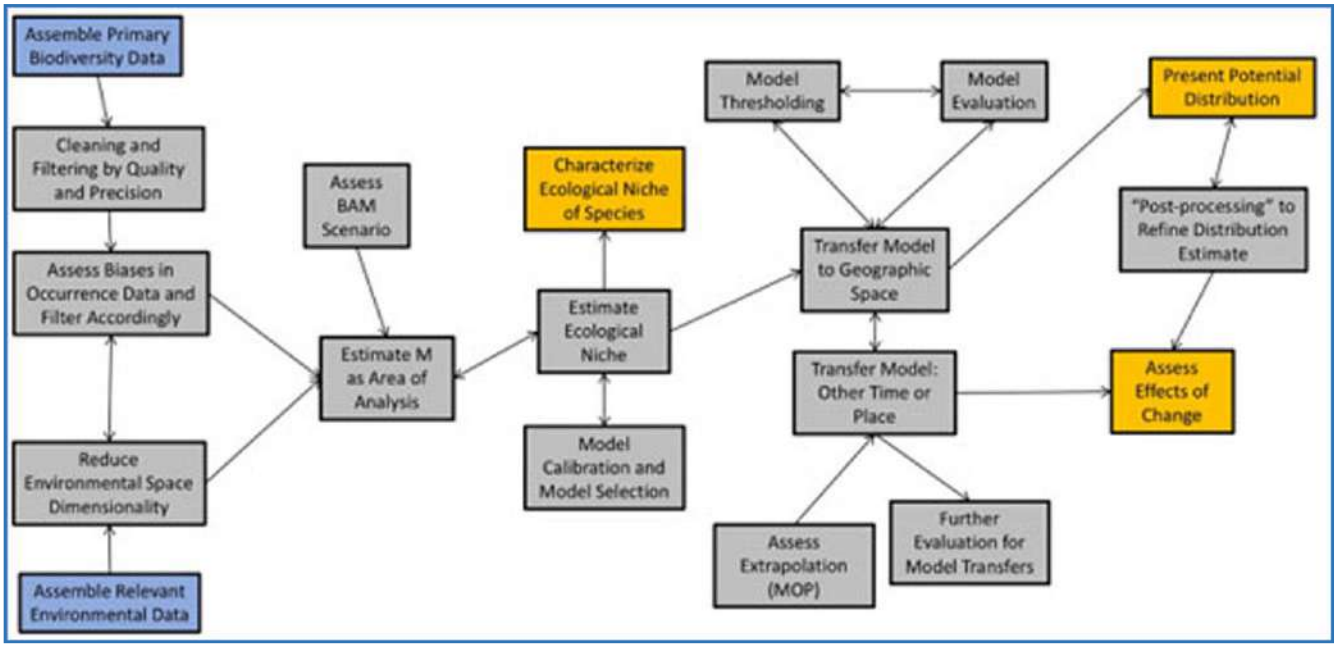

Figure 2. General summary of flow of work, inputs, and products, in ecological niche modeling. Blue boxes indicate data inputs, gray boxes are steps in the process, and gold boxes are outputs. Arrows direction denotes the flow of information. Source: A. T. Peterson, 2019. License: CC BY-NC-SA 4.0.

and indeed most studies have centered on a single climate summary (Hijmans et al., 2005), but one must think more deeply than just that. Rather, in ecological niche modeling, the modeler does not have much freedom to explore massive numbers of environmental dimensions because of problems with model overfitting in too-highly-dimensional environments (Peterson, 2007), so modelers must choose carefully the most interesting or relevant dimensions associated with the persistence of populations of a species. Of course, one approach is simply to "let the data choose," and use the niche modeling algorithm as a sort of data-mining algorithm, but generally a better approach is to assess what is known of the species' natural history, and to pick environmental data layers accordingly.

Once the data streams are identified and prepared, then the niche modeler must begin to **integrate** them. A first step is that of estimating the accessible area **M**, which ends up being the key area over which models should appropriately be calibrated (Barve et al., 2011). A further step is that of assessing or approximating the relative configuration of the **BAM** diagram for that particular species in that particular situation, because certain **BAM** configurations invariably lead to bad models that have little or no predictive power (Saupe et al., 2012; Qiao et al., 2015). A few adjustments can be made, though some situations simply are not appropriate for modeling.

Actual **niche model calibration** is accomplished by means of various algorithms (see illustrations in Figure 3). The algorithms range from the simplest, BIOCLIM, which is an approach to delineating niche estimates as orthogonal tolerance limits in different dimensions based on observed ranges of values, to complex multivariate statistical and machine-learning approaches. Each of this diversity of approaches to estimating niches has its own complexities about how it can and should be calibrated and executed (Muscarella et al., 2014; Sánchez-Tapia et al., 2017). At the end of the model calibration process, the model is generally **evaluated** via some sort of test of its ability to predict independent data sets, usually in geographic space. These tests can be threshold-dependent or threshold-independent, but all devolve into testing how well the model anticipates the independent occurrence data sets in the smallest area possible (Fielding and Bell, 1997). Once models are calibrated and evaluated, they can be **interpreted**, **or transferred** to other times or other regions.

A Worked Example

Here, as an example of the concepts described above, and a bit of an illustration of the inferences that can and cannot be derived from ecological niche modeling of parasites. The wasp *Vespula austriaca* is analyzed as an obligate parasite of its congener *V. rufa* (Taylor, 1939). Occurrence data were gathered for the 2 species from the Global Biodiversity Information Facility (February 28, 2019; queries are available at doi: 10.15468/dl.blijyg and doi: 10.15468/dl.w6spai), and reduced their coverage to western Europe, where point densities were greatest, as a proxy of areas where the species have established successful populations. Figure 3 presents visualizations of the distribution of the 2 species in geographic and environmental spaces.

A first consideration is that of how to characterize the fundamental niches of the species, and many methodological options are available. Focusing for the moment on *Vespula rufa*, the host species, one of the classic approaches to ecological niche modeling is the so-called BIOCLIM approach (Nix, 1986), which basically consists of defining tolerance limits independently in each environmental dimension, creating a multidimensional parallelepiped (Figure 4). This area nicely incorporates all (or nearly all) of the records of the species, but it also tends to include too much environmental space. More modern methods, however, such as Maxent, boosted regression trees, random forests, and general additive models, tend to be more complex in the response types that they reconstruct, which has been seen as an advantage (shown diagrammatically in Figure 4; Elith et al., 2006). However, an emerging realization is that such highly complex reconstructions of response types may not be particularly biologically realistic, as theory and experimental results from physiological studies suggest that fundamental niches should be relatively simple, and effectively convex in environmental space (Maguire, 1973). As such, a more appropriate model of a fundamental niche might enforce the simple and convex nature of these niches (see Figure 4, ellipsoid model).

A final point regards the parasite and its distribution. Several studies in the literature indicate that *Vespula austriaca* is an obligate parasite that focuses on *V. rufa* across its European distributional area. This idea is borne out by the co-distribution of the 2 species, such that no sites are apparent where *V. austriaca* exists in areas where *V. rufa* is not at least close by (Figure 3). As such, one can take the environmental distribution of the host as defining the biotically suitable area **B** for the parasite; the final panel of Figure 4 shows the environmental distributions of the 2 species together and points out some possible niche limitation of *V. austriaca* even within the bounds set by the ecological niche of *V. rufa*. Note that the niche of the parasite remains undefined on 2 sides simply because sites presenting environments in those directions are either 1) not accessible to the parasite or 2) not within the niche of the parasite's obligate host.

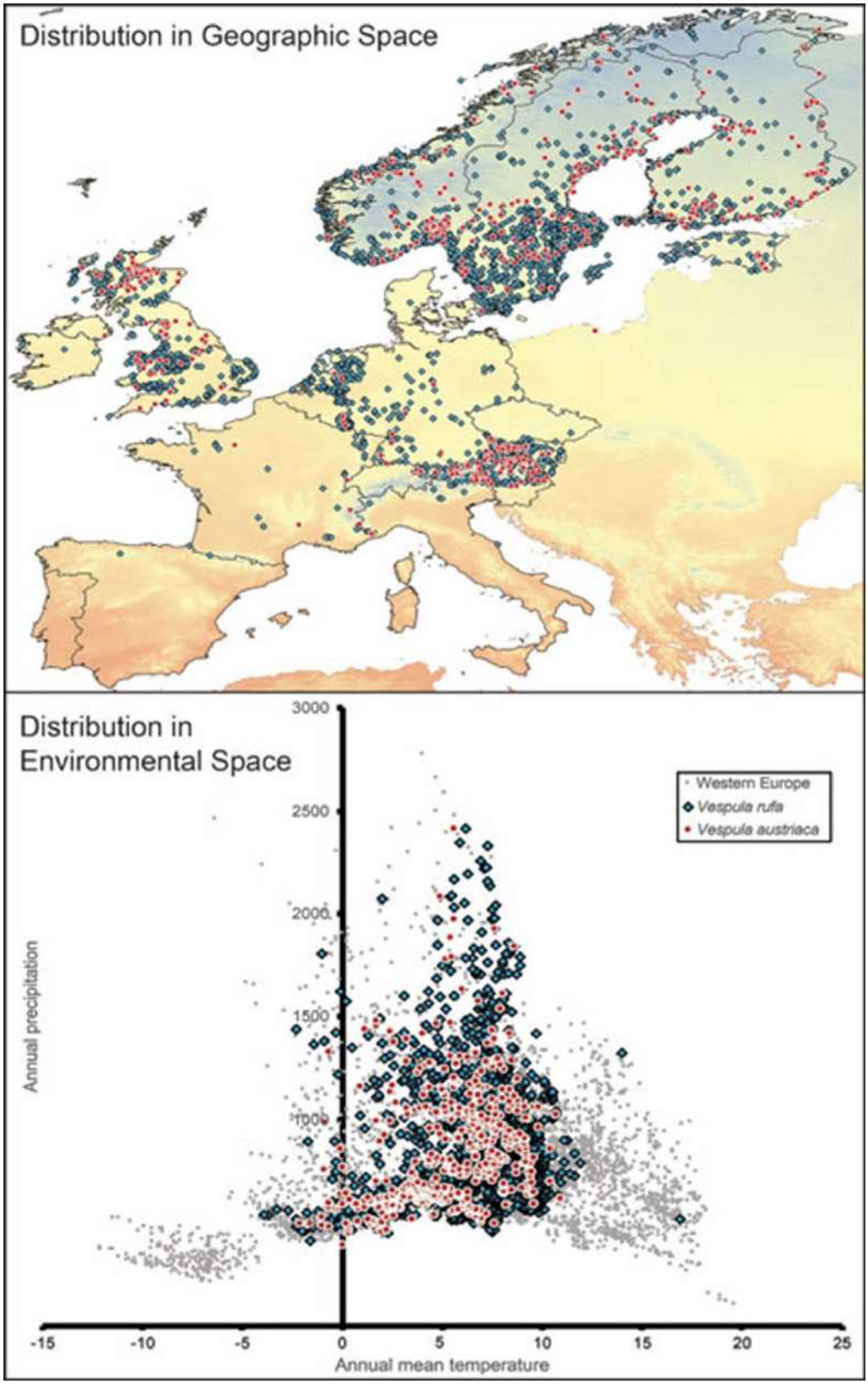

Figure 3. Summary of the distribution of one host-parasite system (*Vespula rufa* and *V. austriaca*, respectively, across western Europe, shown on top of the annual mean temperature data set (**red** = high, **blue** = low) (Hijmans et al., 2005). In the lower panel, the 2 species are shown in relation to the environments available across the region (in medium gray). Source: Adapted from Hijmans et al. (2005). License: CC BY-NC-SA 4.0.

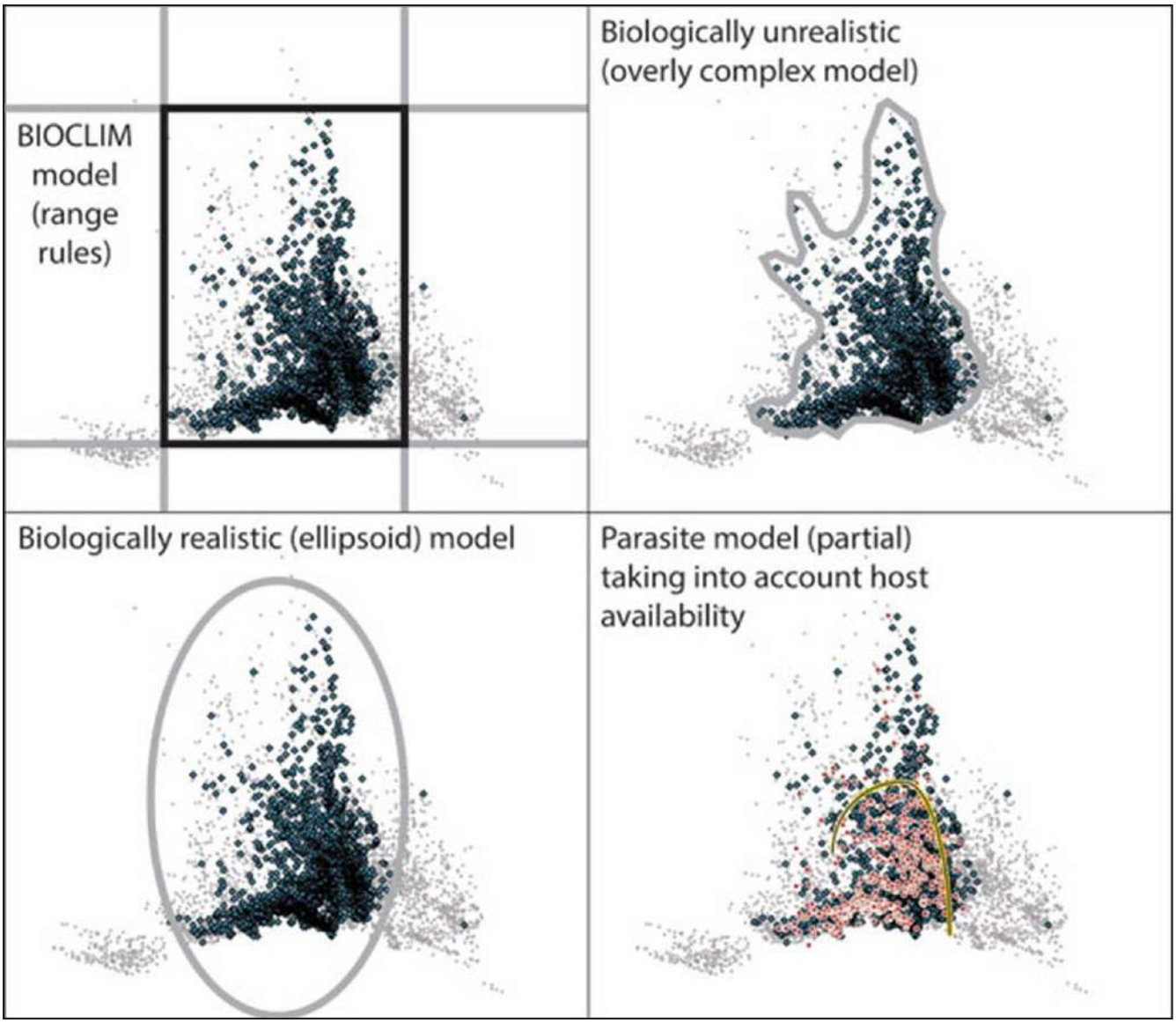

Figure 4. An illustration of methods and some key ideas in ecological niche modeling. Top panels and bottom-left panel are focused on *Vespula rufa* (the host species): Gray dots show the set of environments that is accessible to the species across western Europe, whereas the blue diamonds are the occurrences of the species. The gray and black lines show the set of environments that might be "chosen" as within the species niche under different approaches. Finally, the bottom-right panel shows the parasite (*V. austriaca*) distribution on top of that of the host and the available environments. The **yellow**-and-black line separates the distribution of the parasite (**red** points) from areas in which the host is available (blue diamonds), yet few parasite records are available (note that the great bulk of the parasite records comes from below the **yellow**-and-black curve), suggesting niche limits for the parasite, independent of the host's niche. Source: A. T. Peterson, 2019. License: CC BY-NC-SA 4.0.

Published Examples

Parasitology has a rich history of interest in distributions and environmental constraints on distributions, yet it has not seen an abundance of distributional ecology studies, in the modern, quantitative sense. Where parasites have been analyzed in greatest detail is certainly as regards pathogenic organisms, including viruses (for example, Kearney et al., 2009; Oliveira et al., 2013; Campbell et al., 2015; Escobar et al., 2015a), bacteria (for example, Eisen et al., 2006; Giles et al., 2010; Escobar et al., 2015b), simple eukaryotes (for example, Foley et al., 2008; Kulkarni et al., 2010; Gurgel-Gonçalves et al., 2012; Escobar et al., 2014; Ramsey et al., 2015), and a cutting edge papers on macroparasites (for example, Botero-Cañola et al., 2019; Botero-Cañola and Gardner,

2023; Haverkost et al., 2010; Gentry et al., 2016). However, some of pathogen-related studies mentioned above generally assess the occurrence of the disease per se, and often neglect the independent distributional potentials of the parasite and host. That is, they treat the disease transmission system as a black box that results in human, other (non-human) animal, or plant disease (Peterson, 2014). Black box models have the advantage of integrating over the entire transmission cycle of a parasite or pathogen, but have the failing of not focusing on the ecological niche of any species in particular, and of being easily biased by regional differences in sampling intensity, diagnostic capacities, or reporting frequency (Waller et al., 2007).

Distributions

Most parasite-oriented studies in distributional ecology have focused on distributional questions. That is, most studies have taken known occurrences and have attempted to predict the full geographic distribution of the disease (for example, Sehgal et al., 2010; Machado-Machado, 2012). Rarer are studies that include careful testing with independent data (for example, Escobar et al., 2015a; Botero-Cañola et al., 2019; Botero-Cañola and Gardner, 2023). Other studies include model transfers to future conditions, where distributional shifts are anticipated that will likely manifest eventually as changing disease occurrence patterns (Rödder et al., 2010; Rose and Wall, 2011; Suwannatrai et al., 2017; Alkishe et al., 2018).

Perhaps most interesting is the potential for developing fine-resolution distributional summaries for species, even across complex and poorly sampled landscapes. Here, when fine-resolution occurrence data, such as those that are derived from GPS georeferencing for recent field records, are available, they can be integrated with equally fine-resolution environmental data deriving from remote sensing. The result is a highly precise and detailed mapping of the distributional potential of the species across broad landscapes, thanks to the pairing of fine-resolution data on both occurrence and environment. Examples include applications to understanding the spatial distribution of likely avian influenza risk across Southeast and East Asia (Gilbert et al., 2007; Xiao et al., 2007; Gilbert et al., 2008; Dhingra et al., 2016) and other regions (Bodbyl-Roels et al., 2011), fine-scale predictions of triatomine distributions in Mexico (López-Cárdenas et al., 2005), and others, although exploration of the full diversity of remote-sensing data products is likely still in its infancy in distributional ecological studies.

Finally, it is worth mentioning that studies of this general sort that are specifically interpreted in the context of infection risk—that is, including additional processing beyond just modeling the niche and estimating **A** in the **BAM** diagram—are relatively rare (Ostfeld et al., 2006; Estrada-Peña et al., 2014; Ostfeld et al., 2018). The ideas central to this step (that is, risk mapping) are treated in detail in a book-length contribution (Peterson, 2014).

Niches

On the niche and environment side, this suite of techniques has perhaps seen much less application to those questions. An early contribution (Costa et al., 2014) explored ecological niche variation within a key complex of vector insects that transmit Chagas disease, but failed to distinguish between fundamental and existing niches, which wasn't well appreciated at that time. A later contribution, also focused on Chagas vectors, documented niche differentiation within the *Triatoma dimidiata* complex more rigorously (Gómez-Palacio et al., 2015), including detailed background similarity testing (Warren et al., 2008), to avoid misinterpreting existing niche differentiation as fundamental niche differentiation.

Niches and Distributions

On a more synthetic level, one suite of analyses has gone deep into the interaction between sampling and reporting of pathogen occurrences and their likely geographic distributions (Del Valle et al., 2018), with deep integration of dispersal opportunity and ecological niche, to get at transmission risk more or less rigorously (Escobar et al., 2016). Another study, focused on the plague transmission system, assembled information on human cases, animal detections of the pathogen, and the broader distributions of the host mammal species, to test whether the distribution of plague is a function of the distributions of its hosts, or rather on its own distributional potential (Maher et al., 2010). This work was echoed later in an assessment of a plant-parasite system (Lira-Noriega and Peterson, 2014). Finally, one early analysis focused on using distributional estimates from ecological niche models to predict the mammal hosts of triatomine bugs in the Protracta group of species within the genus *Triatoma*, and the predictions turned out to be quite predictive of host-parasite associations (Peterson et al., 2002). This sort of deeper, and more synthetic, application of distributional ecology tools to parasite distributions is rare, but is quite promising as regards making concrete contributions to understanding parasite distributions.

For macroparasites, early explorations managed to outline the potential of these methods and demonstrate some of the interest in their potential (Haverkost et al., 2010), and regionally focussed studies have recently been published, focusing on a *Echinococcus multilocularis,* a pathogenic cestode by Botero-Cañola et al. (2019) and a general test of latitudinal

variation in parasitism using museum collections based data (Botero-Cañola and Gardner, 2023). Meanwhile global geographic summaries of key groups have also been published (Feidas et al., 2014). Chaiyos et al. (2018) developed detailed niche models for a number of macroparasites in humans in Thailand and explored their results in both geographic and environmental spaces. Lira-Noriega et al. (2013) developed detailed analyses to assess whether biotic drivers (that is, host associations) versus Grinnellian niches drove distributions of parasitic mistletoe distributions.

Future Perspectives

Distributional ecology has progressed from a descriptive effort (for example, making a map by hand) to a quantitative effort, and the quantitative approaches have moved from shots in the dark ("look, this works!") to steps that are firmly based in ecological theory, in just a few decades. As such, the field is exciting and vibrant, and is seeing intensive research attention across many taxa and across many fields. Still, applications in parasitology have lagged somewhat, leaving many opportunities for exciting steps forward in understanding geographic and environmental distributions of many types of parasites.

Parasite applications in distributional ecology may be more complicated than most such studies, because of the frequent negation of the Eltonian Noise Hypotheses—that is, interactions with other species often *do* matter to parasites, at least in many cases. Indeed, one of the most useful testing frameworks has almost never been applied in parasitology: If one has a hypothesis about a biotic interaction, one can build ecological niche models that include and exclude that interacting species (for example, a host). One can then assess quantitatively whether the models with the interactor are better (for example, in predictive challenges, or in terms of maximum likelihood) than the models without the interactor (Atauchi et al., 2018). Such simple assessments have the potential eventually to understand some of the most fundamental elements of distributions of parasites—are their distributions governed by the niches of their hosts, or do they have meaningful niche constraints on their own?

More fundamentally, though, applications of ideas from distributional ecology to questions in parasitology must weigh very carefully the conceptual framework of the question, in order to proceed to deeper and more interesting questions. That is, a world of exciting questions abounds, such as the environmental dimensions of and constraints on the process of host-parasite co-speciation, or micro-scale versus macro-scale niche dimensions that may constrain parasite distributions at multiple scales, and how different types of niches (for example, realized or fundamental) may be broader or narrower at different spatial scales. The challenge, however, is to assemble a methodology that responds first to the conceptual foundations, and then is adapted and applied to the specific case of the parasite in question. Once such conceptual rigor is in hand, exciting distributional ecology results will emerge for parasitology.

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Part II

PROTOZOA, MYXOZOA, MESOZOA

PROTOZOA

9

Protozoa

Apicomplexa

The Coccidia Proper:

Important Apicomplexa Other than Haemoprotozoa

Donald W. Duszynski

Phylum Myzozoa

Subphylum Apicomplexa

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Chapter 9

The Coccidia Proper: Important Apicomplexa Other than Haemoprotozoa

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History of the Term Apicomplexa

Taxonomy addresses the principles of scientific classification by discovering, observing, defining characters, ordering into groups, naming individual organisms that are clearly different within those groups, and archiving type specimens as appropriate in accredited museums. Historically, all living things defined as animals (that is, non-plants), were placed in 1 of 2 groups: Protozoa (meaning single-celled protists) or metazoa (meaning multicellular animals). Omitting hierarchical names (kingdom, phylum, class, and so on) for the moment, all protozoa were ordered into 1 of 4 groups based on how they moved, or didn't: Ciliates (which have cilia), amoeba (which have pseudopodia), flagellates (which have flagella), and a catch-all category called the Sporozoa, most of which (but not all) had spores and some of which (myxosporidia and microsporidia) were not even remotely related to the spore-formers.

As knowledge increased, the name Sporozoa became unwieldy because it did not suggest or represent true evolutionary relationships between the organisms included therein. The widespread use of Transmission Electron Microscopy (TEM) for biological specimens began in the 1950s and continued throughout the 1960s and 1970s; many of these studies examined the fine structure of zoites belonging to a plethora of different protozoans. Eventually, a pattern began to emerge that revealed several common, consistently-shared structures (for example, polar rings, rhoptries, micronemes, and often a conoid) at the more pointed end (now termed anterior) of certain life stages (Figure 1). When present, these structures, in whatever combination, were termed the apical complex. At that time, protozoologists working on parasites sought a more phylogenetically relevant suite of characters to define their organisms, and Norman D. Levine, from the University of Illinois, came up with the name Apicomplexa to unify them. This complex structure is now known to be the focus of events during host cell penetration and establishment of the parasite within the cells of the host.

Introduction to the Apicomplexa

The protozoan group Apicomplexa Adl et al., 2005 (Levine, 1980) contains many obligate intracellular parasites including such diverse organisms as coccidia, gregarines, haemosporoids, piroplasms, and cryptosporids, all united not by their biology or life histories, but by the presence of their unique apical complex. This complex collection of protozoans is subdivided into 2 major assemblages based on the presence or absence of a conoid in their apical complex. The Aconoidasida Mehlhorn et al. 1980 [= Hematozoa Vivier, 1982] all lack a conoid in their asexual motile stages, and include the Haemosporida Danilewsky, 1885 (for example, *Plasmodium, Haemoproteus, Leucocytozoon,* and others) and Piroplasmorida Wenyon, 1926 (for example, *Babesia, Theileria*, and others).

Members of the second major grouping, Conoidasida Levine, 1988, all have a complete apical complex that includes a hollow, truncated conoid in all or most of their asexual motile stages, along with other unifying features. This paraphyletic lineage includes 3 groups: Gregarinasina Dufour, 1828; the monogeneric family Cryptosporididae Tyzzer, 1907; and Coccidia Leuckart, 1879 according to Adl et al. (2012). Of the 2 Conoidasida groups that will not be covered in detail here, the gregarines parasitize invertebrates, and *Cryptosporidium* species, which were once considered to be atypical Coccidia, are most closely related to the gregarines and not the Coccidia (Cavalier-Smith, 2014; Thompson et al., 2016).

Before delving into the Coccidia, the history of taxonomic placement of the former Cryptosporididae will be discussed briefly. Bull et al. (1998) first noticed there was serological cross-reactivity between anti-*Cryptosporidium* monoclonal antibody and sporocysts of the gregarine *Monocystis*, an observation mostly overlooked—or ignored—at the time. The next year, when sequencing SSU rDNA, Carreno et al. (1999) inferred that *Cryptosporidium* was more closely related to gregarines than to Coccidia by phylogenetic analysis of apicomplexan parasites. Based on this and

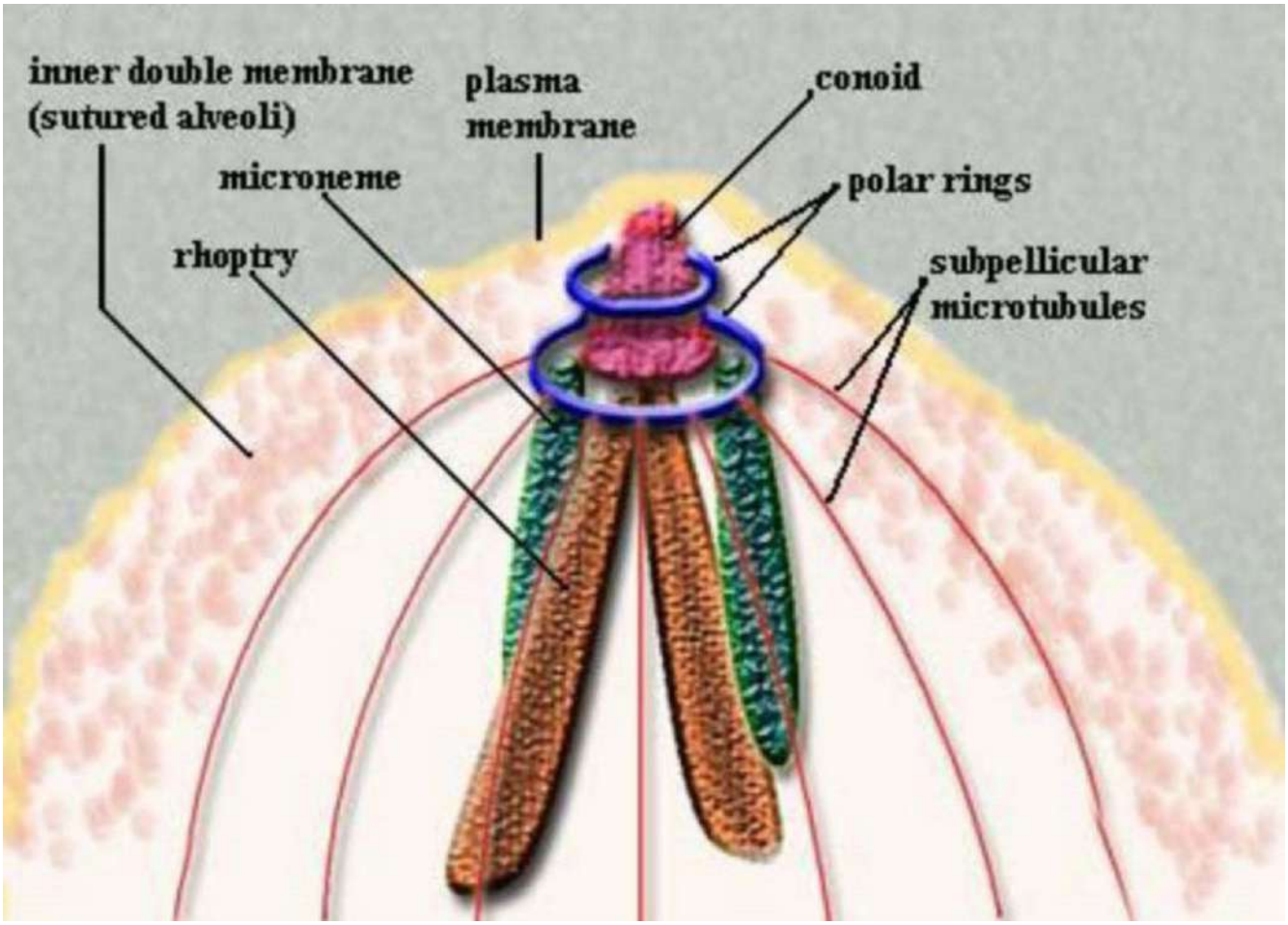


Figure 1. Apical complex structures at the anterior end of a coccidian zoite. Image source: Clowes et al., 2006. License: CC BY.

other molecular congruences, and on biological and behavioral similarities, Cavalier-Smith (2014) established a new subclass, Orthogregarinia, for *Cryptosporidium* and its most closely related gregarines, which include epicellular parasites of vertebrates possessing a gregarine-like feeder organelle and lacking an apicoplast (which is a relict, non-photosynthetic plastid found in most apicomplexan parasites). In addition to the SSU-rDNA sequencing evidence, *Cryptosporidium* shares biological features with gregarines including its epicellular location, connection to the host cell via a myzocytosis-like feeding mechanism, heterogeneity of trophozoite cell shape, and other structural similarities (see Thompson et al., 2016). Gliding movements seen in different trophic stages of *Cryptosporidium* species are behavioral features that also are similar to gliding movements exhibited by some gregarines (Borowski et al., 2008; 2010; Valigurová et al., 2013).

The Coccidia

Coccidia are united by having mature gametes that develop intracellularly, microgametocytes that usually produce many microgametes, and non-motile zygotes that mostly contain sporocysts within their oocysts. There are 2 Coccidia lineages: **Adeleorina Léger, 1911** and **Eimeriorina Léger, 1911**. The Adeleorina has about 7 families, 2 of which each contain a genus of important parasites of vertebrates, Hepatozoidae Wenyon, 1926 (genus *Hepatozoon*) and Klossiellidae Smith and Johnson, 1902 (genus *Klossiella*). The Eimeriorina has 10–12 recognized families, 2 with multiple genera containing important parasites of vertebrates. The Eimeriidae has about 20 genera, but only 6 will be mentioned, to illustrate their diversity, namely, *Acroeimeria*, *Caryospora*, *Choleoeimeria*, *Cyclospora*, *Eimeria*, and *Isospora*. The Sarcocystidae has 7 genera of which 5 have extremely important parasites of humans and/or their domestic animals, namely, *Besnoitia*, *Cystoisospora*, *Neospora*, *Sarcocystis*, and *Toxoplasma*.

Important Genera, Relation to Other Species, and Basic Life Histories

The apicomplexan genera with species that are important parasites of humans and/or their domestic, companion, and wild animals are highlighted in this section in the same taxonomic sequence outlined above:

Conoidasida Levine, 1988
Coccidia Leuckart, 1879
Adeleorina Léger, 1911

The Adeleorina is a poorly understood group of apicomplexan parasites. Members are united biologically by use of syzygy, a characteristic method of gamete formation by which both macro- and microgamonts are pressed together during their development (Adl et al., 2012). The Adeleorina has 7 families of coccidia and includes those with both homoxenous and heteroxenous life cycles (Barta, 2000). In heteroxenous species, the conjugation of gamonts and subsequent sporogony most often occur within an invertebrate definitive host and (mechanical) vector; the oocysts formed contain numerous sporocysts, and sporozoites are found in the hemocoel of the definitive host (Craig, 2001). Once the vector is ingested, sporozoites are released, after which they penetrate the gut of the vertebrate intermediate host and enter the bloodstream to reach leukocytes and cells throughout the body where they undergo merogony. Many of the species in this group have morphologically distinct meronts and merozoites during their asexual reproduction, which occurs in the vertebrate (that is, intermediate) host. The first-generation meronts (M_1) produce large merozoites (m_1) that are thought to initiate a second round of merogony in which the M_2 produce smaller m_2s, which then become the progenitors of gamonts (Barta, 2000). Merogony in the tissues ultimately gives rise to gamonts in white blood cells (WBC) and tissue cysts; these tissue cysts may be a stage that can be transmitted by predation, but this remains to be determined (Craig, 1990; 2001).

Family Hepatozoidae Wenyon, 1926

This family has a single genus, *Hepatozoon* Wenyon, 1926b, with more than 300 described species (Baneth et al., 2007; Ivanov and Tsachev, 2008). Species in this genus infect various vertebrates including amphibians, reptiles, birds, and mammals, which are their intermediate hosts. The definitive hosts for these species are invertebrates that include mites, ticks, and various insects, and infection of the vertebrate host occurs when it ingests the infected invertebrate (not by its bite). Barta (2000) suggested the genus is paraphyletic. One important species in this genus, which can parasitize a favorite companion animal, the domestic dog, will illustrate the biology of these species.

Genus *Hepatozoon* Wenyon, 1926 (Figure 2).

Hepatozoon canis (James, 1905) Wenyon, 1926, can cause serious, life-threatening illness in vertebrates. In addition to dogs, it has been found parasitizing cheetahs, coyotes, jackals, foxes, hyenas, lions, and leopards (each as intermediate hosts) and has a worldwide distribution wherever its definitive host, the brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806), is found. Note that other tick species also can serve as definitive hosts. Its prevalence in infected canid populations often is modest but also may be quite high. For example, Conceição-Silva et al. (1988) found 143 of 301 (48%) red foxes in Portugal to be infected while only 50 of 1,752 (3%) domestic dogs from the same area were infected. O'Dwyer et al. (2001) examined blood smears of dogs from rural areas of 7 counties in Rio de Janeiro state, Brazil, and identified *H. canis* in 98 of 250 (12%) dogs. Cardoso et al. (2014) detected *H. canis* in 68 of 90 (76%) red foxes from 8 districts in Portugal, using both molecular (PCR amplification of 18S rRNA gene fragments) and histopathological sections of multiple tissues (bone marrow, heart, hind leg muscle, jejunum, kidney, liver, lung, popliteal or axillary lymph nodes, spleen, and/or tongue). Furtado et al. (2017) collected blood samples from domestic dogs from 3 regions of Brazil; 81 of 129 (63%) dogs were positive for *H. canis*, as determined by PCR nucleotide sequences of the 18S rRNA gene of *Hepatozoon*.

In the life cycle of *Hepatozoon canis* in vertebrates (Figure 2), monozoic cysts have been found in the spleen, meronts and merozoites in the spleen, lymph nodes, lungs, liver, bone marrow, and gamonts and/or gametocytes in the cytoplasm of neutrophils and monocytes. Once ingested by the tick definitive host, gamonts need about 24 hours to free themselves from vertebrate WBCs and soon thereafter they align side-by-side in syzygy. At 48 hours in the tick, 2 types of cells are present: Elongated cells with an eccentric nucleus, presumed to be microgametes, and more rounded cells, also with an eccentric nucleus, presumed to be macrogametes. At 4 days, zygotes (early oocysts) are formed; by 5 days oocyst wall and sporocyst formation have begun (Baneth et al., 2007) and these stages are extracellular, not within tick host cells. Vincent-Johnson et al. (1997) measured *H. canis* oocysts and said they were mostly spheroidal, 215 × 193 (160–325 × 138–258) µm with sporocysts that were 36 × 26 (29–41 × 17–30) µm.

Infection with *Hepatozoon canis* in dogs (and other vertebrates) ranges from being asymptomatic with low-level parasitemia, to a severe, life-threatening illness with fever lethargy, anemia, and emaciation in animals with very high parasitemia (Baneth et al., 2007). Sakuma et al. (2011) listed the characteristic hematological abnormalities in *H. canis*

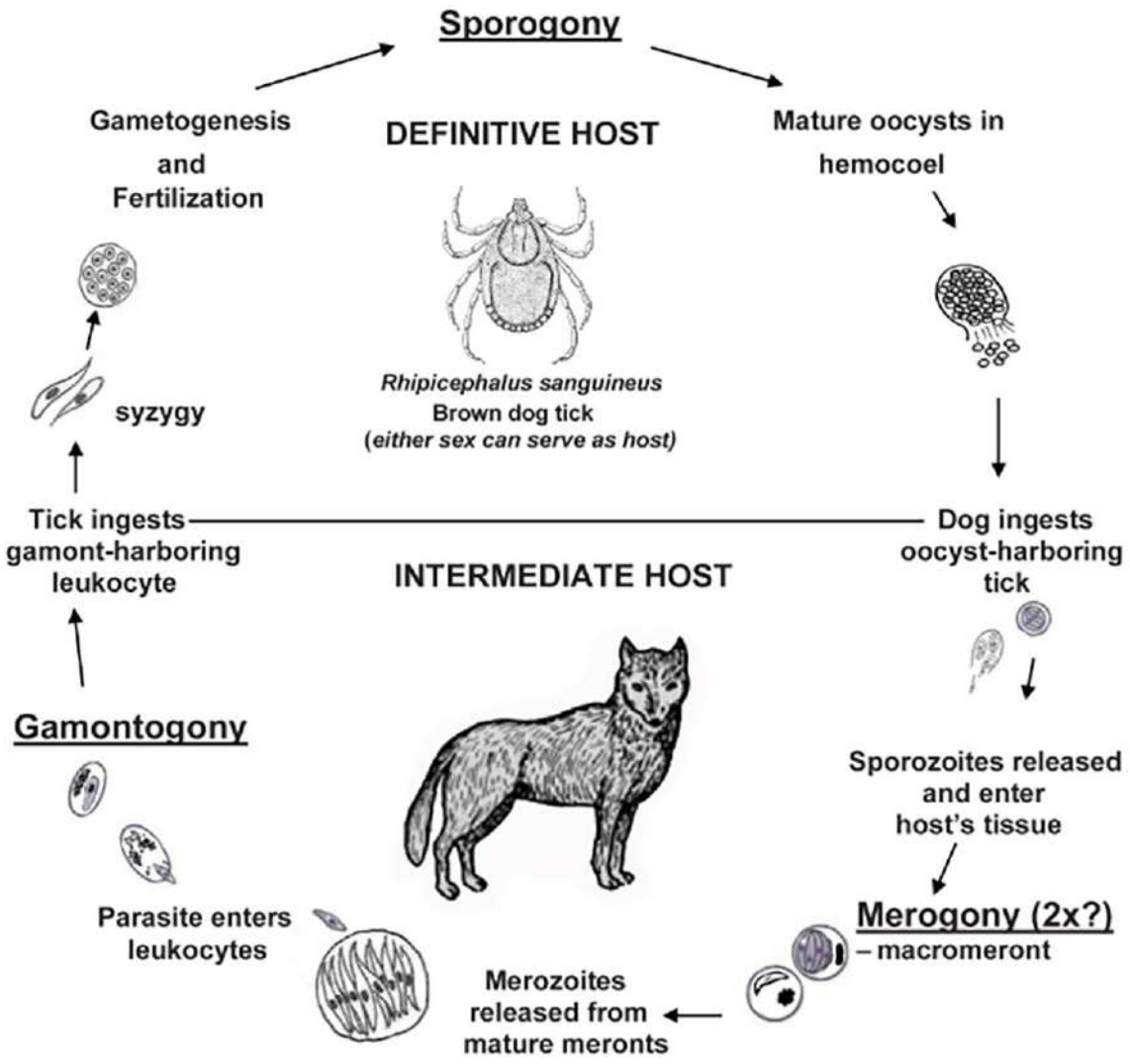


Figure 2. Diagrammatic drawing of the life cycle of *Hepatozoon canis* in dogs. Image sources: Tick, Pratt and Littig, 1962. Dog, V. Rausch, 1952. Other Figures, originals by S. L. Gardner, 2023. Tick image public domain; all other images, CC BY-NC-SA 4.0.

infections to include nonregenerative anemia, thrombocytopenia, neutrophilia, hyperproteinemia, hypoalbuminemia, polyclonal gammopathy, and increased concentrations of serum creatine kinase and alkaline phosphatase.

Family Klossiellidae Smith and Johnson, 1902

This family also has but 1 genus, *Klossiella* Smith and Johnson, 1902, and it contains about 18 named species that infect primarily mammals, in which it invariably undergoes asexual and sexual development in the kidneys. For example, *K. muris* is found in the kidneys of house and lab mice (*Mus musculus*), *K. cobayae* in the capillaries of the guinea pig (*Cavia porcellus*) kidney, and *K. equi* in the kidney of asses (*Equus asinus*) and horses (*Equus caballus*) (Levine, 1973; Levine and Ivens, 1965). Levine and Ivens (1965) reviewed the highlights of Smith and Johnson's (1902) discovery and

Box 1. *Hepatozoon* species — Learn More

Interested readers can find more detailed information on this and other *Hepatozoon* species in dogs, cats, and other carnivores in Duszynski et al. (2018). If interested in various tissue stages, a picture of a meront in the spleen of a dog from the Philippines is shown in Vincent-Johnson et al. (1997; Figure 5); developmental stages in the tick and scanning electron microscopic images of oocysts and sporocysts in ticks are found in Baneth et al. (2007; Figures 2–13 and Figures 14–17, respectively).

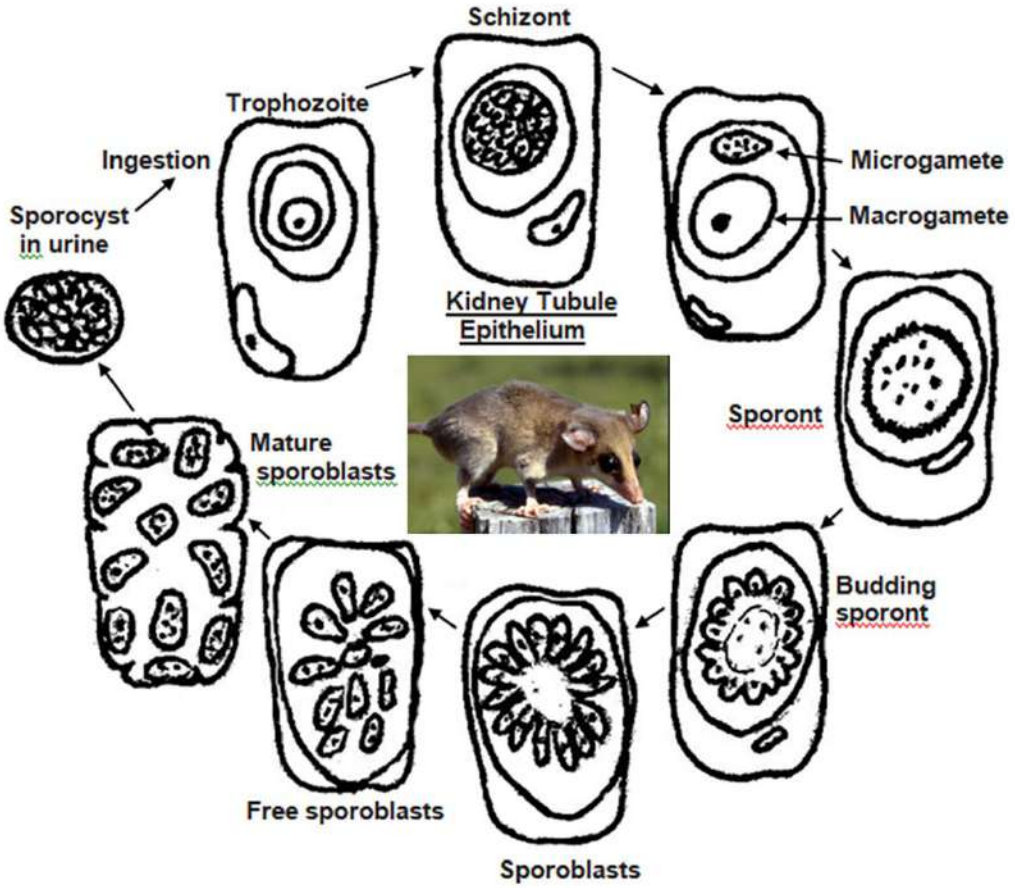


Figure 3. Diagrammatic drawing of the life cycle of *Klossiella tejerai* in opossums. Drawings by Duszynski. Photo by S. L. Gardner, 1993. License for all: CC BY-NC-SA 4.0.

the known developmental stages of this unusual coccidian. One example of a *Klossiella* species that infects the common opossum will suffice to illustrate this very interesting parasite family.

Genus *Klossiella* Smith and Johnson, 1902

Scorza et al. (1957) described *Klossiella tejerai* from a single common opossum, *Didelphis marsupialis* (Linneas, 1758), in Venezuela. To date, *K. tejerai* only has been found in 2 other instances: In 4 of 10 (40%) of *D. marsupialis* in

Box 2. A Cautionary Example — Learn More

Edgcomb et al. (1976) were among the first investigators to talk about pathological changes due to *Klossiella* species. They said, "Passage of schizonts (= meronts) and merozoites through the glomerular membranes occurs without inflammation and hemorrhage. These forms of the parasites evidently have membranes that permit their passage through the entire glomerular wall with restoration of the wall to an intact functional state after passage" (p. 316–317). This seems an odd interpretation from observing just a few tissue sections. It can be envisioned how merozoites can penetrate cell membranes, but not meronts. They went on to say, "The invasion of tubular epithelial cells by gametes, particularly by macrogametes, is associated with ballooning necrosis of the invaded cells" (p. 317). Spitz dos Santos et al. (2014) cautioned that Edgcomb et al. (1976) may have misinterpreted their photomicrographs.

Panama (Edgcomb et al., 1976) and in 1 of 20 (5%) in big-eared opossums, *Didelphis aurita*, from Brazil (Spitz dos Santos, 2014). It is surmised that both asexual (merogony) and sexual (gamogony) stages are found within epithelial cells of the kidneys and associated ducts and tubules. The life cycle is direct (Figure 3) with very large oocysts, 72 × 47 (57–103 × 36–57) µm, that are irregular in shape, sporulation (sporogony) is endogenous producing 12–30 sporocysts, 20.4 × 12.7 (19–22 × 12–14) µm, each with 8–20 sporozoites.

Box 3. *Klossiella* Species — Study It

Clearly, studying *Klossiella* species in the kidneys of vertebrates is an area ripe with potential rewards for new information. Parasitologists should begin to incorporate collecting urine into their field protocols to gain a sense of what oocysts and sporocysts of *Klossiella* really look like, and what variation can exist among species. Collecting kidney and related tissue samples for squash preparations/smears to be stained, and blocks of kidney to be fixed, embedded, sectioned, and prepared for histological examination (light microscopy (LM), transmission electron microscopy (TEM), or scanning electron microscopy (SEM)) will be critical. It will be an innovative milestone when someone finally infects several specimens of a vertebrate species with *Klossiella* oocysts/sporocysts, and then traces the sequential development over time of a complete life cycle within their kidneys. And, of course, it is imperative that DNA be collected and sequenced to gain an exact sense of the nature and affinity of these very interesting parasites—about which so little is known—to other species groups of the Apicomplexa. There are certainly a vast number of potential and obvious research projects available within this system to explore and problems to solve. This presents a wonderful opportunity, especially for graduate students who are teaching, to recruit undergraduates to help them with both field and lab work.

Eimeriorina Léger, 1911

Eimeriidae Minchin, 1903

The Eimeriorina contains species that all undergo merogony (asexual), gamogony (sexual), and sporogony (spore formation) during their life cycle. Members of the Eimeriidae all are homoxenous (direct life cycle), with merogony, gamogony, and the formation of oocysts occurring within the same host. Oocysts then leave the host, via the feces, and usually are unsporulated (= undeveloped, non-infective), but with a few exceptions (*Choleoeimeria*). The development of a genetically determined number of sporocysts and sporozoites within each oocyst occurs outside the host if/when environmental conditions (oxygen, moisture, and temperature) are appropriate (see Figure 4).

Genera *Acroeimeria* Paperna and Landsberg, 1989 and *Choleoeimeria* Paperna and Landsberg, 1989 (Figure 4)

The sporulated oocysts of *Acroeimeria* and *Choleoeimeria* are similar to those of *Eimeria* (see below) in that they all possess 4 sporocysts, each with 2 sporozoites, but all of their sporocysts lack a Stieda body, while most eimerians produce sporocysts with a Stieda body. Bovee and Telford (1965, page 93) were the first to see a possible relationship between the shape of lizard *Eimeria* spp. oocysts and their site of infection when they wrote,

> *Eimeria* spp. of lizards which form nearly spherical or elliptical oocysts with index 1.4 or less are inhabitants of the small intestine (if site of infection is known). Those of greater index, that is, long-ellipsoid or cylindrical form, are parasites of the

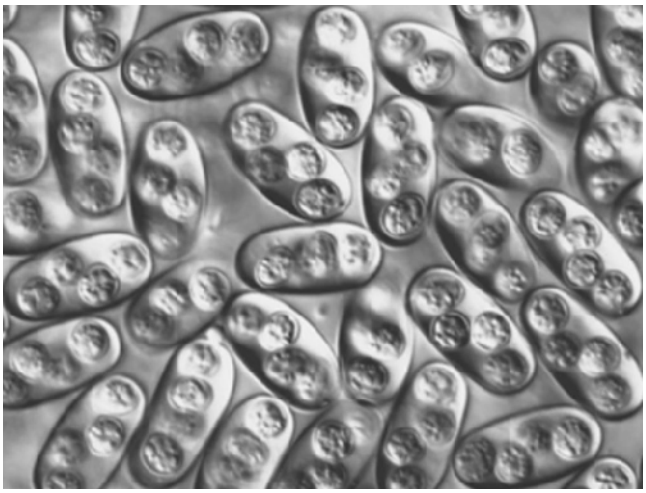
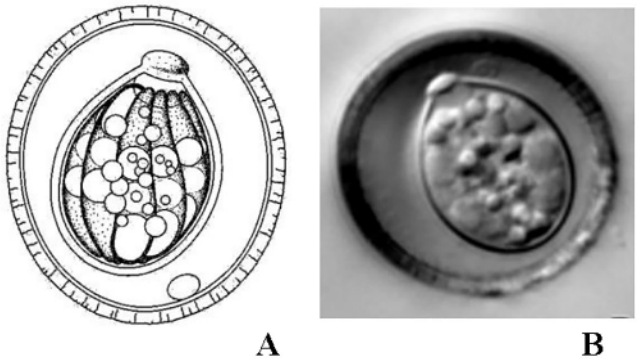


Figure 4. Elongate-ellipsoidal sporulated oocysts of a *Choleoeimeria* species in the bile duct and gallbladder from a colubrid snake, *Masticophis flagellum* from Texas. Original photomicrograph, Duszynski and Upton, 2010. License: CC BY-NC-SA 4.0.

biliary tract and particularly the gall bladder. The significance of this size-shape relationship to site is unknown.

Three decades later, Paperna and Landsberg (1989) reexamined the relationship between location of endogenous development of *Eimeria* spp. in geckos, their shape, and sporocyst structures. To accommodate their observations, they erected 2 new genera, *Acroeimeria* and *Choleoeimeria*. Both of their new genera had the general characteristic of the Eimeriidae (above). However, they defined *Acroeimeria* to have round or ovoidal oocysts with a length/width (L/W) ratio < 1.8, and all of their endogenous development (meronts, gamonts) "at the microvillous zone of the host cell and enclosed in the host cell microvillous boundary, causing the host cell to extend above the intestinal mucosal surface," and sporulation was exogenous. *Choleoeimeria* was defined to have cylindroid to ovoidal oocysts with a L/W ratio always > 1.4 (up to 2.2), endogenous development (meronts, gamonts) in the gallbladder (as far as was known then), development that induced hypertrophy and displacement of epithelial host cells above their original cellular layer, and sporulation was endogenous in the gallbladder and gut lumen. Thus, *Acroeimeria* sporulated oocysts looked like *Eimeria* oocysts, but their endogenous developmental processes were unique.

Localization of endogenous development in the microvillous zone of intestinal epithelial cells was described earlier in fish eimerians by Dyková and Lom (1981) who proposed a new genus, *Epieimeria*, to accommodate these presumably epicytoplasmic piscine eimerians. However, Benajiba et al. (1994) noticed that *Epieimeria* showed both epicytoplasmic and intracytoplasmic endogenous development, and Paperna (1991), using TEM showed both epicytoplasmic and intracytoplasmic endogenous stages that develop within a parasitophorous vacuole, which makes them only intracytoplasmic. Thus, epicytoplasmic endogenous development did not occur in fish *Epieimeria* and this urged Benajiba et al. (1994) to suppress the genus name and reassign all *Epieimeria* species back to the genus *Eimeria.* Several years later, Lainson and Paperna (1999), for unexplained reasons, changed their original definition of *Acroeimeria* slightly by stating that it, "Develops immediately beneath the brush-boarder of the intestinal epithelial cell" (page 151), and this begs the question, whether or not *Acroeimeria*, as defined to have only epicytoplasmic endogenous stages (Paperna and Landsberg, 1989), also should be suppressed. We now know that some species of *Acroeimeria* exhibit both epicytoplasmic (typical of *Acroeimeria*) and intracytoplasmic (typical of *Eimeria*) endogenous development (unpublished data), a situation very similar to the piscine *Epieimeria* story. No one yet has done a careful molecular characterization of *Acroeimeria* versus other coccidians in saurian species.



Figures 5A–B. A) Line drawing of the sporulated oocyst of *Caryospora duszynskii*. B) Photomicrograph of a sporulated oocyst of *C. duszynskii*. Both Figures from a colubrid snake, in Duszynski and Upton, 2010. License: CC BY-NC-SA 4.0.

The *Choleoeimeria* story is much simpler. Development of these species in the gallbladder and associated ducts, with the production of elongate-ellipsoidal or cylindroidal oocysts (Figure 4) that mostly sporulate endogenously seem to be conditions accepted by most of those who study lizard parasites, so far. Assigning species to *Acroeimeria* is more difficult and requires studying not only oocyst morphology, but structural information must be supported by information on the location and stages of endogenous development, and (multiple) gene sequencing whenever possible. We suggest as a practical matter, that unless information on endogenous development and/or partial gene sequence can be obtained to support morphology of saurian coccidia recovered from the feces, the species names used should almost automatically be placed in the *Eimeria*.

Genus *Caryospora* Léger, 1904 (Figures 5A–D)

The *Caryospora* genus is a really intriguing group of apicomplexan parasites. *Caryospora* species are mostly parasites of reptiles (predominantly snakes, but also lizards, turtles) and birds, and 1 species has been reported from mammals. Species assigned to this genus have 2 unusual features. First, their sporulated oocysts (Figures 5A–B) have only 1 sporocyst, which contains 8 sporozoites. Interestingly, those species described from reptiles almost always have a prominent Stieda body/substieda body complex while most of those caryosporans described from birds do not have a Stieda body/substieda body at the more pointed end. Although some species in this genus utilize life cycles similar to *Eimeria* and *Isospora* species, the second unique feature is that several *Caryospora* species from snakes are facultatively

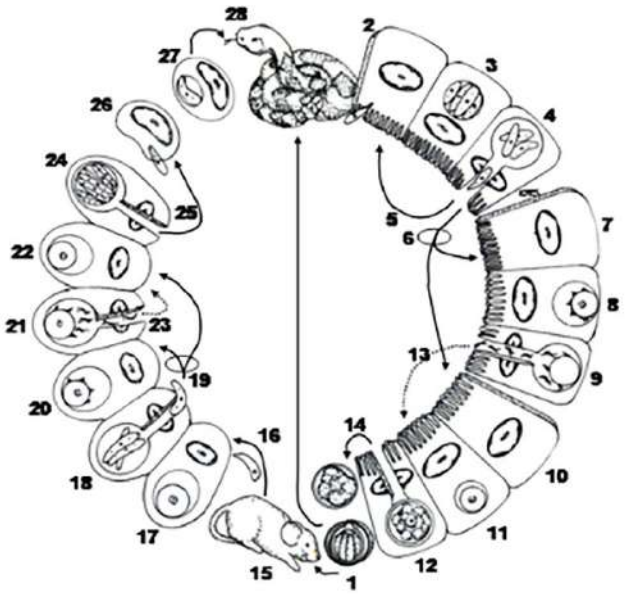


Figure 5C. Life cycle of a *Caryospora* species with both direct and facultatively heteroxenous life cycle components. 1) Typical sporulated oocyst which may be ingested by the snake definitive host in which case the parasite will undergo an enteric life cycle similar to *Eimeria* and *Isospora* species. 2) Sporozoites excyst in the intestine, penetrate epithelial cells, and then form meronts (3) with merozoites that rupture from the host cell (4). These may invade other epithelial cells to undergo several merogonous stages (5), or they may penetrate epithelial cells to produce micro- (6–9) or macrogametocytes (10–12), also typical of enteric coccidia. After fertilization (13) an unsporulated oocyst is formed which then ruptures from the host cell and is shed in the feces of the snake (14). Rodents are the typical secondary host for the facultatively heteroxenous part of the life cycle. When sporulated oocysts are ingested by a rodent (15), sporozoites excyst in the intestine, cross the gut wall, and become disseminated throughout the dermal tissues of the host, probably via the bloodstream. (16) In these cells, at least 2 asexual generations occur (17–19) followed by the sexual stages (20–22). Following fertilization, thin-walled oocysts are formed (24) and 8 sporozoites develop within a membrane, in the absence of a true sporocyst. These rupture from the host cell containing the oocyst, enter macrophages and/or fibroblasts, and are termed caryocysts (25–27). When eaten by the snake host (28), sporozoites are released from caryocysts and development continues in a manner thought to be identical to that known to occur when sporulated oocysts are ingested. Original Figure from Duszynski and Upton, 2010. License: CC BY-NC-SA 4.0.

heteroxenous. In this type of life cycle, both an enteric phase in a snake host and a non-intestinal phase in rodents have been described (Figure 5C).

After asexual and sexual multiplication in snake intestinal epithelial cells, typical of that known for other enteric coccidia, unsporulated oocysts are passed in the feces, but

Figure 5D. Photomicrograph of experimentally-infected (left) and control (right) laboratory mice showing the swelling of dermal tissue about 12 days post-infection with 250,000 sporulated oocysts of *Caryospora simplex*. Source: Duszynski and Upton, 2010. License: CC BY-NC-SA 4.0.

patency may last for months, or even a couple of years; this suggests that either some enteric recycling of asexual stages is occurring or that oocysts are retained deep within host tissues for an unusually long period of time before being released. But the most interesting aspect of the life cycle is what occurs in non-reptile hosts. Rodents are thought to be typical secondary hosts for the non-intestinal phase. Oocysts they ingest undergo excystation, sporozoites cross the gut wall and then become disseminated throughout the dermal tissues. Here, at least 2 asexual generations occur followed by sexual stages. The tissues around the face and neck become edematous (swollen) at this time (Figure 5D). Following fertilization, thin-walled oocysts are formed and 8 sporozoites develop within a membrane, but not a true sporocyst wall. These sporozoites rupture through the thin oocyst wall and enter macrophages and fibroblasts where they become dormant. These modified host cells with dormant sporozoites are termed caryocysts. When eaten by the appropriate snake, sporozoites are liberated from caryocysts and development proceeds in a manner thought to be identical to that which occurs when oocysts are ingested. Although at first glance this type of life cycle may seem unusually complicated, in reality, most developmental stages occurring within the mammalian host appear identical to those occurring in snakes.

Genera *Cyclospora* Schneider, 1881, *Eimeria* Schneider, 1875, and *Isospora* Schneider, 1881 (sensu stricto)

These 3 genera (Figures 6A–F) are considered together because they have mostly identical life cycles, as illustrated in the *Eimeria* cycle shown in Figure 6A. They differ only in the final morphology of their sporulated oocysts. After sporulation, eimerian oocysts have 4 sporocysts, each containing 2 sporozoites (Figures 6B and 6F–G), cyclosporan oocysts

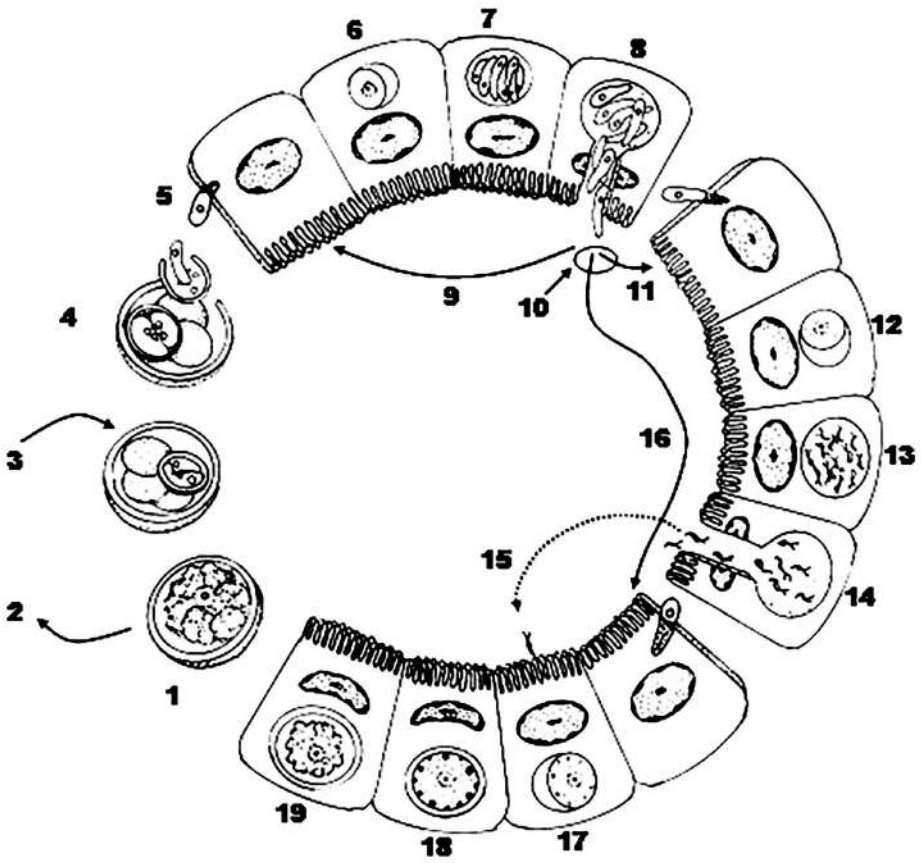
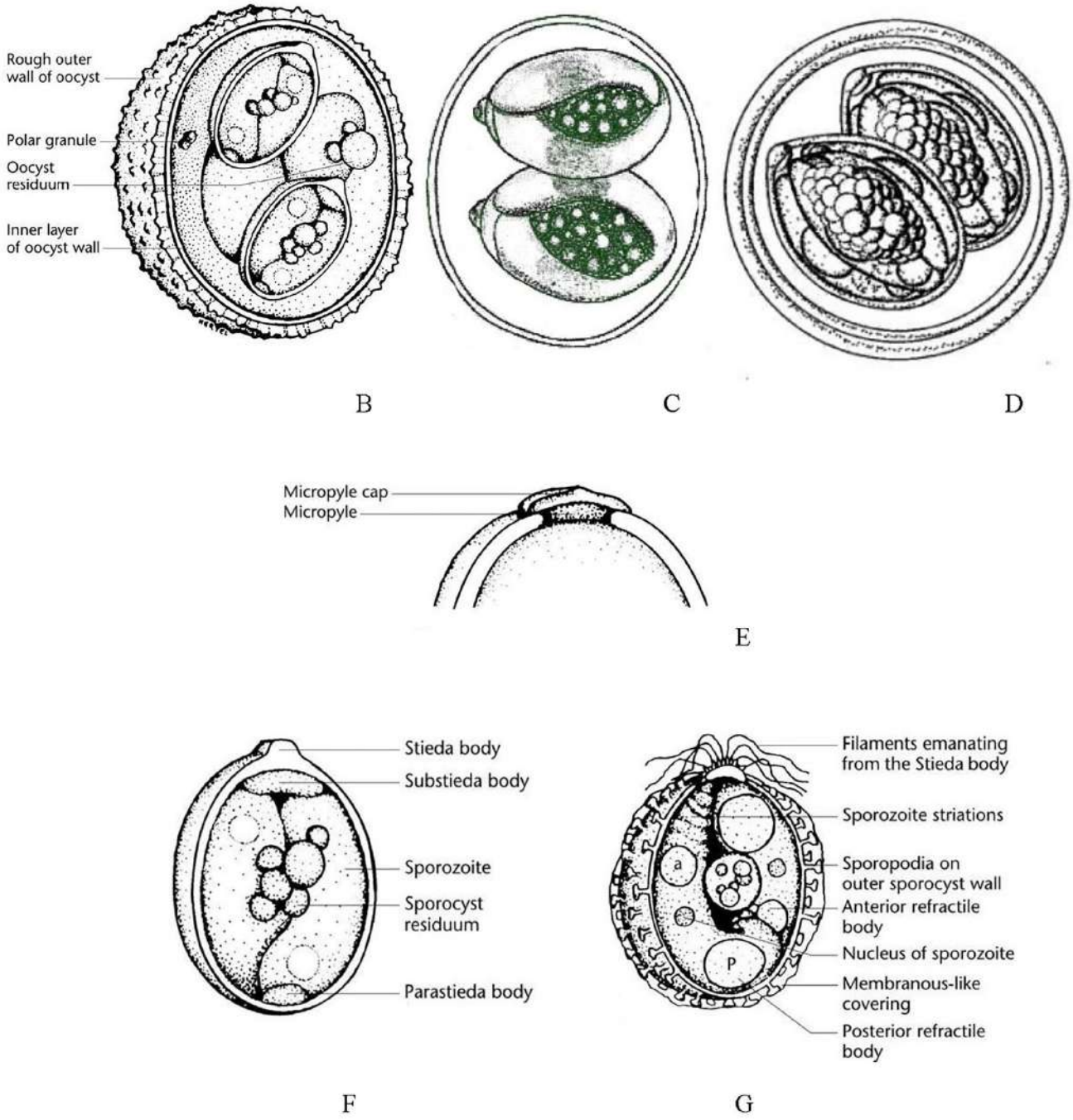


Figure 6A. Homoxenous life cycle of *Eimeria* species with a direct life cycle (*Cyclospora*, *Isospora* species have similar cycles). 1) Unsporulated oocyst leaves the host in its feces. 2) Oocyst needs molecular oxygen, moisture, and a temperature different than the host's body temperature to sporulate. During sporulation, 4 sporocysts, each with 2 sporozoites are formed. 3) Sporulated oocyst is infective to the next host. 4) Sporozoites are released from sporocysts/oocysts in host's gut. 5) Sporozoites penetrate host epithelial cells (6) then round up, enclosed in a parasitophorous vacuole to begin merogony. 7) Meront contains several to hundreds to thousands of merozoites. 8) Merozoites destroy host cell and may infect other cells (9) to produce more merogonous stages or (10) last generation of merozoites penetrate enterocytes to begin gamogony. 11) Microgametogony: the merozoite rounds up (12), many bi-flagellated microgametes are produced (13), rupture from their cell (14) and find a host cell with a developing macrogamont (15). 16) Macrogametogony: merozoite rounds up, producing a young macrogamete. After the microgamete penetrates the host cell (15) and fertilizes the macrogamete a young zygote is produced (17). Soon after, wall forming bodies (18) migrate to periphery of cell where they eventually coalesce to form the resistant oocyst wall; once wall is formed and the sporoplasm condenses, (19) the unsporulated oocyst ruptures from the host epithelial cell (1) to be discharged from the host in its feces. Source: Duszynski and Upton. License: CC BY-NC-SA 4.0.

have 2 sporocysts each containing 2 sporozoites (Figure 6C), and isosporan oocysts have 2 sporocysts each containing 4 sporozoites (Figure 6D). Numerous variations may be seen in different species on the surface structures of the oocyst and sporocyst walls (Figures 6B–G).

It is likely that an *Eimeria* species was 1 of the first protozoa visualized when Antonie van Leeuwenhoek saw what surely were oocysts of *Eimeria stiedai* Lindemann, 1895 in the bile of a rabbit in 1674. Since the oocyst is the stage that leaves the host, usually in the feces, it is the structure in the life cycle that is readily available to the veterinarian, wildlife biologist, or parasitologist who needs to identify the species without having to kill the host. As a result, about 98% of all *Eimeria*, *Isospora*, and *Cyclospora* species are known only from this 1 life cycle stage, the sporulated oocyst. *Eimeria*, with perhaps 2,000 named species to date, is the largest apicomplexan genus and may be the most speciose genus of all parasite genera (see Figures 7 and 8), and *Isospora* has about 250 named species; both have been reported in amphibians, reptiles, mammals, and birds and many *Eimeria* species (but not *Isospora*) have been reported in fishes. Fewer than 20 *Cyclospora* species have been named to date, most in mammals



Figures 6B–G. Line drawings of oocyst and sporocyst structures. B) Typical *Eimeria* oocyst, 4 sporocysts, each with 2 sporozoites. C) *Cyclospora* oocyst, 2 sporocysts, each with 2 sporozoites. D) *Isospora* oocyst, 2 sporocysts, each with 4 sporozoites. E) One end of an oocyst with a smooth outer surface and showing other possible structures, a micropyle and micropyle cap. F) Sporulated sporocyst showing major structural features including 2 sporozoites and the Stieda body/substieda body complex. G) Another sporulated sporocyst showing a variety of structural features, some of which may be present on sporocysts of different species. Source of all images: Duszynski and Upton, 2010. License: CC BY-NC-SA 4.0.

(insectivores, rodents, and primates) and a few in arthropods and reptiles. This genus is best known for 1 species, *Cyclospora cayetanensis* Ortega, Gilman and Sterling, 1994, a pathogenic coccidium transmitted by fecal contamination of food (fruits and vegetables) and water, that can cause diarrhea in humans and other primates.

The complete life cycle stages of a typical *Eimeria* species are shown in Figure 6A (see the figure legend for details) and

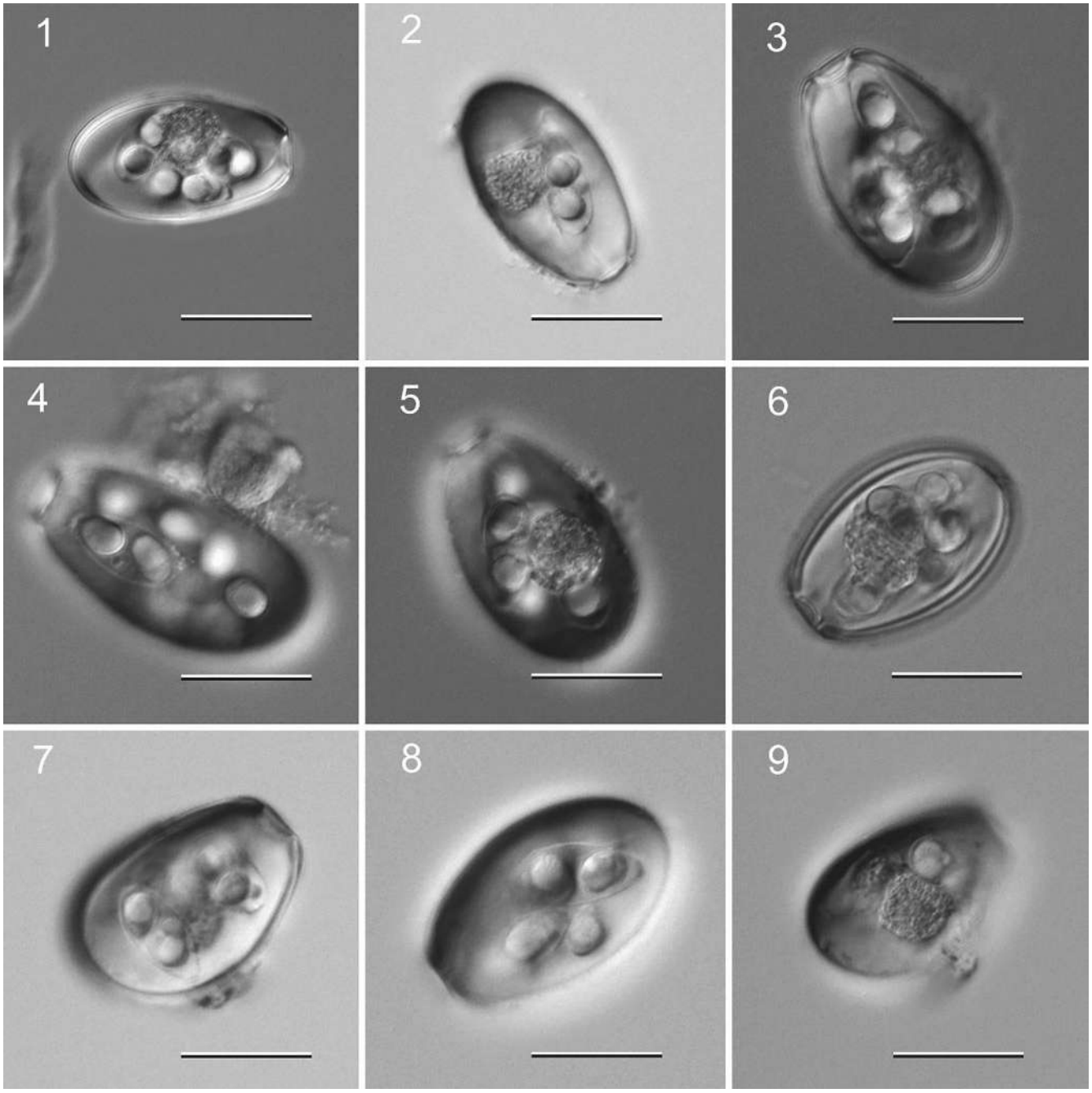


Figure 7. Examples of species of *Eimeria* from a Mongolian hare *Lepus tolai* from Mongolia. Scale bar = 25 μm. Source: S. L. Gardner, HWML. License: CC BY.

similar life histories are used by *Isospora* and *Cyclospora* species. To reiterate briefly, after a sporulated oocyst is ingested by a suitable host, sporozoites excyst and do so by both mechanical (via muscular contractions) and enzymatic (via trypsin or bile salts) digestive processes of the upper gastrointestinal tract in their host. These make the sporocyst and oocyst walls more permeable. Eventually, certain parts of each may be digested, or they may collapse or are broken, releasing their sporozoites so they can penetrate host epithelial cells. Invasion of the host cell is complicated, involving a sequential series of steps including recognition of a host cell, attachment to surface components, formation of a tight junction, entry into the cell (facilitated by organelles of the apical complex), and formation of a parasitophorous vacuole (PV) around the sporozoite (Sam-Yellowe, 1996). Inside its PV, the sporozoite initiates merogony (that is, asexual multiple fission).

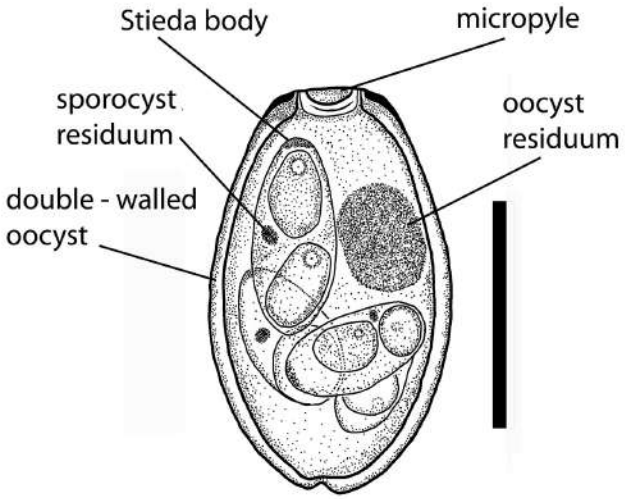


Figure 8. An oocyst of *Eimeria gobiensis* from a Mongolian hare *Lepus tolai* in Mongolia. Source: S. L. Gardner, HWML, 2009. License: CC BY.

During merogony, as few as 2, or up to as many as 100,000, merozoites may be formed by each sporozoite, depending on the species. Once mature, merozoites rupture the host cell, each seeking to penetrate a new epithelial cell to begin merogony again. It is believed that each species is genetically programmed for a specific number of merogonous generations. This was first demonstrated by Levine (1940). In this classic paper, he transferred merozoites of *Eimeria necatrix* from the intestine of 1 chicken to a second, coccidia-free chicken and showed that the time required for development of oocysts in the second bird was equal to that required in a single host, thus, showing that the length of the life cycle was determined not by increasing resistance of the host, but was inherent in each species of *Eimeria*. For the few coccidia species of which we know the actual number of asexual generations, it most often varies from 2 to 4 generations. Whatever the number, tremendous biological magnification of the parasite results from these developmental stages.

When the last generation of merozoites enter host epithelial cells, they develop not into additional meronts, but into gamonts. The vast majority develop into macrogametocytes (macrogamonts) to form uninucleate macrogametes, whereas the remaining merozoites develop into microgametocytes, each of which will undergo multiple fissions to produce thousands of motile, flagellated microgametes, but the precise mechanism that regulates if and when a merozoite will become a macrogamete or microgamete is unknown. Microgametes all are similar in structure with an elongate nucleus, an equally elongate mitochondrion, and 2 or 3 flagella (Scholtyseck, 1979). The nucleus occupies most of the space in the microgamete, which averages 4–7 mm-long. The elongate mitochondrion, about 2–5 mm-long, lies closely adjacent to and often in a groove of the nucleus. When they are mature, microgametes exit their host cell to seek out and penetrate cells with a mature macrogamete, but how microgametes find cells with developed macrogametes inside them, and details of the fertilization process, are unknown and warrant further study. When fertilization does occur, the diploid (2n) condition is restored. Thus, infections with these 3 genera are self-limiting as asexual reproduction does not continue indefinitely.

Family Sarcocystidae Poche, 1913

A second major family in the Eimeriorina, Sarcocystidae Poche, 1913, has 3 subfamilies, Cystoisosporinae Frenkel et al., 1987, Sarcocystinae Poche, 1913, and Toxoplasmatinae Biocca, 1957. All have *Isospora*-like oocysts in their life cycles with 2 sporocysts, each containing 4 sporozoites, but none of the sporocysts ever have a Stieda body. Instead, their sporocysts have longitudinal sutures that divide the surface into 4 or more plates.

Subfamily Cystoisosporinae Frenkel et al., 1987.

Frenkel et al. (1987) noted, “How we classify the heretofore unthought of cycles and stages is a scientific problem of taxonomy rather than of nomenclature” (page 250). Their new taxonomic ideas on these genera with heteroxenous life cycles reflects on the reproductive and transmission strategies of the parasites while maintaining the nomenclature. For this reason, they created 3 separate taxonomic concepts (subfamilies) for the isosporid coccidia without Stieda bodies in the interest of stability, uniqueness, and distinction.

Genus *Cystoisospora* Frenkel, 1977. Frenkel (1977, pages 620 and 625) erected the genus *Cystoisospora* to include those mammalian *Isospora* species with no Stieda body complex in their sporocysts, and with the ability to produce unique monozoic tissue cysts (MZTC) in intermediate or paratenic hosts, and these MZTC stages are a defining character of *Cystoisospora* species. Earlier, Frenkel and Dubey (1972) discovered the occurrence of tissue cyst stages of 2 intestinal coccidia of cats, *C. felis* and *C. rivolta*, in rodent paratenic hosts. They (1972) and others (Dubey, 1975; 1978a; 1978b; Rommel and Zielasko, 1981) also demonstrated that extraintestinal (EIN) stages can occur in the tissues (mesenteric lymph nodes, liver, spleen, lungs, brain, and musculature) of cats and dogs (which are definitive hosts) when fed sporulated oocysts of *C. felis* and *C. rivolta*, or *C. canis*, respectively. When either sporulated oocysts or infected intermediate hosts are ingested, these

parasites undergo merogony and gamogony in the intestinal epithelial cells of the carnivore definitive host and, ultimately, they discharge **un**sporulated oocysts with relatively thick walls (for example, *C. felis* and *C. rivolta* of felids; *C. canis, C. ohioensis*, and *C. vulpina* of canids). Thus, oocysts of *Cystoisospora* species look identical to *Isospora* species except for their ability to infect additional host species (Fayer and Dubey, 1987).

Subfamily Sarcocystinae Poche, 1913.

This is the second subfamily within the Sarcocystidae Poche, 1913 and contains 2 genera (*Sarcocystis* and *Frenkelia*). Votýpka et al. (1998), Modrý et al. (2004), and others consider *Sarcocystis* and *Frenkelia* as synonyms.

Genus *Sarcocystis* Lancaster, 1882. Miescher (1843) was the first to see what he called milky white threads (which were actually sarcocysts) in the skeletal muscles of a house mouse in Switzerland, and Huet (1882) saw the first sarcocysts in the muscles of a carnivore, a sea lion that died in the Jardin des Plantes de Paris, France. Lankester (1882) introduced the genus name for these Miescher's tubules to reflect what he saw, muscle (in Greek, **sarco** means flesh or muscle) and cyst (in Greek, **cyst** means bladder or bag), and

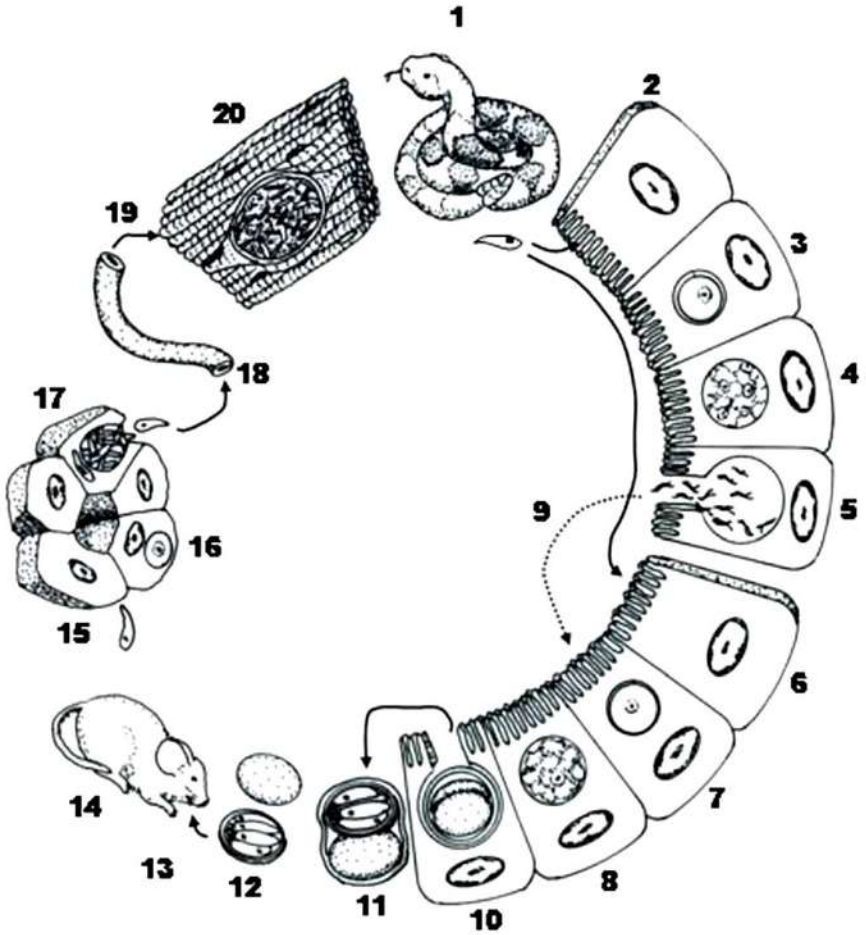


Figure 9. Typical life cycle of a *Sarcocystis* species with its obligate indirect life cycle. 1) Definitive host ingests infected prey items with sarcocysts in their tissues, bradyzoites are released and penetrate enterocytes of small intestine (2) where they develop directly into micro- (3–5) or macrogametocytes (6–8). After fertilization (5–7), sporogony occurs (8) in the lamina propria and sporulated oocysts are slowly released into the gut lumen fully formed and infective. (10) Oocyst wall is thin, it often ruptures during transit down the intestinal tract releasing 2 sporocysts in the feces, each with 4 sporozoites, rather than intact *Isospora*-type oocysts. 11) When oocysts and/or sporocysts are ingested by intermediate hosts, excystation occurs in the small intestine, sporozoites penetrate gut wall and enter a variety of extraintestinal tissues (12–14). 15–18) Precystic merogony usually occurs in tissues and merozoites from the last generation to enter the blood and are carried to striated muscles throughout body where they become bradyzoites and initiate sarcocyst formation. 19) Sarcocysts, with thousands of infective bradyzoites (20), are infective to the definitive host when it ingests an infected prey animal. Source: Duszynski and Upton, 2010. License: CC BY-NC-SA 4.0.

Blanchard (1885) named the organism *Miescheria hueti*. Finally, Labbé (1899) transferred this parasite to the genus *Sarcocystis*. The seminal work by Fayer (1970; 1972) first reported the transformation of bradyzoites from muscle cysts in grackles (*Quiscalus quiscula*) into gametocytes and oocysts in cell culture, and this was soon followed by Rommel et al. (1972) who described the shedding of sporulated sporocysts from cats after they ingested sarcocyst-infected mutton (also known as *Sarcocystis tenella*). Thus, the life cycle of all *Sarcocystis* species is now known to be an obligate, indirect cycle in which the definitive host is a carnivore in which only gametogony occurs, with the release of thin-walled sporulated oocysts or individual infective sporocysts; these stages must be ingested by a suitable intermediate host, in which tissue sarcocysts develop, and only these sarcocysts are infective for the definitive host (but not oocysts/sporocysts) (Figure 9).

Dubey et al. (2015) published an extensive treatise on *Sarcocystis* species in humans and other animals and listed 195 names as valid (Table 24.1 in Dubey et al., 2015), 49 *Sarcocystis* species names as invalid (Table 24.2 in Dubey et al., 2015), and 83 names (*Sarcocystis* sp.) that have never received a binomial. Students, and all interested readers, in all disciplines, should use these references when interested in maximizing *Sarcocystis* species data for any particular host species group.

Box 4. *Sarcocystis muris* Transmission — Learn More

Smith and Frenkel (1978) found sarcocysts in skeletal muscles of some lab mice housed in the same room as cats that had shed sporulated sporocysts of *Sarcocystis muris*. They noted that cat feces never came in proximity with mouse cages, but they saw German cockroaches (*Blatella germanica*) in the same room from time to time. To assess the role of *B. germanica* and the American cockroach (*Periplaneta americana*) in transmission, cockroaches were exposed to cat feces that contained oocysts/sporocysts of *S. muris, Isospora felis,* and *Toxoplasma gondii*. They found that *S. muris* sporocysts, which remained infectious in cat feces for at least 20 days, were transmitted to mice by *P. americana* for at least 20 days, and by *B. germanica* for 5 days post-exposure to infected cat feces.

Subfamily Toxoplasmatinae Biocca, 1957.

There are 3 important genera within this third subfamily, Toxoplasmatinae, which need to be mentioned. All of them have somewhat unusual complicated life histories and all of them have *Isospora*-type oocysts, but they are small and their sporocysts do not have Stieda bodies. An overview of each genus is covered below.

Genus *Besnoitia* Henry, 1913. Darling (1910), in Panama, found unusual cysts in an opossum, *Didelphis marsupialis*, and thought the parasite was a species of *Sarcocystis*, even though he expressed concern with some of the features in his cysts from the defining characteristics of the genus. Besnoit and Robin (1912), in France, found a protozoan that caused cutaneous and internal lesions in cattle associated with subspheroidal cysts. They also tentatively referred to their organism as *Sarcocystis*, but did not propose a binomial. Marotel (1912), unaware of Darling's (1910) paper, discussed Besnoit and Robin's (1912) work, and wrote, "Nothing similar has been found in animals ... and this is why I propose to designate their parasite with the name *Sarcocystis besnoiti*" (Jellison, 1956). The next year, Henry (1913) reexamined the characteristics of the organism, and the nomenclature assigned to it, and used the genus name *Besnoitia*.

Besnoitia species are obligatory heteroxenous coccidia, similar to those of *Sarcocystis* species, but they differ from *Sarcocystis* in 2 unique ways: 1) Oocysts are shed unsporulated by their definitive hosts and have relatively thick walls; and 2) these species can be successfully propagated asexually by mechanical transmission from intermediate host to intermediate host by blood-sucking arthropods. Their life cycles are similar to those of *Sarcocystis* species because the completion of the sexual cycle in the definitive host is dependent upon ingestion of tissue cysts from a suitable intermediate host—that is, the ability of oocysts to initiate gametogenesis in the definitive host also has been lost. Other details of what little is known about the life cycle of various *Besnoitia* species are summarized elsewhere (Leighton and Gajadhar, 2001; Dubey et al., 2003; Houk et al., 2011; Charles et al., 2011; and Duszynski and Couch, 2013). *Besnoitia* is the fifth apicomplexan to be a mammalian tissue parasite, along with *Cystoisospora, Hammondia, Sarcocystis,* and *Toxoplasma*. There are now approximately 10 valid species in this genus; the definitive host, which is a carnivore, is only known for 4 or 5 of these species and it is the domestic cat (*Felis silvestris catus*).

Genus *Neospora* Dubey et al., 1988. In 1984, a neuromuscular syndrome in dogs that simulated toxoplasmosis was documented by 3 Norwegian veterinarians (Bjerkås et al., 1984), who reported a protozoan causing severe encephalomyelitis in 6 Norwegian pups, but which had no antibodies to *Toxoplasma gondii*. All dogs originated from 3 litters from a single Boxer female. The pups appeared healthy until 2 months old. Five of these pups had neurological signs for several months, and all 6 were examined at necropsy and diagnosed with encephalitis and myositis with protozoa found in the lesions, including numerous tachyzoites and a few tissue cysts in their brains. Ultrastructural examination of tachyzoites showed them to be similar to those of *T. gondii*, but with more rhoptries. This confirmed the vertical transmission of this new, unnamed protozoan parasite.

Dubey et al. (1988) examined tissue sections and case histories from all dogs and cats that had died of a *Toxoplasma gondii*-like illness from 1952 to 1987 and were archived at the Angell Memorial Animal Hospital (AMAH), Boston, Massachusetts, the largest hospital for dogs and cats in the United States, which keeps meticulous records of pathology cases. Together, they examined thousands of slides from dogs and cats, and concluded that the syndrome recognized by Bjerkås et al. (1984) was not toxoplasmosis (see the review in Dubey et al., 2017). The records also showed that, in addition to neuromuscular clinical signs, dogs suffered severe disease involving the heart, lungs, liver, and the skin. Dubey et al. (1988) found a similar parasite in formalin-fixed tissues from 10 dogs in the United States, named a new genus, *Neospora*. *Neospora caninum* Dubey et al., 1988 later became the type species.

A decade later, McAllister et al. (1998) firmly established dogs as the definitive host of *Neospora caninum* and their genus definition included: 1) Tissue cysts in several cell types, but primarily in the neural tissues; 2) a tissue cyst wall up to 4 μm thick, much thicker than *Toxoplasma gondii* tissue cysts (~ 0.5 μm); 3) numerous bradyzoites, not separated by septa; 4) tachyzoites with numerous electron-dense rhoptries, some posterior to the nucleus; 5) canids (dog, coyote, and wolf) as definitive hosts and many intermediate hosts, including dogs, cattle, horses, goats, deer, water buffaloes, coyotes, red foxes, and camels (see also Dubey, 1999; Lindsay and Dubey, 2000); 6) tachyzoites and tissue cysts in both intermediate and definitive hosts; 7) oocysts excreted unsporulated; 8) antibodies to *T. gondii* not present in infected dogs and the parasites not reacting to *T. gondii* antibodies in immunohistochemical tests; 9) transmission by carnivorism, transplacental and fecal; and 10) tachyzoites, tissue cysts, and oocysts all infectious to both intermediate and definitive hosts.

Box 5. Neosporosis — Learn More

Considerable progress in understanding the biology of neosporosis has been made in the last 30+ years, re-sulting in more than 2,000 scientific publications! For the interested reader, Dubey et al. (2017) have written a comprehensive, well-organized, easily-read book on this subject.

Genus *Toxoplasma* Nicolle and Manceaux, 1909. There may be several *Toxoplasma* species in poikilotherms (Duszynski and Upton, 2010), but most parasitologists who work in this area believe there is only 1 species, *T. gondii*, in mammals, and it has worldwide distribution. Prior to the early 1970s it was thought that *T. gondii* might be transmitted by blood sucking arthropods, but it is now known that felids are the definitive host. Clinical toxoplasmosis has been reported in virtually all species of warm-blooded animals, including humans, and domestic and wild animals (Dubey and Beattie, 1988; Dubey, 2010). In fact, *T. gondii* may be the most ubiquitous parasite on Earth because it can be transmitted directly (fecal/oral, including using arthropods as mechanical vectors), transplacentally, and by carnivorism (see Chapter 8, p. 133–140 in Duszynski, 2016 for a brief review).

Toxoplasma gondii has an indirect life cycle with only felids serving as definitive hosts in which the parasite goes through both asexual and sexual endogenous development in intestinal epithelial cells. All other vertebrate animals that ingest sporulated oocysts are susceptible to infection but, in them, *T. gondii* forms cysts in cells of virtually any tissue in the body. If these tissue cysts are eaten by another omnivore or non-felid carnivore, the process can be repeated, with the development of tissue cysts in the new host. When cats consume a host animal harboring mature tissue cysts, endogenous development in the gut can be initiated (depending upon the cat's immune status to *T. gondii* from a previous infection), and/or bradyzoites from the ingested cysts can go on to develop in the tissues of the cat, too.

Dubey and Frenkel (1972) outlined the sequence of events in the epithelial cells of cats inoculated orally with tissue cysts of *Toxoplasma gondii* and found 5 new structural stages they designated as types A–D. Interestingly, the feeding of each of the 3 principal *T. gondii* stages to cats results in different prepatent periods. If chronically-infected mice

(characterized by older tissue cysts with bradyzoites) are fed to cats, oocysts can be found in cat feces 3–5 days post-infection (dpi). Cats fed acutely-infected mice (characterized by young tissue cysts with tachyzoites) won't shed unsporulated oocysts until 5–10 dpi, and cats fed sporulated oocysts usually do not begin to shed oocysts until at least 20–24 dpi.

The mechanisms by which *Toxoplasma gondii* is transmitted in nature to maintain its ubiquity as an infectious agent still are not completely understood because they are so highly varied. Insects in nature can become infected and, if ingested by mammals or birds, insects may be important transport or paratenic hosts. Wallace (1971) demonstrated the potential of both *Musca domestica* (common house fly) and *Chrysomya megacephala* (latrine fly) to be able to transmit sporulated oocysts of *T. gondii* for at least 24 and 48 hours, respectively, and *Periplaneta americana* (American cockroach) and *Rhyparobia maderae* (Madeira cockroach) for up to 12 days post-infection. However, to be of practical interest, it needed to be determined that some of the more prevalent cockroaches were prone to ingest cat feces. Chinchilla and Ruiz (1976) worked with 3 of the most common cockroaches in Costa Rica, *P. americana, P. australasiae,* and *R. maderae*, by experimentally showing that both *Periplaneta* species ate cat feces even in the presence of common foods (for example, dough, sugar, bread, cheese) found in most Costa Rican homes, and that *R. maderae* showed the greatest tendency to ingest cat feces. Their results suggest that these insects are potential transport hosts for oocysts of *T. gondii* in cat feces. They also noted that these 3 cockroach species are the most common in city markets, where cats also abound. Also, Smith and Frenkel (1978) found *P. americana* and, to a lesser extent, German cockroaches (*Blatella germanica*), transmitted *T. gondii* oocysts to mice for up to 10 days post-exposure to infected cat feces.

Oocysts of *Toxoplasma gondii* also can last a long time in the external environment. Frenkel and Dubey (1973) determined that sporulated oocysts suffer little attrition after constant or intermittent freezing at −6 °C, but greater attrition at −21 °C, and that sporulated oocysts survive −20 °C for 28 days, indicating that freezing weather alone does not eliminate oocyst infectivity from soil contaminated by cat feces. Frenkel et al. (1975) looked at the effects of freezing and soil storage in Costa Rica and Kansas, United States. In Costa Rica, infectivity persisted for 1 year in 3 shaded sites, 2 moist sites, and 1 relatively dry site in the soil, and in Kansas infectivity lasted up to 18 months, including 2 winters. Frenkel et al. (1975) also recovered oocysts from the surface of 1 *Musca*, several soil isopods, and earthworms. Dubey (1998) looked at the survival of sporulated *T. gondii* oocysts under defined temperatures, and then tested their infectivity by mouse bioassay. There was no marked loss of infectivity of oocysts stored at 10-, 15-, 20-, and 25 °C for 200 days; oocysts stored at 35 °C were infective for 32, but not at 62 days, those at 40 °C were infective for 9, but not 28 days, those at 45 °C were infective for 1 day, but not for 2 days. Sporulated oocysts remained infective up to 54 months at 4 °C, and no loss of infectivity was seen in oocysts stored for 106 days at −5 °C and −10 °C, and for 13 months at 0 °C.

There have been thousands of surveys around the world looking for oocysts in cat feces and testing blood for antibodies in a variety of in vitro tests, and inspecting tissues for cysts in many other vertebrates, including many carnivores. Dubey (1976) pointed out that even though > 60% of cats in the United States and elsewhere have antibodies to *Toxoplasma gondii*, only about 1% or less are found to be shedding unsporulated oocysts at any given time. Weiss and Kim (2007) contributed a definitive textbook on the perspectives and methods of *T. gondii* as a model apicomplexan.

Cryptosporididae Tyzzer, 1907

The taxonomy of this group has changed considerably since it was discovered by Tyzzer (1907; 1910) because it possesses features of both coccidians and gregarines. It was initially classified with the Coccidia, but it was found later to be phylogenetically more closely related to Gregarinasina (Carreno et al., 1999; Barta and Thompson, 2006; Kuo et al., 2008). Currently, it is a distinct group of the Conoidasida, on equal status with the Coccidia and the Gregarinasina (Adl et al., 2012).

Genus *Cryptosporidium* Tyzzer, 1907

Formerly, *Cryptosporidium* was thought to be a monospecific genus (Tzipori et al., 1980; Tzipori and Campbell, 1981) because of its presumed lack of host specificity, nearly identical life cycle developmental stages (both exogenous and endogenous), and their shared antigenicity (see Figure 8). However, with the advent of gene sequencing and other molecular innovations that tease apart subtle genetic differences, it is now believed there may be at least 30 valid species, and > 50 genotypes, many of which may be mostly adapted to a narrow spectrum of hosts (Lucio-Forster et al., 2010; Osman et al., 2015; Lihua Xiao, personal communication). However, this area of study is still a work in progress and no definitive documentation exists yet regarding the exact number of *Cryptosporidium* species (Plutzer and Karanis, 2009; Fayer et al., 2010). Many isolates have been classified as "genotypes," without species definitions (Fayer, 2010) or binomials, and may simply represent cryptic species.

Cryptosporidium species are obligate, monoxenous, intracellular, but extracytoplasmic, parasites. They have been found to infect a wide variety of vertebrate species worldwide, including humans, their domestic food and companion animals, and many species of wild and laboratory animals. Since recognition of the seeming ubiquity of *Cryptosporidium* oocysts in host feces, searching for them has historically followed 2 paths. First, their oocysts are intentionally sought out in cases of chronic or acute clinical illness, especially in our domesticated and companion animals. Second, general, non-invasive surveys of larger sample sizes of various vertebrate populations have been conducted worldwide to determine prevalence. But prevalence studies rely principally on morphology of the oocysts found, and therein lies the problem. Oocysts are so small, nondescript, difficult to find, and they lack in mensural characters such that they are virtually impossible to use to identify species. At present, fecal flotation, several staining procedures, and immunofluorescence assays of fecal samples are the most commonly-used laboratory techniques for diagnosing *Cryptosporidium*. Thus, light microscopy (LM) is routinely used for diagnostics; however, it does not allow the identification of species because of the morphological uniformity of the oocysts. Moreover, LM suffers from low sensitivity, because the oocysts: 1) May be shed in small numbers, often under detectable levels; 2) are translucent and small (~ 4–7 μm-wide); and 3) may be confused with yeasts, fungal spores, and/or other structures in fecal samples. Thus, examination using LM requires a trained technician because the oocysts may be overlooked easily, or may be misdiagnosed, and lead to false-positive diagnoses. Moreover, since oocysts are shed intermittently, 1 negative fecal exam may not necessarily mean that the host individual is not parasitized. Therefore, repeated fecal exams should be undertaken when possible.

Oocysts already are sporulated when passed, thus immediately infective. They remain infective in the environment for a long period of time, are resistant to most common disinfectants, and also are able to survive routine wastewater treatment (Fayer et al., 2000; Ryan and Power, 2012). The most common environmental and alimentary sources of *Cryptosporidium* are water treatment facilities, raw sewage discharge, especially into rivers, wells, ditches, and oceans, where molluscs, oysters, and vegetables become exposed (Meireles, 2010). When sporulated oocysts have been ingested by a suitable host (Figure 10), the infection is usually self-limiting in immunocompetent individuals, but may become acute leading to morbidity and mortality in immunocompromised ones. Therefore, *Cryptosporidium* species can and do have a great influence on public health.

Cryptosporidium Diagnosis and Genotyping

Due to their small size, intermittent shedding, and limited morphological variation, only molecular and immunological methods can begin to tease apart the subtle sequence or genetic differences between *Cryptosporidium* species and genotypes. Of particular relevance in evaluating, detecting, resolving, and differentiating the identity of *Cryptosporidium* species, the following diagnostic and genotyping methods are particularly useful: Polymerase chain reaction (PCR), real-time PCR, nested PCR-RFLP, IMS-qPCR, qPCR-MCA assay, enzyme immunoassays, and sequencing of specific genes or regions (Feng et al., 2009; Gao et al., 2013; Homem et al., 2012; Jiang and Xiao, 2003; Lalonde et al., 2013; Leoni et al., 2006; Lindergard et al., 2003; Silva et al., 2013; Xiao et al., 2004). In the case of *Cryptosporidium* species, several markers or loci are now commonly employed to determine species or genotype differences including, but not limited to, partial and full sequences of 18S rRNA, *Cryptosporidium* oocyst wall protein (COWP), 70 kDa heat shock protein (Hsp70), glycoprotein 60 (gp60), and actin genes, with partial 18S rRNA gene sequences being the most commonly used marker. Clearly, combining as many of these techniques as possible is much more sensitive in detecting *Cryptosporidium*-positive fecal samples (Morgan and Thompson, 1998; McGlade et al., 2003; Scorza et al., 2003; Fayer et al., 2006; others) than could be expected under only LM. However, a positive PCR does not provide information on the viability and infectivity of the pathogen. Thus, a combination of methods (LM, TEM, molecular detection, and immunological methods) is recommended and vital, especially in cases where only a few oocysts are present in the feces, or when any doubts are raised regarding the diagnosis, especially in the isolates involved in human outbreaks and/or epidemiological studies.

The diversity demonstrated by *Cryptosporidium* species is not surprising. Gregarines are ubiquitous, incredibly diverse parasites, with thousands of species so far described and a heterogeneity of life cycle patterns and developmental forms. The recognition of *Cryptosporidium* affinities with this group helps to explain the increasing numbers of novel genotypes that are being discovered and emphasizes that the specificity of environmental detection procedures for *Cryptosporidium* could be compromised by cross-reactivity with gregarine protozoa that are ubiquitous in freshwater environments (Bull et al., 1998; Hijjawi et al., 2002; Tenter et al., 2002).

A better understanding of the developmental biology of *Cryptosporidium* in its host can now be achieved by a more comparative approach with what is known of some higher gregarines. This applies to the parasite's relationship with

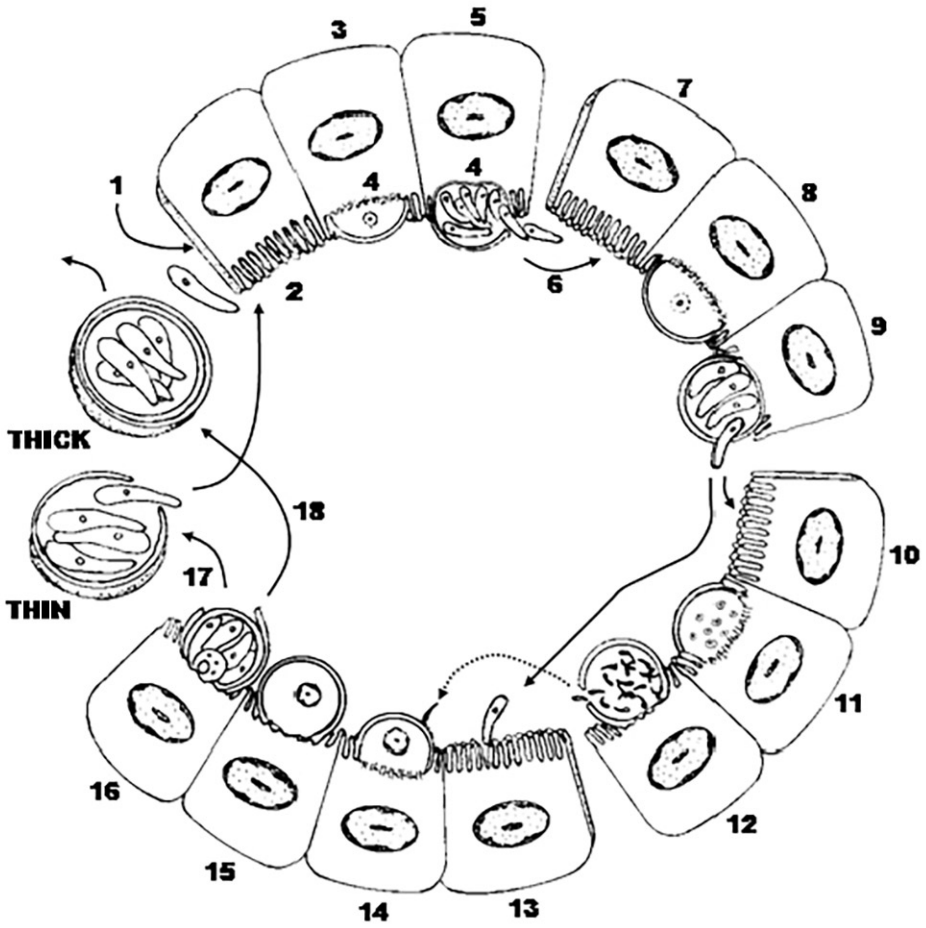


Figure 10. Direct life cycle of a *Cryptosporidium* species. 1) Ingestion of sporulated (thick-walled) oocyst (4 sporozoites) with contaminated food and/or water. 2) Sporozoites excyst from oocyst and penetrate the microvillus layer of epithelial cell and become enclosed by a thin layer of host cell cytoplasm and membranes (3). 4) A desmosome-like attachment organelle and folding of the parasite membranes develop at the interface between parasite and host cell cytoplasm. 5) Merogony forms 8 merozoites in Type I meront. 6) The meront ruptures the host cell releasing merozoites, which penetrate new host cells (7) forming Type II meronts (8). 9 and 10) Type II merozoites enter other epithelial cells to become microgametocytes (11) that undergo multiple fission (12) to produce ~16 non-flagellated microgametes. (13) Most Type II merozoites penetrate epithelial cells enlarging into a macrogametocyte/macrogamont to become a macrogamete (14). 15) Cells with macrogamonts are penetrated by microgametes which penetrate a macrogamete to form a zygote. 16) Sporogony occurs releasing sporulated oocysts into the environment of the intestinal lumen and the feces. About 20% of oocysts fail to form an oocyst wall (17) and only a series of membranes surround the sporozoites. Sporozoites from these thin-walled oocysts are thought to excyst within the gut and infect new epithelial cells (1 and 2). The remaining 80% of thick-walled oocysts exit the host in the feces to potentially contaminate food and water of future hosts. Source: Duszynski and Upton. License: CC BY-NC-SA 4.0.

its host cell and whether *Cryptosporidium*'s epimerite-like feeder organelle obtains nutrients in a way that is truly analogous to myzocytosis, as utilized by many gregarines, through which host cell contents are obtained. In this respect, it is interesting that the feeder organelle has been observed in extracellular stages in a biofilm environment and thus may be able to acquire nutrients in such a host cell-free environment (see Koh et al., 2014).

Discussion, Conclusions, and Difficulties of Working with Apicomplexa

Species Identifications

Accurate species identification is fundamental to every biological investigation. Taxonomists who work with the Coccidia face numerous challenges when defining new species because these parasites undergo a sequential series of

structural changes, both inside (endogenous) and outside (exogenous) their host species. Endogenous developmental stages (multiple stages of merogony, merozoites, micro- and macrogametocytes, developing zygotes/oocysts) exhibit sequential structural changes and to find and measure them requires killing the host. Sporulated oocysts outside the host have been studied the most, historically, because they are resistant to environmental extremes, can be collected by non-invasive means (fecal collection/preservation), and usually can be maintained for long periods of time. Unfortunately, however, oocysts have only a small suite of qualitative and structural characteristics that are quantifiable in the Eimeriidae, but especially in the Cryptosporididae. Generally, the identification of *Eimeria*, *Isospora*, *Caryospora*, and other species in the family is based primarily on their oocyst features without other supporting information (Jirků et al., 2009). Thus, to date, ~ 2,000 nominal species of these genera have been described, with ~ 98% of them identified only by their oocyst's morphology (Asmundsson et al., 2006; Ghimire, 2010). The morphology of oocyst structures both within and between host species can be quite diverse to the point that it becomes confusing and is sometimes difficult to distinguish species based entirely on morphological features. Thus, morphology alone is no longer sufficient to confidently identify many coccidian species, especially those in genera with very small oocysts and sporocysts. These identifications should be supplemented by multiple data sets with information collected from, but not limited to, site of sporulation (endogenous versus exogenous), information on the location and sizes of some or all of the endogenous developmental stages, and sequence data to conduct phylogenetic analyses that will allow the investigator to more robustly assign a parasite to a group, genus, or even species (for example, see Merino et al., 2008; 2009; 2010).

When Do Oocysts Become Sexual?

There is only limited information on sexual differentiation in endogenous and/or exogenous life cycle stages. Canning (1963), Klimes et al. (1972), and others (Jeffers, 1978; Cornelissen et al., 1984; Cornelissen and Overdulve, 1985) showed that merozoites were sexually differentiated. This may happen either between the sporozoite and the first generation meront/merozoites or the first generation merozoites could be sexually undifferentiated, and gene expression is responsible for the formation of either male or female type second generation meronts/merozoites. Cornelissen et al. (1984) called these merozoites macro- or microgamontoblasts. Microgamontoblast merozoites were reported to have only a few granules of polysaccharide reserves and their nuclei lack nucleoli, while those giving rise to macrogamonts had abundant coarse granules of polysaccharide and the nuclei each have a conspicuous nucleolus. Both gamontoblasts contained the haploid amount of DNA and none has been found to be synthesizing DNA.

There is no good evidence that fertilization of a macrogamete is a necessary stimulus to form the oocyst wall, but in oocysts which do sporulate, the zygote sporoplasm is the only stage to possess a diploid nucleus. During the first nuclear division of sporogony, chromosome reduction occurs in a single meiotic division, whereas 2 subsequent nuclear divisions within the zygote are thought to be mitotic. Thus, sporozoites in each sporocyst, as products of a meiosis, would be genetically identical; since infection with either a single sporocyst, or even a single sporozoite, produces viable oocysts in the right host, suggesting that sexual differentiation occurs at a stage in the life cycle later than sporogony. This makes the 2 sexually undifferentiated sporozoites in each sporocyst the basic unit of propagation. It is clearly the advantage of the parasite to remain sexually undifferentiated until it is well established in the host, thus avoiding the possibility of unsuccessful infections due to the loss of sporozoites of the opposite type (Lee et al., 1977).

One sporulated oocyst doesn't necessarily represent a population of genetically identical organisms because it may contain recombination characters from 2 different parental lines. Once ingested and the sporozoites are released, they penetrate epithelial cells and merogony begins. The question then arises, when and where does sexual differentiation occur? The sexuality of individual sporozoites was debated throughout the 1960s and 1970s, but sufficient work was done to indicate they most likely are bisexual (Haberkorn, 1970; Shirley and Millard, 1976; Jeffers, 1978; Cornelissen et al., 1984; Cornelissen and Overdulve, 1985). That is, sexual differentiation is influenced by environmental stimuli responsible for their expression, but the exact nature of exogenous stimuli is unclear. This demonstrates that true clones of *Eimeria* can be established only from individual sporozoites or sporocysts. If sex were determined by genetic factors which segregate during zygotic meiosis, individual sporocysts would contain sporozoites of like sex and would be incapable of producing a complete infection that could produce zygotes.

Oocyst Production

The time between when a suitable host ingests a sporulated oocyst and when oocysts leave that host in its feces is termed the **prepatent period**. During this interval, which can vary from 3 to 10 days (or more), no oocysts are found in the feces because only merogony and the beginning of gamogony are occurring in the host. The time interval during which oocysts are discharged from an infected host is

termed the **patent period** and lasts until all the fertilized and unfertilized macrogametes have been released from their host cells. Both time periods vary between host and coccidian species and are dependent on many factors including: Coccidian species, number of oocysts ingested, number of endogenous stages for that species, depth within the tissues where merogony, gamogony, and fertilization occur concurrent infection with other parasites, host age, nutritional and immune status, and other ecological and physiological factors that are not yet understood.

Once outside the host, the oocyst must sporulate in many species before it is infective to another suitable host. The presence of oxygen, moisture, shade (direct exposure to ultraviolet radiation—sunlight—will kill oocysts quickly) and, generally, a temperature less than the body temperature of the host, all are necessary for oocyst survival. If these conditions are met, complete sporulation occurs and the fully formed oocyst and sporocysts are resistant to environmental extremes, and the sporozoites therein are immediately infective to the next suitable hosts that may ingest them. Each oocyst has a suite of structural characters, unique to its species, that can help the experienced taxonomist distinguish one species from the next in many instances. Unfortunately, because this suite of characters is so small, sometimes sporulated oocysts from different host species look very nearly identical in size and structure and may not be easily or reliably differentiated by morphological features alone. In these instances, life history information (for example, tissue stages as in *Choleoeimeria*) and molecular techniques (such as gene sequencing followed by phylogenetic analysis) is necessary to assist in final identification of the parasite under scrutiny.

Survival of Oocysts

Our understanding of the survival of oocysts in the external environment and the mechanisms by which they reach an appropriate definitive host is minimal and requires additional study. Moisture, temperature, and direct exposure to sunlight all influence the ability of oocysts to sporulate in the external environment (or not), but the interactions of these and other factors (for example, mechanical vectors such as invertebrates) are not well understood. In general, oocysts sporulate more rapidly at higher temperatures and slower at lower temperatures; exposure to temperatures less than 10 ºC or greater than 50 ºC is lethal to unsporulated oocysts. Between these extremes, the sporulation of oocysts in a field-collected fecal sample is dependent on at least the following factors: 1) The parasite species, 2) the time and temperature between collection and arrival of the sample at the laboratory, 3) the medium in which the sample was stored, 4) the amount of molecular oxygen available to the stored oocysts, and 5) the concentration of oocysts in the sample. Under optimal laboratory conditions, sporulation of oocysts from mammals occurs best between 20 °C and 25 °C, but this will vary among vertebrate classes (Duszynski and Wilber, 1997). Interestingly, a few oocysts of some *Eimeria* species (normally, 4 sporocysts each with 2 sporozoites) can be induced to change into *Isospora*-like oocysts (2 sporocysts each with 4 sporozoites) when fresh, unsporulated oocysts are first heated to 50 ºC for 30–60 seconds before incubation at 25 °C for a week (Matsui et al., 1989).

Once sporulated, oocysts of some species remain viable and infective in 2% aqueous potassium dichromate (kills bacteria, prevents putrification) at 4–5 ºC for up to 24 years (Williams et al., 2010)! In their natural external environment, oocysts remain viable and infective from as little as 49 days up to 86 weeks, dependent upon the species and the interplay of abiotic and biotic environmental parameters.

Other Means of Transmission?

The role that naturally occurring soil (for example, mites, ticks, earthworms, and so on) or household organisms (such as house flies and cockroaches) can serve as mechanical vectors has been little studied, but it is known that in many instances invertebrates can be important contributors to the continuation of coccidian life cycles. In *Hepatozoon* species we know that many invertebrate species (such as mites, ticks, and so on) serve as the definitive host while a vertebrate becomes the intermediate host, and gamonts of the parasite in its red blood cells must be ingested by the intermediate host for the cycle to complete. We know that *Besnoitia* species can be propagated asexually by mechanical transmission from intermediate host to intermediate host by blood sucking arthropods. Cockroaches are known to transmit oocysts/sporocysts of *Sarcocystis* species to mice in which sarcocysts can and/or will form. Goodwin and Waltman (1996) demonstrated that darkling beetles (*Alphitobius diaperimus*) could transmit sporulated oocysts of *Eimeria* species to chicks inoculated with beetle homogenates (also see Markus, 1974; 1980; Clubb and Frenkel, 1992).

It has been demonstrated experimentally that at least a few bird and mammalian *Eimeria* may form extraintestinal tissue stages (Carpenter, 1993; Mottalei et al., 1992). Apparently, sporozoites excyst from oocysts ingested by these hosts, infect cells in various places in the body and become dormant. The infected host may or may not be the ‘normal’ host for that *Eimeria* species; if the host with such tissue stages is eaten by the appropriate host, these dormant sporozoites become active, infect enterocytes (which are intestinal epithelial cells) and initiate their typical life cycle. It is not known if such a cycle is functional in natural

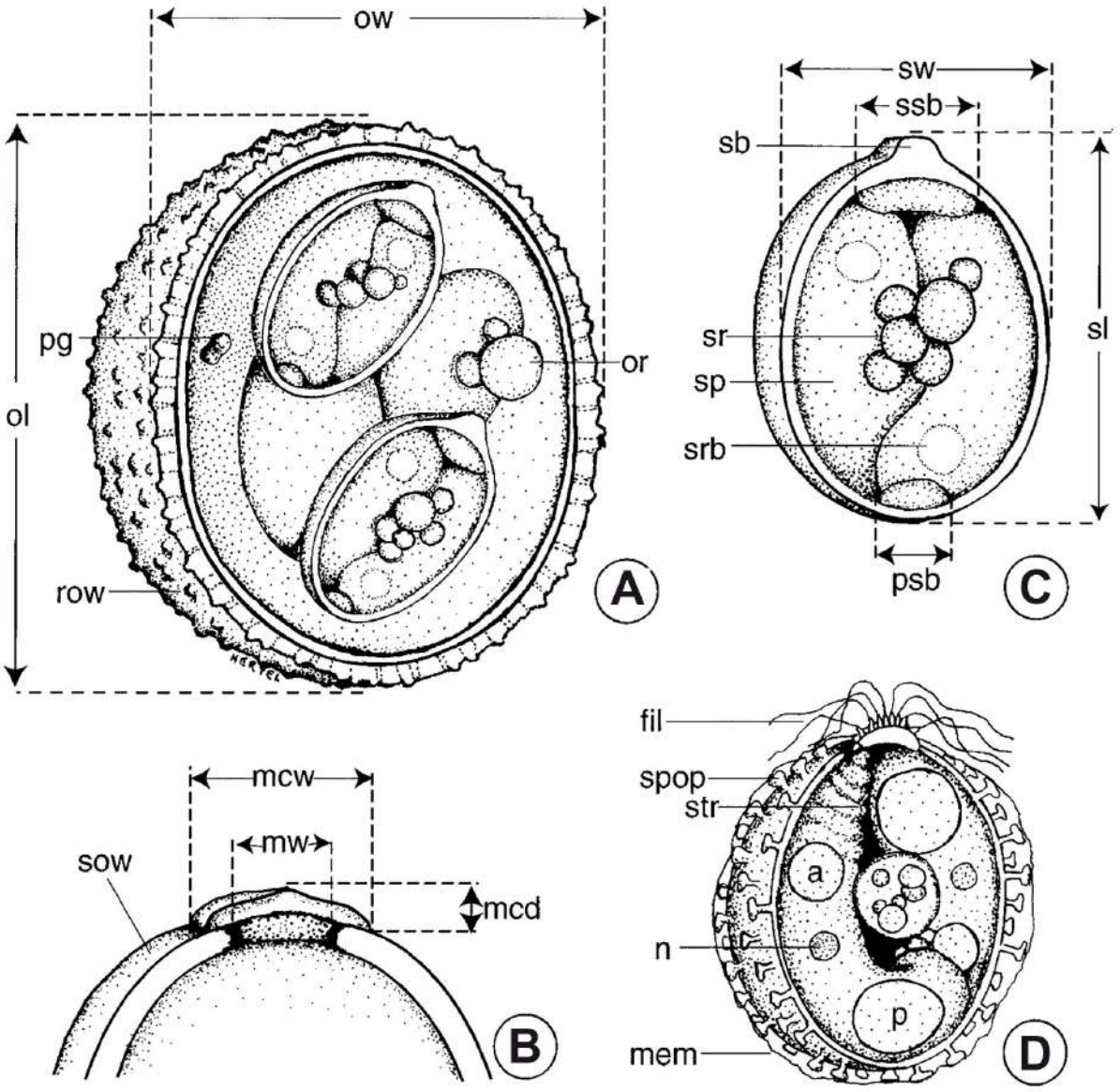


Figure 11A–D. Line drawings of the parts of sporulated oocysts (Eimeriidae: *Eimeria*, *Isospora*, et al.) that should be measured and carefully documented when submitting a new species description for publication. A) Sporulated oocyst of an *Eimeria* sp., drawn in optical cross section, showing essential structural parts that should be measured/documented in the species description: **ow**, oocyst width, measure the widest part when the oocyst is in good optical cross section under oil immersion; **ol**, oocyst length; **pg**, polar granule, note shape and size; **or**, oocyst residuum, note shape, structure, size, and whether or not it may be membrane bounded; **row**, rough outer wall, note this feature, if present, as well as its thickness relative to the inner wall (if present). B) The top of an oocyst that has a micropyle, micropyle cap, and a smooth, 1-layered wall: **sow**, smooth outer wall; **mw**, width of the micropyle; **mcw**, width of the micropyle cap; **mcd**, depth (= height) of the micropyle cap. C) Composite sporulated sporocyst (hypothetical) from an oocyst of *Eimeria* sp., drawn in optical cross section, and enlarged to show detail: **sw**, sporocyst width, measure the widest part when the sporocyst is in optical cross section under oil immersion; **sl**, sporocyst length; **sb**, Stieda body; **ssb**, substieda body, measure width and note relationship to sb (for example, 2 × wider); **psb**, parastieda body, measure width and height (if possible); **sr**, sporocyst residuum, note shape, structure, size, and whether or not it may be membrane bounded; **sp**, sporozoite, note any peculiar or unique features; **srb**, sporozoite refractile body, note size, number, and relative locations in sp. D) Composite sporulated sporocyst (hypothetical) showing a number of unique structural features that may be present in/on the sporocysts/sporozoites of certain eimeriid species: **fil**, filaments emanating from the area of the Stieda body; **spop**, sporopodia extending from the outer surface of sporocyst wall; **mem**, membranous-like covering sometimes associated with sporopodia; **n**, a nucleus sometimes is visible within sporozoite; **str**, sporozoites sometimes have striations at their anterior end; although some sporozoites have only 1 refractile body (Figure 9C), others have both anterior (a) and posterior (p) refractile bodies as shown here. Source: Original by L. A. Hertel; adapted from Duszynski and Wilber, 1997.

communities. And, of course, some *Caryospora* species are facultatively heteroxenous. *Cystoisospora*, *Besnoitia*, and *Toxoplasma* species form tissue cysts in intermediate hosts that can continue these cycles where those intermediate hosts are ingested.

Finally, another area that needs further study is to determine the mechanisms of how *Eimeria* overwinter in hibernating animals and the importance of these mechanisms to their maintenance in natural populations.

Ubiquitous, Neglected, and Complex: Untapped Biodiversity

The number of species of eimeriid coccidia is potentially staggering because these parasites have been found to infect all vertebrate and some invertebrate species that have been sampled for them. Unfortunately, most parasite surveys of vertebrates have concentrated only on their helminth and/or arthropod companions and largely have ignored their *Eimeria* (and other protozoan) parasites. For example, looking at the 5 classes of vertebrates we know only the following about their coccidia to date:

Amphibia (Frogs, Toads, and Salamanders)

This class has 3 orders, 56 families, 464 genera, and 6,009 species. Only 14 of 56 (25%) extant families, 28 of 464 (6%) genera, and 45 of 6,009 (< 1%) species have ever been examined for Coccidia. From these surveys 89 identifications were made. These include 52 coccidia species described and given binomials: 38 *Eimeria*, 11 *Isospora*, 2 *Goussia*, and 1 *Hyaloklossia* species. In addition, 37 additional names appeared that researchers believe are not valid including: 10 species inquirendae (which are species of doubtful identity), 22 incertae sedis (which have been placed in an uncertain taxonomic position), and 5 nomen nuda (which are nude names without formal descriptions) are entered into the literature (Duszynski et al., 2007).

Aves (Birds)

No definitive summary exists yet for *Eimeria* or other Coccidia genera from the 2 superorders and 29 orders of about 10,000 extant bird species, but it is known that there are many *Eimeria* and *Isospora* species already described (along with at least 6 other coccidian genera) and that some of these species, especially some eimerians from chickens and turkeys, can be exceptionally pathogenic to their hosts.

Mammals

There are about 5,416 mammal species organized into 1,229 genera in 53 families placed into 29 orders (Wilson and Reeder, 2005). It is noteworthy that 13 of 29 (45%) orders have been looked at in detail for their coccidia including:

Soricomorpha (Insectivores)

In the insectivores, 4 of 7 (57%) families, 19 of 66 (29%) genera, and 37 of 428 (9%) species have been examined for their coccidia. From these surveys, 120 coccidia species were described including: 48 *Eimeria*, 22 *Isospora*, 5 *Cyclospora*, and 45 species inquirendae including *Coccidium*, *Cyclospora*, *Eimeria*, *Gousseffia*, *Isospora*, and "Coccidia" species (Duszynski and Upton, 2000).

Primates (Monkeys)

Only 7 of 13 (54%) families, 14 of 60 (23%) genera, and 18 of 233 (8%) species have been examined for their coccidia. From these surveys 28 coccidia species were described including: 7 *Eimeria*, 8 *Isospora*, 1 *Cyclospora*, and 12 species inquirendae (Duszynski et al., 1999).

Scandentia (Tree Shrews)

Tree shrews all are placed in 1 family. Only 2 of 5 (40%) genera and 4 of 19 (21%) species in this family have been examined for coccidians. From these surveys only 4 *Eimeria* species have been described (Duszynski et al., 1999).

Chiroptera (Bats)

Only 6 of 17 (35%) families, 37 of 177 (21%) genera, and 86 of 925 (9%) species have been examined for their coccidia. From these surveys 39 coccidia species were described including 31 *Eimeria* and 8 species inquirendae (Duszynski, 2002).

Lagomorpha (Rabbits)

When compared to other mammalian orders, the Lagomorpha is not diverse and contains only 2 extant families, but even with such a tractable group not much is known about their coccidian parasites, except for just a few species. Although species in both extant families have been studied (a little), only 5 of 12 (42%) extant genera and 25 of 96 (26%) species have been examined. From these surveys, 87 coccidia species were described including 3 *Besnoitia*, 3 *Cryptosporidium*, 73 *Eimeria*, 2 *Isospora*, 5 *Sarcocystis*, and *Toxoplasma gondii*, and 33 species inquirendae (Duszynski and Couch, 2013).

Marsupialia (Marsupials)

In most earlier classifications of mammals (for example, Nowak, 1991) all marsupials were placed in a single order, but results from molecular and genetic research tools in the last 15 years have directed mammalogists to partition them into 7 orders within 2 superorders: Ameridelphia (Didelphimorphia, Microbiotheria, Paucituberculata), the American marsupials, and the Australidelphia (Dasyuromorphia, Diprotodontia, Notoryctemorphia, Peramelemorphia) the Australian marsupials (Wilson and Reeder, 2005). Duszynski combines the parasite data for these 7 orders into the original

Marsupialia, within which there are 21 families, 92 genera, and 331 species. From all pertinent surveys 154 coccidia species are named including: 1 *Besnoitia*, 6 *Cryptosporidium*, 56 *Eimeria*, 1 *Isospora*, 11 *Klossiella,* 10 *Sarcocystis* species, *Toxoplasma gondii*, and 68 species inquirendae from 85 of 331 (26%) marsupial species examined. These species are found in 14 of 21 (67%) families examined for coccidian parasites, in 45 of 92 (49%) genera, and in only 85 of 331 (26%) marsupial species that have been examined for coccidian parasites (Duszynski, 2016).

Carnivora (Carnivores, Cats, and Dogs)

This order is separated into 2 suborders, Caniformia (9 families) and Feliformia (6 families), and these 15 families have 126 genera and 287 species (Wilson and Reeder, 2005). There are about 207 valid coccidian species named including 5 *Besnoitia*, 1 *Caryospora*, 10 *Cryptosporidium*, 1 *Cyclospora*, 53 *Cystoisospora*, 39 *Eimeria*, 3 *Hammondia*, 6 *Hepatozoon*, 9 *Isospora*, 1 *Neospora*, 78 *Sarcocystis*, and *Toxoplasma gondii*. There also are about 483 incompletely named species (species inquirendae) recorded from carnivores that fit taxonomically into these taxa as genus names only, or less. These species are found in 11 of 15 (73%) families, in 30 of 126 (24%) genera, and in only 48 of 287 (17%) carnivore species that have been examined for coccidian parasites.

Rodentia (Mice, Rats, Squirrels, etc.)

There are 33 families, 481 genera, and 2,277 extant species of rodents (Wilson and Reeder, 2005). Although there is no group-by-group summary to date, it is estimated that only about 15% of all rodent species have been surveyed for coccidia. From these surveys about 450 *Eimeria* and *Isospora* species have been described (Duszynski and Upton, 2001).

Remaining 15 Mammalian Orders

No definitive summaries exist to date although some coccidia species in 8 genera have already been described from a few of their species.

Pisces (Fish)

No definitive summary exists yet for the 32,500 extant fish species although many coccidian species in at least 6 genera already have been described (unpublished data).

Reptiles (Snakes)

Only 6 of 17 families (35%), 110 of 457 genera (24%), and 208 of 3,108 snake species (7%) have been examined for their coccidia. From these surveys, 302 coccidia species were described including 52 *Caryospora*, 2 *Cryptosporidium*, 4 *Cyclospora*, 66 *Eimeria*, 7 *Isospora*, 22 *Sarcocystis*, 1 *Wenyonella*, 2 *Tyzzeria*, and 148 species inquirendae, the latter including 3 additional genera (*Dorisiella, Globidium*, and *Pythonella*) (Duszynski and Upton, 2010).

Reptiles (Turtles)

The order Testudines is separated into 2 suborders, Cryptodira (11 families) and Pleurodira (3 families), and these 14 families have 96 genera and 351 species (Uetz, et al., 2018). Surprisingly, at least 1 species in 10 of the 14 (71%) families has been examined for coccidian parasites, but only 30 of 96 (31%) genera and 61 of 351 (17%) turtle species have been examined for coccidia and 100 coccidia species in 7 genera are known: 2 *Caryospora,* 66 *Eimeria,* 3 *Isospora,* 1 *Sarcocystis*, and 28 species inquirendae (4 *Coccidium,* 1 *Caryospora*, 9 *Cryptosporidium*, 5 *Eimeria,* 1 *Manotella*, and 8 *Sarcocystis* species [?]) (Duszynski and Morrow, 2014).

Reptiles (Alligators)

The order Crocodylia includes 27 species of alligators, caimans, crocodiles, and gharials. Duszynski et al. (2020) reported the blood and intestinal apicomplexans know to date from these reptiles and concluded that 17/27 (63%) had 16 apicomplexan species unique to them including: 8 Eimeria, 1 Haemogregarina, 4 Hepatozoon, 2 Isospora, and 1 Progarnia species; they also reported an additional 46 apicomplexan-like forms that were considered species inquirendae that await further study.

Reptiles (Lizards)

No definitive summary exists yet for all lizards, although many apicomplexans in 8 genera already have been described (unpublished data). This is now a work in progress.

There are approximately 62,150 extant vertebrate species known on Earth and, to date, there is comprehensive, systematic survey data on 16 vertebrate groups, (amphibians, 13 mammalian orders, snakes, turtles, crocodiles) which comprise 11,787 species, but only 634 (5.3%) of these species have been examined for coccidia and from them about 1,146 species have been named in the literature or about 1.8 coccidia species per host species examined. Given that some host species (for example, chickens, rabbits, and others) have 10 or more *Eimeria* species that may be unique to them, and that even domestic animals, whose parasites have been studied for decades, have had new *Eimeria* species described from them recently, it is clear that only a fraction of the number of *Eimeria* (and other coccidia) species that occur in vertebrates have been described to date. Using numbers that are now out of date, Levine (1973) estimated that more than 45,000 species of *Eimeria* would be found if all vertebrate species were examined. This is a gross underestimation, but it points to the urgent need for more work in this area, especially given the alarming rate of habitat destruction

and vertebrate species extinctions occurring worldwide. If we assume, conservatively, that every vertebrate species on Earth is host to (minimally) 2 coccidia species unique to it, we could expect to find at least 124,300 total coccidia species. The 1,800 or so coccidia species currently known is only 1.4% of the number of species that likely exist in Earth's vertebrates. In other words, 98.6% (or more) of the coccidian parasites of vertebrates are yet to be discovered! Clearly, there is lots of work to be done.

Ubiquitous, Neglected, and Complex: Specificity

Eimeria species demonstrate both site and host specificity, but to somewhat different degrees. The majority of species for which endogenous development is known undergo development within certain cells of the gastrointestinal tract, but not all species are found in this location. *Eimeria stiedai* undergoes development in epithelial cells of the bile duct and parenchymal cells of the liver of rabbits. Other species have been found to develop in cells of the gallbladder (goat), placenta (hippopotamus), epididymis (elk), uterus (impala), genitalia of both sexes (hamsters), bile duct (chamois), liver parenchyma (wallaby), and pyloric antrum (kangaroo) (Duszynski and Upton, 2001). *Hepatozoon* species develop in the blood of vertebrates and in arthropods, *Klossiella* species develop in kidney epithelial cells, and *Cystoisospora, Sarcocystis, Besnoitia,* and *Toxoplasma* have heteroxenous life histories. Once within their specific organ system of choice, *Eimeria* species seem to be limited to specific zones, specific cells within that zone, and specific locations within those cells. Thus, 1 species may be found only in the middle third of the small intestine and another only in the cells of the cecum. Within their specific region 1 species may be found only in cells at the base of the crypts of Lieberkühn, a second species in epithelial cells along the villi, and a third species in endothelial cells of the lacteals in the villi. Some species develop below the striated (microvillus) border of endothelial cells, but above the nucleus, others below the nucleus and a few within the nucleus. And a few other species or genera associate closely with the brush border of the epithelial cells and may even be extracytoplasmic (for example, *Cryptosporidium* species).

The degree of host specificity seems to vary from host group to host group; it's been studied best in mammals, and to a lesser degree in birds, especially domesticated stock or flock animals. *Eimeria* species from goats cannot be transmitted to sheep and vice versa (Lindsay and Todd, 1993), but *Eimeria* from cattle (*Bos*) are found to infect American bison (*Bison*). *Eimeria* species from certain rodents (Sciuridae) seem to cross host generic boundaries easily (Wilber et al., 1998), whereas other rodent species (Muridae) may cross species, but not genus boundaries (Hnida and Duszynski, 1999). In the Lagomorpha, 6 of 17 (35%) *Eimeria* species reported from cottontails (*Sylvilagus* spp.) are experimentally infective for the tame rabbit (*Oryctolagus cuniculus*). Similarly, some species from gallinaceous birds can be transmitted only to congeners, whereas others can be cross-transmitted between genera. One species has even been reported to cross familial lines, but this seems rare (De Vos, 1970). It also is known that *Eimeria separata* Becker and Hall, 1931, from rats will infect certain genetic strains of mice and that genetically altered or immunosuppressed mammals are susceptible to infection with *Eimeria* species to which they otherwise might be naturally refractory. Thus, numerous biotic interactions, particularly the genome of both parasite and host, must contribute to the host specificity, or lack thereof, attributed to each *Eimeria* species.

Ubiquitous, Neglected, and Complex: Significance in Biomedical Research

All members of the protozoan phylum Apicomplexa are obligate intracellular parasites. In addition to the *Eimeria*, many of their closely related cousins (for example, *Isospora*, *Sarcocystis*, *Tyzzeria*, and others) can cause economically important diseases in domesticated, and sometimes wild, animals. Other related forms in the phylum (for example, *Toxoplasma*, *Cyclospora*, *Cryptosporidium*, and *Plasmodium*) cause human disease in hundreds of millions of people worldwide. Classical genetic studies have been limited by the intracellular habitats of all these organisms and/or by the complex life cycles of some of them. However, development of pulsed-field electrophoresis, DNA sequencing, PCR, and related techniques has allowed good progress in understanding of the genomes of these parasites. In fact, the complete genome sequence of the most pathogenic human malaria parasite, *P. falciparum*, is now known (Gardner et al., 2002).

Recently, use of some of these molecular techniques has shown that a number of apicomplexan parasites (*Eimeria*, *Plasmodium*, and *Toxoplasma* species) have 2 extrachromosomal DNAs: 1) A small mitochondrial genome and 2) a unique 35 kb circular DNA. Sequencing and other molecular data suggest that the 35 kb DNA may be related to plastid DNA (plDNA). This plDNA should be of keen interest to researchers because its true origin, cellular location, function within these parasite cells, and relation to their nuclear genomes, are still a mystery. The exciting appeal for studying the plDNAs is their potential as specific targets for chemotherapeutics: Potential "silver bullets" to control the undesirable parasites of humans and their domesticated animals

that reside within the Apicomplexa. If the sequence of plDNA genes differs significantly from the genes for similar functions in their hosts, then such a function-mediated gene may prove an ideal target for development of chemotherapeutics that could be efficacious and nontoxic, while not inducing resistance. If plDNAs prove to be a common or universal feature of members of the Apicomplexa, they also have great potential for use as a phylogenetic yardstick to determine the evolutionary origin and history of apicomplexan parasites.

Ubiquitous, Neglected, and Complex: Human and Veterinary Medicine

Unlike members of the closely related genus *Cyclospora* and its more distantly related cousin *Cryptosporidium*, there is no evidence that any *Eimeria* species infect humans. In fact, both the nearest relatives to the primates, the Scandentia and the prosimians within the Primates are infected only by *Eimeria* species, whereas the anthropoid primates, which include the hominids, are only infected by *Cystoisospora* species, such as *Cystoisospora belli*, an important human pathogen.

Wild animals other than anthropoids (for example, mice, rabbits, and moles) almost always are infected with 1 or more *Eimeria* species at one or more times during their life and some might be infected during their entire lives with several species that cycle through them constantly. Given their ubiquitous nature, *Eimeria* species probably do not often cause discernable pathology or disease under natural conditions, but exceptions exist. For example, *E. bovis* (Züblin, 1908) Fiebiger, 1912 in cattle, *E. tenella* (Raillet and Lucet, 1891) Fantham, 1909 in chickens, and *E. stiedai* (Lindemann, 1865) Kisskalt and Hartmann, 1907 in rabbits all are known to be highly pathogenic in their respective hosts and, recently, another pathogenic species, *E. brachylagia* Duszynski et al., 2005, was found to cause heavy intestinal infections, some of which resulted in deaths in the endangered Columbia Basin pygmy rabbit, *Brachylagus idahoensis*, in Washington and Oregon, United States (Duszynski et al., 2005). *Eimeria chinchillae* has a broad host range and is also highly pathogenic, causing bloody diarrhea, anorexia, severe lesions in the intestines, and ultimately leads to the death of infected animals. It may also cause neurological symptoms (De Vos and Westhuizen, 1968; De Vos, 1970).

When animals are concentrated together, enhancing transmission of *Eimeria* via its rapid, direct life cycle, some species will cause a disease condition, coccidiosis. Coccidiosis is recognized as a major health hazard: During intensive husbandry of domestic animals; in wild, captive animals such as those in breeding and research facilities and zoos; in wild animal populations when habitat is lost and crowding occurs; and in wild animal species that have great reproductive potential and are protected by laws so that their populations increase inordinately (for example, kangaroos in Australia). All of these conditions are the result of human intervention or perturbation.

Coccidiosis is a serious problem in the poultry industry. In the United States alone, more than 4 *trillion* birds are raised annually and the United States Department of Agriculture estimated that loss to poultry farmers in the mid-1980s exceeded US$ 80 million when deaths, medicated feeds, and all added labor costs were considered. Worldwide expenditures, just for coccidiostats added to broiler feed, are estimated to be US$ 250–300 million annually. Once a flock becomes infected, especially with 1 or several of the more pathogenic species, a large percentage of the flock can die rapidly. Birds not killed outright by their infection become listless and are more susceptible to predators and other diseases. Even if they survive their infection, they have reduced feed efficiencies. In addition, *Eimeria* species are becoming widely resistant to the coccidiostats in feed. Similar morbidity and/or mortality and related events occur in cattle feedlots or wherever meat animals are congregated in large numbers.

Coccidiosis is also well documented in some wild species. *Eimeria gruis* Yakimoff and Matschoulsky, 1935 and *E. reichenowi* Yakimoff and Matschoulsky, 1935, for example, are common parasites of both whooping cranes and Sandhill cranes in North America and have been reported in other crane species in captivity. These species are considered an important cause of mortality in captive cranes and, during migrations when large numbers of several species of cranes congregate for lengthy periods at watering holes, can be responsible for illness and death in wild populations. Although the disease is generally limited to the intestinal tract in most animals, *Eimeria* infection in cranes may result in disseminated visceral coccidiosis, where endogenous stages from the gastrointestinal tract become disseminated throughout the body, via the blood or lymphatic systems. Nodules with meront and gamont stages are found in many organs, including lungs, air sacs, trachea, and nares. This disseminated visceral coccidiosis has caused the death of a number of captive Sandhill cranes and whooping cranes. Of the few *Eimeria* species known to have extraintestinal developmental stages, only the species in cranes can complete their life cycle in both the digestive and respiratory tracts (Carpenter, 1993).

Habitat Destruction, Coccidian Transmission, and Disease

Finally, as the human population continues to grow and agricultural development accelerates to try keep pace, natural places and their endemic faunas will decrease dramatically. The immediate effect of shrinking ecosystems (for example,

tropical rain forests, coastal estuaries/wetlands, temperate old-growth forests) is to concentrate both species and individuals into restricted, fragmented areas promoting increased transmission and exchange of parasites, especially those with direct life cycles with resistant oocysts, like many coccidians. Such close contact between host species and their parasites could allow these organisms to become agents of extinction as the host range(s) contract.

Fragmentation increases the edge-effect and can bring an influx of new host species into disturbed or agricultural habitats between fragments, introducing new coccidians and possibly leading to the development of new and more pathogenic strains. Changes in parasite species, intensities, or pathogenicity can have repercussions on the whole food web. The potential, either for domestic animals to become infected by coccidian parasites maintained in wild reservoir host populations, or the reverse, is a strong possibility. For example, we know that deer, elk, or bison can serve as reservoirs for *Eimeria* and other parasites for domestic livestock and wild rabbits can serve as reservoirs of *Eimeria* species capable of infecting domesticated rabbits. As humans breed themselves to the brink of extinction and habitat disappears globally at an ever-alarming rate, the potential for biological disaster from the exchange of ubiquitous protozoan parasites, like *Eimeria* species and its close relatives, may destabilize food webs. Environmental stressors (for example, PCBs), which may compromise host immune systems, global climate change, which challenges the adaptability of host organisms, and the invasion of new parasites, from edge-dwelling hosts, all increase the potential for many apicomplexan parasites to become pathogenic; thus, the importance of disease should be expected to increase in shrinking ecosystems as a consequence of habitat destruction.

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10

Protozoa

Apicomplexa

Haemosporida (Order): The "Malaria Parasites"

Susan L. Perkins and Spencer C. Galen

Phylum Myzozoa

Subphylum Apicomplexa

Order Haemosporida

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Chapter 10

Haemosporida (Order): The "Malaria Parasites"

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Introduction

The term malaria refers specifically to a disease of humans that is caused by an infection of red blood cells (erythrocytes) and other cells by protozoan parasites in the genus *Plasmodium* and transmitted by mosquitoes in the genus *Anopheles*. The pathology of this disease results from 3 primary sources: 1) Episodic fevers that are caused by the cyclic rupturing of host erythrocytes; 2) anemia that follows from infection of host erythrocytes and their subsequent death; and 3) clogged capillaries from the combination of a loss of elasticity of infected erythrocytes as well as parasites triggering host erythrocytes to express surface proteins that make them likely to cling to one another. Nearly half a million people still die from malaria worldwide every year (Cibulskis et al., 2016; WHO, 2021), primarily in sub-Saharan Africa and other tropical regions. No vaccine is yet available on a global scale, but an RTS,S vaccine against the most pathogenic species (*Plasmodium falciparum*) has recently been approved and is being deployed in Ghana (WHO, 2023). Several drugs have been used as prophylaxis or treatment, but the parasites have evolved resistance to these compounds in many regions of the world. The human parasites are only a tiny fraction of the very diverse clade Haemosporida (sometimes called Haemospororida) which contains over 600 described species of these protozoan parasites occurring in many different species of reptiles, birds, and other mammals. All haemosporidians use a vertebrate host and a biting dipteran (fly) vector during different stages of their life cycles. Non-human haemosporidians are sometimes colloquially referred to as the malaria parasites, due to their close relationships and similar life cycle to the parasites that cause this disease in humans.

History of Knowledge of Malaria

Symptoms of malaria, particularly its regularly spaced fevers, have been described by writers in antiquity going back as far as 5,000 BCE in China and more than 3,000 years ago in India, Sumeria, and Egypt. The writings of ancient Greece describe characteristic symptoms of *Plasmodium falciparum*, *P. vivax*, and *P. malariae* and Alexander the Great is believed to have died from *P. falciparum* while attempting to travel to India in 323 BCE (Carter and Mendis, 2002). It is thought that both European colonists and the enslaved West Africans that they transported to the New World brought malaria parasites, and by the mid-19th century, malaria was a common and endemic disease throughout the tropical and temperate regions of North America and South America (Carter and Mendis, 2002).

Despite the ubiquity and severity of malaria, the root cause of this disease remained largely a mystery, with only the link between smelly, swampy regions and the resulting symptoms as a clue (the word malaria stems from the Italian words for bad air, namely, **mal** + **aria**). Eventually, in the late 1800s a series of scientists, most notably Charles Laveran, began to piece together that tiny specks in the blood of sick humans, later known to be the blood stages of the parasites, were associated with the characteristic fevers of the disease. How it could pass from one human to another was not known until 1897, when Ronald Ross, a British Army doctor, showed that there were cells that could be found in the saliva of *Anopheles* mosquitoes that had fed on birds that were somewhat similar to those that he observed in the blood of sick human patients. Ross was awarded the Nobel Prize in Physiology or Medicine in 1902 for this major piece of the discovery, but the other aspects of the malaria life cycle remained unknown for many decades to come, namely, where the sporozoites went between the time they were injected into the host by the mosquito and when they appeared in the bloodstream of the same host.

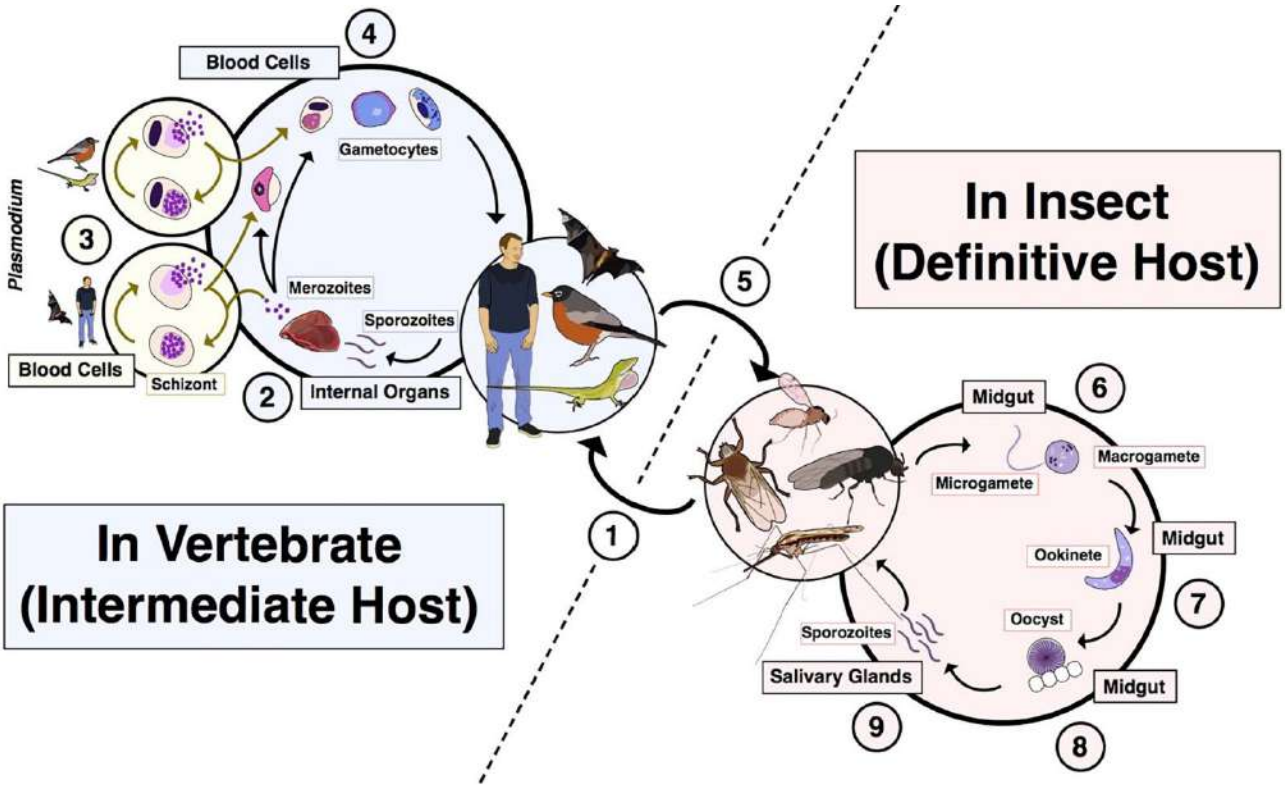

Figure 1. Generalized life cycle for haemosporidian (malaria) parasites. 1) An infected dipteran takes a blood meal from a vertebrate and sporozoite stages are injected into the bloodstream along with its saliva. 2) The sporozoites travel through the blood until they reach specific cells in internal organs where they will undergo rounds of asexual division. In human malaria parasites and many others of mammals, this occurs in cells of the liver. 3) For *Plasmodium* parasites, there are additional rounds of asexual division in blood cells as well. This can occur in both anucleated red blood cells of mammals (bottom) and in nucleated blood cells of birds and squamates (top). 4) Eventually, a developmental switch triggers the development of gametocytes, or the transmission stages. These stages are either of future male function (microgametocytes) or female function (macrogametocytes). These stages are highly variable across the diversity of haemosporidians. The illustration depicts gametocytes of *Plasmodium falciparum* (lower left), a lizard *Plasmodium* (second from left), a *Leucocytozoon* (third from left), and a *Haemoproteus* (fourth from left). 5) If a dipteran vector feeds from an infected vertebrate host, she will pick up gametocytes as part of that blood meal. 6). Once inside the fly's midgut, the gametes undergo exflagellation, forming the macrogamete (female) and multiple microgametes (male). 7) After fertilization, the zygote transforms into a motile stage known as the ookinete, which penetrates the wall of the fly's midgut. 8) A cyst, known as the oocyst forms and meiosis occurs at this point. 9) Sporozoites rupture from the oocyst and travel to the fly's head, coming to reside in the salivary glands to await the next blood meal. Source: S. C. Galen, 2019. License: CC BY-NC-SA 4.0.

Malaria Today

Today, malaria is still one of the largest public health burdens in the world (Sachs and Malaney, 2002; WHO, 2021; 2023) with as many as a quarter of a billion new cases arising each year. The number of cases and particularly the number of deaths has fallen recently due to improved prevention measures such as insecticide-infused bed nets, better diagnostics, and improved treatments, however, there is still plenty of reason to be cautious. The drug artemisinin (and its derivatives), which is the first line of treatment in all malaria endemic-countries, is now at risk of lower efficacy as resistance has evolved in several endemic regions and insecticide resistance remains a looming and potential problem.

General Life Cycle

All members of the order Haemosporida follow the same generalized life cycle, which is obligately heteroxenous (multiple hosts), alternating between a vertebrate and a biting fly such as a mosquito, midge, louse fly, sand fly, or black fly (Figure 1). Take as an example the bite of an already infected insect (Figure 1, point 1). Several groups of flies use blood as a source of proteins and lipids with which to make their eggs. If a fly is infected with haemosporidians, when she feeds from the vertebrate, the stages of the parasite known as sporozoites will be injected into the vertebrate bloodstream along with her saliva. Sporozoites will travel through the bloodstream until they come to the liver or other

target tissues, where they will invade the host cell and begin to asexually divide (Figure 1, point 2), often making thousands of daughter cells within just a few days. In some groups of haemosporidians, namely the genus *Plasmodium*, after this first round of tissue schizogony some of the parasites will leave the tissue stages and invade blood cells (typically red blood cells; Figure 1, point 3), where they transition into the asexual feeding stages known as trophozoites. During this point in the life cycle, the parasites digest the hemoglobin present in the hosts' red blood cells, eventually forming a crystalline compound known as hemozoin. These cells will then undergo additional asexual division and burst from the host cell, going on to infect new blood cells. However, the majority of the genera in the order Haemosporida do not asexually replicate in the host's blood cells, but instead continue their cycle in other tissues such as the epithelium of the lungs or spleen. All members of the order will at some point show the transmission stages, known as gametocytes, in the host blood cells (Figure 1, point 4). These cells exist as different sexes; females are known as macrogametocytes and males are known as microgametocytes. When another biting fly comes to feed on this host, the gametocytes will be taken up as part of her blood meal (Figure 1, point 5). Within the midgut of the fly, the gametocytes undergo a process called exflagellation, as the blood cells themselves begin to be digested. The microgametocytes split into several smaller microgametes, each of which can fertilize an exposed macrogamete (Figure 1, point 6). Once fused, the parasite exists as a motile stage called an ookinete (Figure 1, point 7). These cells push through the cells of the insect's gut and encyst on the outer edge, in a structure called the oocyst (Figure 1, point 8). Thousands of sporozoites emerge from each oocyst and migrate to the insect's salivary glands, where they wait until her next blood meal to infect a new vertebrate host (Figure 1, point 9).

Because the sexual component of the malaria parasite's life cycle occurs within the insect, technically it is the insect that should be referred to as the definitive host, with the human or other vertebrate host referred to as the intermediate host. The dipteran insect is, however, often referred to as the vector of the malaria parasite as it does transmit the parasite between humans or other vertebrate hosts. The complete life cycle has been worked out in detail for only a few of the species of haemosporidians that infect non-human hosts, with most assumed to follow the same stages as their close relatives in the same genus. Although the use of different insect host groups (Table 1) is known for some of these parasites, the vast majority of haemosporidians are transmitted by unknown species of flies.

Diversity of and Relationships within the Family Haemosporida

The species of *Plasmodium* that commonly infect humans are the best known and most intensively studied haemosporidians, though they are of very minor importance in terms of the overall diversity of these parasites. Birds or their dinosaur precursors are most likely the original vertebrate hosts of the malaria parasites and still harbor the greatest diversity of the haemosporidians, both in terms of the number of described species that use birds as hosts, but also their geographic range (Valkiūnas, 2004). Numerous other vertebrate groups are hosts to species of haemosporidians, including monkeys, bats, lizards, and turtles. Haemosporidians are typically not capable of infecting hosts outside of their major host group, that is, bird malaria parasites cannot infect humans and vice versa. However, within their major host groups some malaria parasites (especially the avian malaria parasites) can infect many different species. For example, the cosmopolitan avian malaria parasite *P. relictum* has been recorded to infect over 300 different species of birds throughout the world (Valkiūnas et al., 2018).

Table 1. Major groups of haemosporidians, with vertebrate and dipteran hosts used.

Genus	Vertebrate hosts used	Dipteran insect hosts used
Plasmodium	Primates, rodents, bats, birds,* squamates,* ungulates*	Anopheline and culicine mosquitoes
Hepatocystis	Primates, bats	Culicoides midges, others?
Polychromophilus	Bats	Nycterbid flies
Nycteria	Bats	Unknown
Haemoproteus	Birds	Hippoboscid flies
Parahaemoproteus	Birds	Culicoides midges
Haemocystidium	Squamates, turtles	Tabanid flies, others?
Leucocytozoon	Birds	Simulids (black flies)

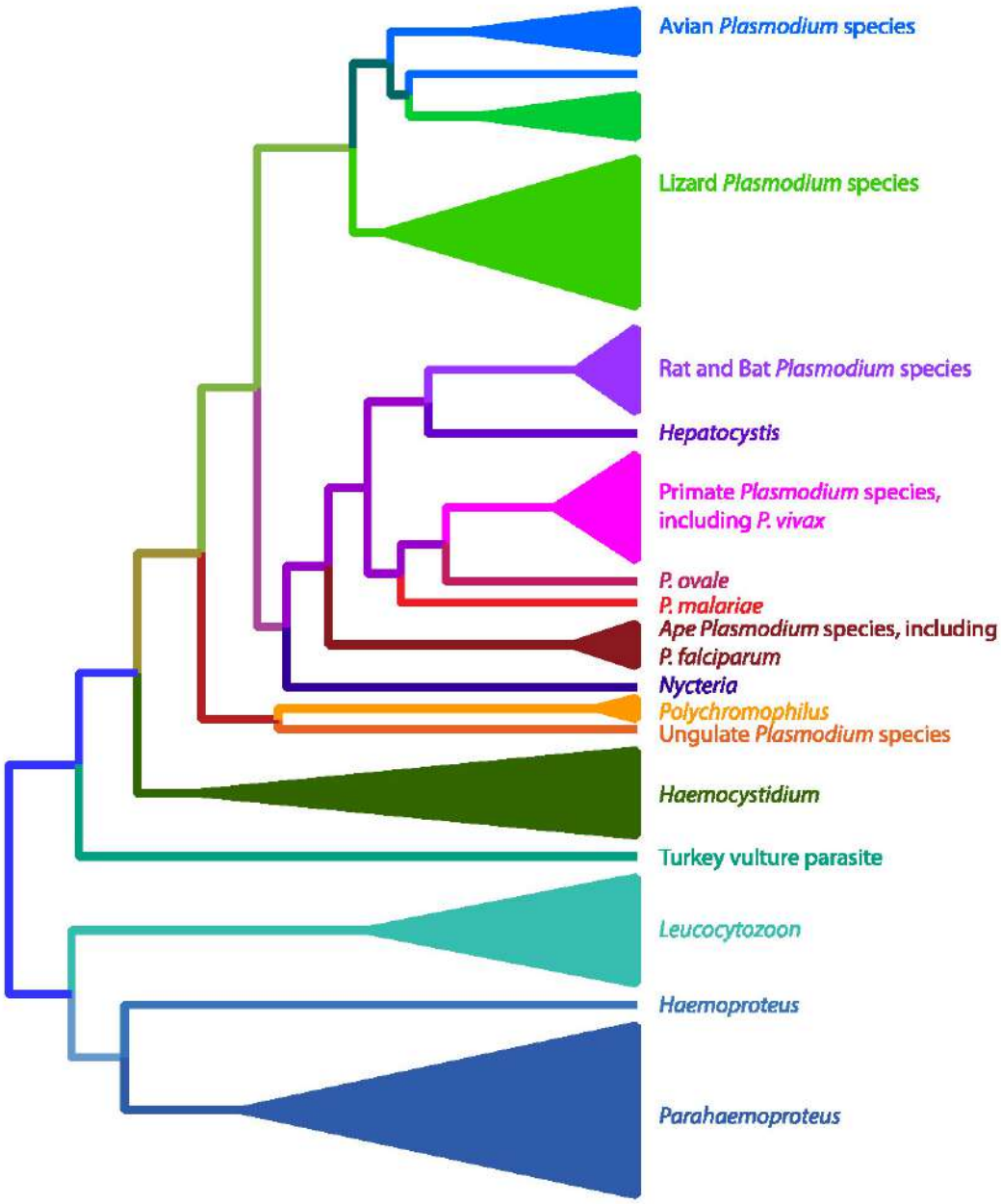

Figure 2. Phylogenetic relationships of the haemosporidian (malaria) parasites. An analysis of more than 20 genes (Galen et al., 2018) resulted in this hypothesis of the evolutionary relationships of various clades of haemosporidians. This tree supports that birds were the original hosts of these parasites with either a single introduction into mammalian hosts and a subsequent reinfection of birds and lizards by *Plasmodium* parasites, or that there were 2 invasions into mammals. This tree would make the genus *Plasmodium* polyphyletic as not all members share a common ancestor, however this would mean that significant taxonomic changes need to occur. Source: Galen et al., 2018. License: CC BY-NC-SA 4.0.

Classification

The classification of the malaria parasites has been extremely fluid throughout history. For the bulk of this time, taxonomic groups including genera and families were primarily structured around 2 primary characteristics of the parasite species: 1) If the parasite reproduced asexually in the host blood (known as erythrocytic schizogony) and 2) whether or not hemozoin pigment was visible in the blood stages of the parasite. These traits were considered to be the most important in the separation of the parasites into genera and served as the basis for the creation of 4 families within the order (Levine, 1988).

The advent of using DNA sequences as characters with which to understand the evolutionary history and

relationships among organisms drastically changed the hypotheses of the relationships within Haemosporida. The first molecular systematic study of these parasites used the 18S ribosomal small-subunit gene and a small set of taxa including the important human parasites, *Plasmodium falciparum* and *P. vivax*, as well as parasites found in rodents and 2 species from birds (Waters et al., 1991). The resulting topology showed a close relationship between *P. falciparum* and *P. gallinaceum*, a parasite that infects chickens, and the authors naturally concluded that humans had acquired the virulent *P. falciparum* following a host switch after chickens were domesticated. However, subsequent studies, particularly those using other genes, did not support the human/chicken connection (Ayala et al., 1999; Perkins and Schall, 2002) and instead showed that the human- and bird-infecting *Plasmodium* lineages are distantly related. These later studies also established that the avian parasites in the genus *Leucocytozoon* were likely an early-diverging lineage (Perkins and Schall, 2002). Recently, multiple genes from a large number of different haemosporidian parasites were sequenced and used to create the most comprehensive phylogeny to date (Figure 2) (Galen et al., 2018). These results highlighted the complex evolution of the Haemosporida and show that the original characters used to define clades have likely evolved more than once. This updated phylogeny also showed that the taxonomy of this group of parasites needed to be revised. For instance, most recent analyses have recovered parasites that have been classified as the distinct genus *Hepatocystis* as closely related to the human-infecting *Plasmodium* species (Perkins and Schall, 2002; Galen et al., 2018). Conversely, many parasites classified as *Plasmodium* because they show schizogony in blood cells and clear hemozoin pigment, have been shown not to be part of a monophyletic group that contains the other *Plasmodium* species, including the type species of the genus.

Malaria Parasites of Birds

Malaria parasites are practically ubiquitous in birds with a cosmpolitan distribution. Several hundred species have been described from the genera *Plasmodium*, *Haemoproteus*, *Parahaemoproteus*, and *Leucocytozoon* (Table 1; Figure 2), making the bird-infecting malaria parasites the most species rich group within the Haemosporida.

Avian malaria has been instrumental in studies of the disease ever since it was first discovered in the late 1800s. Ronald Ross (Figure 3), who won the Nobel Prize for his discovery that mosquitoes were responsible for transmitting the parasites from person to person, first did experiments on birds infected with *Plasmodium* parasites (Rivero and Gandon, 2018). Bird systems were also what allowed the discovery that the parasite first completes 1 or more exoerythrocytic stages before it begins to infect the blood cells of the host (Huff and Coulston, 1946). Also, experiments using avian malaria were useful for understanding immunity to *Plasmodium* infection by testing the efficacy of early anti-malarial drugs (Tonkin and Hawking, 1947; Rivero and Gandon, 2018). The method of inoculating naive hosts with sporozoite stages that had been rendered inactive, one that is being tested in humans now (2019), was first developed in an avian malaria system (Rivero and Gandon, 2018). A large number of researchers continue to use avian malaria as a model system for studying parasite-host interactions and diversification. The attractiveness of avian malaria as a system lies in the fact that it is relatively easy and cost-effective to sample large numbers of birds from a variety of species in a given habitat via mist-netting and drawing a small blood sample. Haemosporidian-specific primers are available that allow the samples to be rapidly screened for the presence of parasites and identified to lineages by sequencing. Comprehensive and publicly accessible databases can then be assembled (Bensch et al., 2009) so that comparative studies of host use and diversification are possible. Through this type of molecular work on avian systems, over 3,000 different lineages of malaria parasites have been reported from all over the world, with some authors estimating that the number of species of avian malaria parasites may be as high as 10,000 (Bensch et al., 2004). One pattern that has emerged from this work is that avian malaria parasites can exhibit host generalism (with a broad host range), infecting large numbers of distantly related species of birds, or host specialization (with a narrow host range), infecting a small number of closely related host species (Martínez-de la Puente et al., 2011; Svensson-Coelho et al., 2014; Ellis et al., 2015). The reasons for the higher abundance, diversity, and variation in host infection patterns exhibited by the avian malaria parasites relative to other malaria parasites are poorly understood and have led to an increased interest in avian haemosporidian research in recent years (Bensch et al., 2009).

Although important in early laboratory studies of malaria, the popularity of using birds as a model system waned substantially when the rodent malaria parasites were successfully cycled in laboratory mice. However, because the rodent malaria system involves just a small set of closely related parasite species that are used to infect an unnatural host species, there has been a recent resurgence in using birds as experimental systems with which to study the biology of malaria parasites (Rivero and Gandon, 2018). These studies have been accelerated by the ability to sequence the first genomes of avian malaria parasites (Bensch et al., 2016; Lutz et al., 2016a; Böhme et al., 2018) as well as transcriptome studies

Figure 3. Ronald Ross. Sir Ronald Ross (1857–1932) was a British medical doctor whose work in India on both avian and human malaria parasites resulted in the discovery that mosquitoes transmit infective stages between vertebrates. He won the Nobel Prize in Medicine in 1902. Photo source: United States National Library of Medicine Digital Collections, https://collections.nlm.nih.gov/catalog/nlm:nlmuid-101427700-img. Public domain.

that can be done quite easily in bird hosts (Videvall et al., 2015; Weinberg et al., 2018).

Examples of some of the questions that are easily addressed using avian malaria parasites include studies on parasite virulence in relation to parasitemia in the host (Palinauskas et al., 2018) and costs to the reproduction and survival of parasites in the mosquito vector (Pigeault et al., 2015; Yan et al., 2018; Vézilier et al., 2012). One of the most exciting recent discoveries involving avian malaria parasites was that infected birds showed a marked shortening in their telomeres, the ends of chromosomes, which are thought to be related to life span in vertebrates in general (Asghar et al., 2016; Remot et al., 2022). This discovery prompted similar examination of telomere length in malaria-infected humans and showed that cell-death is induced by *Plasmodium* infection in our species as well (Asghar et al., 2017).

Avian malaria parasites have also been shown to have negative impacts on naïve host populations in at least one tragic case where the parasite was accidentally introduced to a region. In the early 1800s, *Culex* mosquitoes were accidentally introduced to the Hawaiian Islands and a few decades later, *Plasmodium relictum* was also brought there. An endemic transmission cycle was established, which quickly spilled into the native Hawaiian avifauna and likely contributed to their extinctions of some species (van Riper et al., 1986; Atkinson and Samuel, 2010; Samuel, et al., 2011).

Malaria Parasites of Squamate Reptiles (Class Reptilia: Order Squamata)

As with birds, the malaria parasites of reptiles are also geographically widespread (occurring on every continent except for Antarctica) and diverse, with over 100 described species (Telford, 2008). They infect a large number of squamates as hosts including over 10 different families of lizards and 3 species in snakes.

In lizards, the pathology of haemosporidians has only been well studied in 2 different systems, with varying results. In western fence lizards (*Sceloporus occidentalis*) that are infected with *Plasmodium mexicanum*, serious fitness consequences from infection were observed (Schall, 1990). Male lizards with these parasites were less likely to be able to defend a territory and infected female lizards laid fewer eggs per clutch. However, in another system, the Saban anole and its *Plasmodium* parasites in the Caribbean, these results were not found—infected and uninfected lizards showed similar reproductive success and survival (Schall and Staats, 2002).

The malaria parasites of lizards have been used as a model system to study a variety of host-parasite relationships, including the role of these parasites on sexual selection (Schall, 1983; Schall and Staats, 1997), the evolution of sex ratios for optimal transmission (Schall, 2000; 2009; Osgood and Schall, 2004; Neal and Schall, 2010; 2014; Neal, 2011), and island biogeography and parasite diversification (Mahrt, 1987; Perkins, 2001; Falk et al., 2015).

Malaria Parasites of Rodents

The rodent malaria parasites represent an unusual case where the parasite was first discovered in the insect host as opposed to the vertebrate one. In the 1940s entomological surveys in what is now the Democratic Republic of the Congo discovered a new species of *Anopheles* mosquito and some of them were found to contain sporozoites in their salivary glands. Given that this was long before DNA sequencing

could be used to identify the hosts that they had fed on, assays that tested interactions with blood proteins were used, and rodents were identified as the likely source. A few years later, *Grammomys surdaster* (order Rodentia: family Muridae), the African woodland thicket rat were found infected with the parasites and the *Plasmodium* was successfully inoculated into white laboratory mice—and the rodent-malaria model system was born (Killick-Kendrick and Peters, 1978).

In a short span of time, 4 main species of rodent malaria were described and established as culture systems in laboratory mice. This model system played major roles in the early laboratory studies and characterization of malaria parasites, including early cell biology as well as genetic and immunological studies. The system was so important that the genome of a rodent malaria parasite, *Plasmodium yoelii*, was the very next to be sequenced following the publication of the *P. falciparum* genome (Carlton et al., 2002).

Malaria Parasites of Other Mammals

There are several other groups of mammals that are natural hosts for malaria parasites, including other primates, bats, and ungulates. However, generally these malaria parasites of other mammal groups have been less intensively studied than the model malaria parasites of humans and rodents.

Bats played an important role in the discovery of malaria parasites as it was Dionisi who first observed the cells in their blood as far back as 1898 (Perkins and Schaer, 2016). Given the dispersed nature of bats as hosts in the phylogeny of haemosporidians (Figure 2), they are also likely to be important transition hosts between bird hosts and other mammal hosts (Lutz et al., 2016b; Perkins and Schaer, 2016). Four primary genera have been found in various groups of bats worldwide. These include *Plasmodium* in Africa, *Hepatocystis* in Africa and Asia, *Nycteria* in Africa, and *Polychromophilus* from Africa, Europe, Central America, and South America. Several other monotypic genera (that is, a genus with a single species) have also been described from bat hosts, but their status will remain uncertain until genetic data can be collected. What was most interesting about the first molecular systematic studies of bat malaria is that they showed a very close relationship with the rodent malaria parasites that are so popular now as laboratory models (Schaer et al., 2013). The bat hosts of these parasites roost in trees that likely overlap ecologically with the arboreal thicket rats that serve as the natural hosts for the rodent-infecting *Plasmodium* species.

Several species of *Plasmodium* have been described from various ungulates including buffalo, goats, and small antelope. *Plasmodium* was also identified in a single white-tailed deer that had had its spleen removed in the southern United States (Garnham and Kuttler, 1980). Although deer are abundant in the eastern United States, the parasites in deer were not observed again until just recently, when several animals in Washington, DC, and other sites were shown to be infected by this parasite, now named *P. odocoilei* (Martinsen et al., 2016). Around the same time, other researchers also reported malaria parasites in hooved hosts ranging from goats in Africa to water buffalo in Thailand (Templeton et al., 2016). Phylogenetic analyses show that all ungulate malaria parasites discovered thus far are part of the same clade, but also that this clade is not truly part of the genus *Plasmodium*, but rather likely a distinct genus (Galen et al., 2018). The pathology of the white-tailed deer parasites and their tendency to infect and be virulent in very young animals is of interest to biomedical researchers, however, as although they are distantly related, this life history may mean that these parasites could serve as a model for *P. falciparum* infection in humans (Guggisberg et al., 2018; Perkins, 2018).

Malaria parasites have also been reported in 2 other groups of mammals: The colugos of Africa and elephant shrews of Malaysia (Perkins and Schaer, 2016). In both of these cases, there are many other species of haemosporidians known from the region, however, suggesting expanded host range. Nonetheless, the distribution of malaria parasites in mammals worldwide presents a puzzling pattern. There are no known haemosporidians from major groups of mammals such as carnivores or lagomorphs, and relatively few malaria parasite species have been described from the 2 largest orders of mammals (rodents and bats) and those that are known from these mammals have restricted geographic distributions.

Malaria Parasites of Humans

There are several species of *Plasmodium* that use humans as their hosts. In the past decade, many closely related lineages of parasites have been discovered to infect wild apes or other primates with a potential to also infect humans. The genetic divergences and host specificity among these novel ape malaria parasites are subjects of much study, thus it is likely imprudent to give an exact number of the taxa that do or could infect humans (McFadden, 2019). The 5 most common species that are found in humans are discussed below.

Plasmodium falciparum

Plasmodium falciparum, sometimes referred to as malignant tertian malaria, is the most virulent of the human-infecting species (the term tertian stems from the fact that the fevers from this infection become synchronized to every 2 days, and the Romans who first classified it as such did not use the concept of zero). It is widely distributed throughout

Figure 4. *Anopheles arabiensis*, one of the primary insect hosts of *Plasmodium falciparum*. Source: United States Centers for Disease Control and Prevention Public Health Image Library, image 18749; J. Gathany, 2014. Public domain.

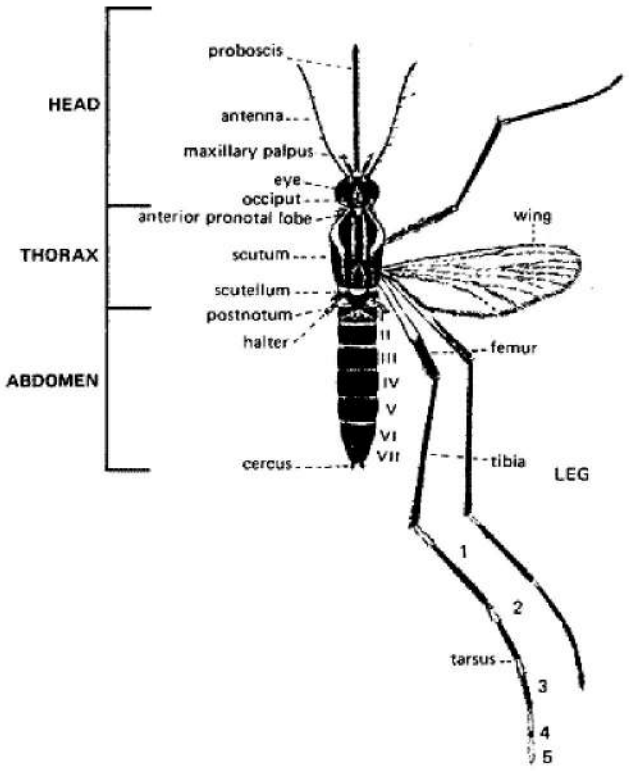

Figure 5. Mosquito morphology (female). Source: United States Centers for Disease Control and Prevention. Public domain.

the tropics, but is primarily concentrated in sub-Saharan Africa, Southeast Asia, and Oceania with known foci in South America. The reasons for the high virulence of this species are 2-fold. First, *P. falciparum* will invade any type of red blood cells and so can reach higher parasitemia than the other human parasites. Second, cells infected with *P. falciparum* become what might be thought of as sticky due to proteins expressed onto their surface as well as rigid and unable to bend, making it highly likely that they accumulate in capillaries causing a phenomenon known as sequestration. Sequestration in the brain or other vital tissues can cause death.

Plasmodium falciparum is transmitted among people primarily by the mosquito *Anopheles gambiae*, though many other species of *Anopheles*, such as *A. arabiensis* (Figure 4; see also Figure 5), are capable of transmission, depending on the geographic region (Molina-Cruz et al., 2016). In infected people the early trophozoite stages, which are called ring stages, are sometimes observed on thin blood smears but the mature stages are typically not observed due to the tendency of this species to sequester. *Plasmodium falciparum* is unusual amongst most mammal-infecting haemosporidians in that its gametocytes are crescent-shaped, rather than rounded (Figure 6A).

Virtually all human deaths attributed to malaria are caused by *Plasmodium falciparum*. It is currently present on all continents except for Europe (and Antarctica), but the largest proportion of fatalities is in children under 5 years-old who are living in sub-Saharan Africa. Because of its enormous global health importance, *P. falciparum* was the first malaria parasite—and one of the first organisms—to have its genome completely sequenced (Gardner et al., 2002).

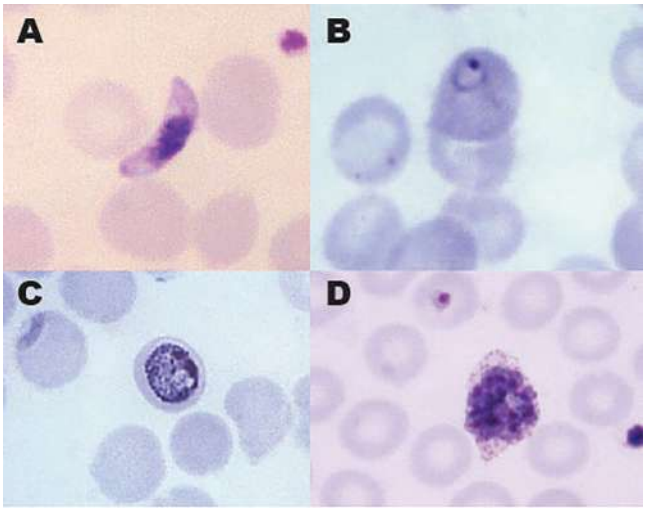

Figure 6. Stages of the 4 most common human malaria parasites. A) Gametocyte of *Plasmodium falciparum*. B) Ring stage of *P. vivax*. C) Trophozoite of *P. malariae*. D) Schizont of *P. ovale*. Source of photos: United States Centers for Disease Control and Prevention Public Health Image Library (A, image 4905; M. Melvin, 1966; C, image 5838; S. Glenn, 1979; D, image 5846; S. Glenn, 1979). Public domain.

Plasmodium vivax

Plasmodium vivax, also known as benign tertian malaria, is also globally distributed and in the not-so-distant past, was even present in eastern cities of the United States such as Washington, DC, Philadelphia, and New York City. Because it is so geographically widespread, the economic burden of this parasite is very large—almost 3 billion people worldwide live in areas where *P. vivax* is present (Battle et al., 2012).

Although *Plasmodium vivax* is colloquially referred to as benign, it also has major health effects on its hosts, similar to those of *P. falciparum*, including anemia, jaundice, and even cerebral malaria (Bourgard et al., 2018). However, from an evolutionary standpoint *P. vivax* is more closely related to what are typically referred to as the macaque malarias, including *P. cynomolgi* and *P. knowlesi* (Galen et al., 2018). Unlike *P. falciparum* which is flexible in the blood cells it infects, *P. vivax* has a strong preference for using the reticulocytes of the host (Galinski and Barnwell, 1996; Galen et al., 2018) and so has a much lower parasitemia with only ring stages typically present in circulating blood (Figure 6B). It also produces the gametocyte stages much earlier in the vertebrate host, as early as 4 days even before clinical symptoms might present, a factor that could promote its transmission to new mosquitoes (Bourgard et al., 2018). What is perhaps most notable about *P. vivax*'s life cycle, however, is its presence of stages that remain viable in the liver called hypnozoites (Markus, 1980) that can later trigger a relapse in the disease.

Unlike *Plasmodium falciparum*, it has not been possible to culture *P. vivax* parasites in vitro in the laboratory, therefore it has been more challenging to work on this species. However, because of its great importance, the complete genome of *P. vivax* was one of the first malaria parasite genomes to be sequenced and was completed in 2008 (Carlton et al., 2008; Bourgard et al., 2018), opening up many new approaches to studying the biology of the parasite for its control.

Plasmodium malariae

Plasmodium malariae (Figure 4C), or benign quartan malaria has a 3-day periodicity (again, remember the lack of zero when it was given this name by the Romans). Recognized by the ancient Greeks, it was not until the late 19th century that Golgi made careful note that there seemed to be 2 parasites infecting people—1 with fevers every 48 hours (that is, tertian malaria) and 1 that had a slightly different periodicity, which he correlated with slight differences in the parasites that he observed in the patients' blood (Garnham, 1966). *Plasmodium malariae* occurs throughout the world as well, but is most common in sub-Saharan Africa and the southwest Pacific though it can be very challenging to detect with just examination of blood films and thus molecular techniques such as PCR (polymerase chain reaction) are important to use (Garnham, 1966; Mueller et al., 2007).

In the early 1900s, a circus monkey was subjected to studies of its blood and a malaria parasite, similar in morphology to *Plasmodium malariae*, which was discovered and subsequently described as *P. brasilianum* (Garnham, 1966). These parasites were later observed in many wild monkeys in Central America and South America and for over a century were considered to be close relatives of—but not the same as—the human parasite. However, recent genetic results showed that these parasites were extremely similar (Fandeur et al., 2000), and in 2015, parasites that were genetically identical to *P. brasilianum* in wild howler monkeys were isolated from Indigenous Yanomami people living in Venezuela (Garnham, 1966; Lalremruata et al., 2015). When the complete genomes were sequenced, *P. malariae* and *P. brasilianum* were found to be the same species. A separate parasite, termed *P. malariae*-like, which was isolated from chimpanzees has since been found to be distinct (Rutledge et al., 2017).

Although *Plasmodium malariae* is typically considered a more benign form of malaria, it should not be dismissed as a public health concern. Because it can be difficult to diagnose with microscopy alone, it often goes undetected and may result in a fatal kidney disease (Eiam-Ong, 2003; Rutledge et al., 2017).

Plasmodium ovale

Until recently, *Plasmodium ovale* (Figure 6D) was generally considered to be the rarest form of the malaria parasites infecting humans. *Plasmodium ovale* has a rather scattered geographic distribution that primarily consists of western Africa, eastern Indonesia and New Guinea, and the Philippines, though of course due to the high mobility of humans, these parasites have also been reported in many other parts of the world (Mueller et al., 2007). Like *P. malariae*, *P. ovale* has also had a somewhat tumultuous taxonomic history. It was originally considered to be a variant of *P. vivax*, but was eventually described as a distinct species and named for the oval shape that some infected erythrocytes assume (Collins and Jeffery, 2005). Recently it was further split into 2 nominal subspecies on the basis of genetic data, *P. o. wallikeri*, and *P. o. curtisi* (see Sutherland et al., 2010).

Plasmodium knowlesi

In 2004, after a large number of malaria cases in Malaysian Borneo that were thought to have been *Plasmodium malariae* failed to amplify with species-specific primers, additional genetic testing confirmed that they were, in

fact, naturally acquired infections of *P. knowlesi*, a parasite thought to be confined to macaques (Singh et al., 2004). *Plasmodium knowlesi* was later reported from mainland Malaysia as well as in isolated cases in Thailand and in China, though likely the latter was acquired in Myanmar (Singh et al., 2004; Cox-Singh et al., 2008). It has the shortest of periodicities of the human parasites, completing a cycle in just 24 hours and can reach extremely high, even fatal, human parasitemias and so proper diagnosis of this species and distinguishing it from *P. malariae* is very important (Cox-Singh et al., 2008).

Malaria Parasites in Apes

In the early part of the 20th century, parasitologists working in western Africa discovered 3 species of *Plasmodium* infecting both wild chimpanzees and gorillas. Although there were similarities to the species known to infect humans, distinct names were given to these taxa nonetheless. In 1 of these cases, *P. reichenowi*, genetic material was available as the parasite was isolated and cultured from a chimpanzee that had been imported into the United States. When molecular systematic analyses using parasite DNA were first attempted, the resulting phylogenetic trees supported the idea that *P. reichenowi* was closely related to *P. falciparum*, but nonetheless was a distinct species (Escalante et al., 1998; Perkins and Schall, 2001). However, the larger picture of malaria parasites in apes was largely unknown until around 2010. Understanding of malaria parasites in apes began to change during this period, as new samples were collected, first from captive apes and then via the screening of a large number of non-invasively collected ape fecal samples. These results showed that there were many genetically divergent malaria parasite lineages present in African chimpanzees and in western gorillas (though interestingly, never in bonobos nor eastern gorillas even though these host species were very well sampled; Liu et al., 2010). A total of 4 species of ape malaria parasite have now been named that appear to be close relatives. The phylogenetic relationships amongst the 6 species of *Plasmodium* (*Laverania*) suggest that these parasites have shifted among human, gorilla, and chimpanzee hosts several times, although previous transferal experiments had suggested that they were largely host specific. The possibility that wild ape malaria parasites might be able to jump into human hosts as zoonoses is worrisome not only to public health officials, who see the apes as a large host population that is not treatable and may represent a reservoir of the parasites, but also to conservation biologists, who are concerned that parasites that have undergone selective pressure in humans might be more virulent in the wild ape hosts.

Impact on Human Genetics

Because of the enormous impact on human health, it is not at all surprising that malaria has served as an important selective force throughout our history and in fact, the disease is thought to have been the strongest source of natural selection on human evolution at least in recent times (Kwiatkowski, 2005). Two prominent examples are often discussed, sickle cell anemia and Duffy coat receptors.

Sickle Cell Anemia

The primary molecule inside red blood cells—and in fact, the only significant protein inside mammalian red blood cells—is hemoglobin. This molecule is made up of 4 chains of amino acids with an iron group in the center. Hemoglobin is adept at binding to the 2 key molecules of aerobic respiration, oxygen and carbon dioxide, and serves as the transporter of these gases throughout the bloodstream of vertebrates. Mutations in the hemoglobin molecule have been identified that alter its function. One of these, known as HbS, can disrupt the structure of the red blood cell, making it fragile and likely to collapse into a sort of sickle shape as opposed to the normal round shape if the tension of the respiratory gases is abnormal. If a person has 2 copies of the hemoglobin gene with this mutation, they will suffer from sickle cell anemia, a painful, largely untreatable, and sometimes fatal condition. One would predict, therefore, that natural selection would have removed these alleles from the human genome. And yet, they persist to this day. The reason is that people who are heterozygous for the variant hemoglobin gene have about a 10-fold higher protection from the forms of malaria infection most likely to cause death (Allison, 1954; Ackerman et al., 2005). If a red blood cell of a person who is heterozygous for sickle cell is infected with *P. falciparum*, the S type hemoglobin polymerizes, which then stalls the growth of the parasites (Archer et al., 2018).

Duffy coat Receptors

In human populations native to sub-Saharan Africa, there has been an almost complete fixation of a mutation that causes red blood cells to not express a protein that is necessary for the merozoite stage of *Plasmodium vivax* parasites to invade them (Kwiatkowski, 2005). This is known as the Duffy blood group-negative phenotype and makes those who have it essentially immune from *P. vivax* and *P. knowlesi*-caused forms of malaria.

Fighting Malaria as a Disease: Nets and Drugs

When Jesuit priests returned from missions in South America in the early 17th century, they brought bark from trees that indigenous Americans chewed to prevent shivering

and other ailments, hypothesizing that it might also be helpful with the shivering that accompanied the fevers of malaria (Meshnick and Dobson, 2001). It did have some success in treating malaria patients (remember that at this point, they still did not know exactly how the disease was transmitted from person to person) and became widely known in Europe as the fever tree, Jesuits' bark, or Peruvian bark. Around 1820, French chemists successfully extracted the main chemical component of the bark—quinine. Explorers searched the New World for trees that produced the highest concentrations of quinine and eventually seeds of *Cinchona ledgeriana* were used to start large plantations in Indonesia by the Dutch, and they controlled most of the world's production of quinine. (An interesting side note is that because of its bitterness, it was often mixed with spirits to make it more palatable—this may have inspired the gin and tonic cocktail!)

Synthetic antimalarial drugs, particularly the drug chloroquine, became widely used especially in World War II. Chloroquine was very effective; it worked by disrupting the parasite's ability to break down hemoglobin in the host cell and it was so widely used that in fact at one point it was sometimes mixed with table salt for mass distribution in malaria-endemic parts of the world. However, by the early 1960s *Plasmodium falciparum* parasites evolved resistance to chloroquine and the resistance quickly swept throughout most of the world. Mefloquine, sometimes known as Lariam®, and atovoquinone, often referred to as Malarone®, are 2 commonly used drugs that travelers to malarious parts of the world might be prescribed, though they are not safe to take for long periods of time (thus not usable for those people that live in malaria-endemic regions) and the parasites have evolved resistance to these compounds as well. In fact, there is not an anti-malarial drug that has been found or synthesized that the parasites have not evolved resistance to (Haldar et al., 2018). A compound known as qinghaosu or artemisinin (and its derivatives) that was used for centuries in China is now being produced and marketed, particularly in regions of Asia where parasites have evolved resistance to most of the other common anti-malarial compounds. It is a very effective drug and can even help patients in which the parasites have begun to sequester in capillaries. In most uses, it is administered along with other antimalarial drugs that have longer half lives in the body in an approach known as artemisin-combination therapy (White, 2008).

The other side of the malaria control coin is preventing people from acquiring the parasites in the first place, by stopping them from getting bitten by the mosquito vectors. This has been attempted through 3 main tactics: 1) Spraying insecticides, 2) poisoning the water sources where mosquito larvae are found, and 3) encouraging people, particularly children, to sleep under bed nets (Figure 7). All of these have had challenges or risks. In the 1950s there was a massive campaign to spray the insecticide DDT as a means of controlling mosquitoes and other insects. Although it worked very well to decrease mosquito populations, scientists quickly learned that this chemical exhibited bioaccumulation, or increased concentration as it moved up the food chain, ultimately being banned as a substance in the United States because of its severe environmental consequences. And, like the antimalarial drugs, the mosquitoes often quickly evolve resistance to these insecticides, causing them to lose their effectiveness (Hemingway et al., 2016). It is clear that combinations of methods and coordinated public health programs are going to have to be employed if malaria cases are going to be controlled, let alone if there is any hope of eradication of the disease. Some have argued that in order to be successful, evolutionary theory will need to be deployed into models of malaria control because these rapidly reproducing insects can more readily adapt to human-made chemicals than can be discovered and brought market (Read et al., 2009; Hemingway et al., 2016).

Figure 7. Children sleeping under a bed net. Ambitious programs to distribute insecticide-treated bed nets in malaria endemic areas has resulted in many fewer deaths in the past decade. Source: United States Global Health Initiative, 2006, https://commons.wikimedia.org/wiki/File:Malaria_prevention-Insecticide_treated_bed_net-PMI.jpg. Public domain.

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11

Protozoa

Trypanosomatidae

Trypanosoma (Genus)

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Phylum Euglenozoa

Class Kinetoplastea

Order Trypanosomatida

Family Trypanosomatidae

Genus *Trypanosoma*

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Chapter 11

Trypanosoma (Genus)

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Introduction

It is difficult to know exactly where to begin the introduction of the flagellated eukaryotic parasites that are classified in the genus *Trypanosoma*. This is because there is so much that can be learned about parasitism from the study of the morphological characters of these organisms; or by studies of how their populations cycle through individual mammals; or how they grow and reproduce in their insect vectors; or how these parasites are maintained in populations of mammals and their arthropod vectors; there is even a species that jumps from host to host without the inconvenience of having to use a vector; it has evolved past the need for a vector and instead uses the behavior of its infected host to transfer to a new potential host. However, starting off with a general classification of the group is generally a good idea, so that students who are just beginning, or even an advanced student, can see where the parasite belongs in the general scheme of life.

The trypanosomes are a **monophyletic** group (meaning, having a common origin from a single ancestral species) of single-celled eukaryotes (**eu** = true, **karyon** = nucleus; Greek), or those organisms that have a true nucleus. Obligate parasites of the genus *Trypanosoma* are included within the superorder Kinetoplastida. One of the current classifications looks like this:

Domain Eukaryota
Phylum Euglenozoa
Class Kinetoplastida
Order Trypanosomatida
Family Trypanosomatidae
Genus *Trypanosoma*

As seen in the list above, all known trypanosomatids are classified in the order Trypanosomatida, family Trypanosomatidae (see Moreira et al., 2004).

Members of this unique family are characterized by the single celled organism having an elongated cell body containing one flagellum emerging at the anterior part of the parasite and a single mitochondrion that is distributed throughout the cell body (see Figure 1 for details about the general morphology). The compressed DNA of this mitochondrion is called a **kinetoplast**, which is composed of **maxi-circles** and **mini-circles**, and is situated close to the base of the **flagellar pocket** (the place in the body from which the flagellum originates). Maxi-circles encode the genes required to perform the physiological function of oxidative phosphorylation that occurs, for example, in the midgut of the tsetse fly, and mini-circles are responsible for mRNA editing (Shlomai, 2004).

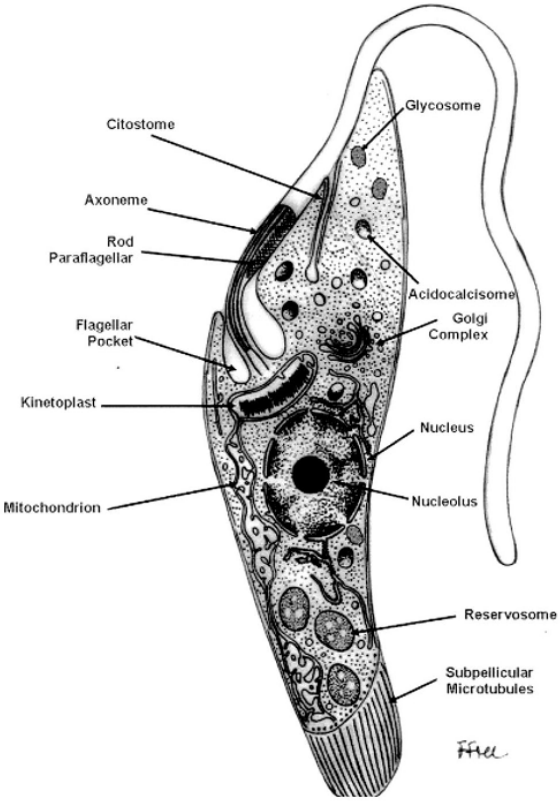

Figure 1. Schematic drawing, based on information obtained with the transmission electron microscope, showing the various structures found in the epimastigote form of *Trypanosoma cruzi*. Source of diagram: Souza, 1999. License: CC BY 4.0.

While species included in the family Trypanosomatidae have variable and distinct life histories, all species are obligate parasites. This family includes predominantly **monoxenic** forms (meaning, living on one host species), but also includes 4 genera with **heteroxenic** species (meaning, living in more than one host species) that are parasites of: 1) Plants (*Phytomonas* spp.); 2) sloths (*Endotrypanum* spp.); 3) other mammals and lizards (*Leishmania* spp.); and 4) all vertebrate classes (*Trypanosoma* spp.). Relatively recently, a fifth genus was proposed (*Porcisia* sp.) to include Neotropical porcupine-infecting species, previously known as *Leishmania hertigi* and *L. deane* (see Espinosa et al., 2018).

Concerning the monoxenic trypanosomatids, currently from 14 to 17 genera of parasites have been recognized, including those parasitizing Diptera, Hymenoptera, Siphonaptera, and Hemiptera. These monoxenic forms are distributed among the genera: *Angomonas*, *Blechomonas*, *Blastocrithidia*, *Crithidia*, *Herpetomonas*, *Kentomonas*, *Leptomonas*, *Lotmaria*, *Novymonas*, *Paratrypanosoma*, *Sergeia*, *Strigomonas*, *Wallacemonas*, and *Zelonia* (see Kaufer et al., 2017), as well as *Jaeinimonas, Lafontella*, and *Rhynchoidomonas* (see D'Ávila-Levy et al., 2015). Although described as monoxenic insect parasites, this trait (occurring in insects) seems not to be strict, since some of them have been reported parasitizing mammals, probably after being transmitted by insects. In fact, co-infections of *Leptomonas seymouri* and *Leishmania donovani* have been reported in patients with visceral leishmaniasis (Ghosh et al., 2012), and records of concomitant infections by *Leptomonas* sp. and *Herpetomonas samuelpessoai* in a HIV-positive human patient (Morio et al., 2008). In addition, there are records of *Herpetomonas* species infecting plants (Borghesan et al., 2013), and reports of *Blastocrithidia* sp. and *Chritidia mellificae* occurring in bats (Hodo et al., 2016; Rangel et al., unpublished data). Interestingly, when experimentally injected in the scent glands of opossums, *Leptomonas* sp. and *Chritidia* sp. not only established the infection, but also multiplied. The parasitism of scent glands of *Didelphis* spp. by monoxenic trypanosomatids was interpreted by Deane et al. (1984) as being the stepping-stone of trypanosomatids on their way to adapt to a mammal host.

Species of the genus *Trypanosoma* are parasites of vertebrates that are, with a few exceptions, transmitted among vertebrates by invertebrate vector hosts (Figure 2). Four primary morphological stages are recognized among trypanosomes: The extracellular trypomastigote, epimastigote, and spheromastiogote forms, and the intracellular amastigote form. These morphological forms occur in various stages among trypanosomatids with different species sometimes manifesting these forms differently. In 1972, the British protozoologist Cecil Hoare, in his foundational monograph, proposed the separation of the genus *Trypanosoma* in 2 **sections** based on the **transmission mode** of these parasites: **Stercoraria** and **Salivaria** (see Hoare, 1972). The divergence between these 2 sections would have occurred in the Cretaceous period (approximately 100 Ma = million years ago), accompanying the breakup of Gondwanaland and the separation of Africa from South America, Antarctica, and Australia. According to this classification that was widely accepted and used up to the current time, there are 3 sub-genera in **Stercoraria section**: *T.* (*Herpetosoma*) sp., *T.* (*Megatrypanum*) sp., and *T.* (*Schizotrypanum*) sp.; and 4 in **Salivaria section**: *T.* (*Dutonella*) sp., *T.* (*Nannomonas*) sp., *T.* (*Trypanozon*) sp., and *T.* (*Pycnomonas*). An eighth subgenus, *Tejeraia*, was proposed at a later date to include *Trypanosoma rangeli,* a South American parasite previously included in the *Herpetosoma* subgenus, but, as it later became clear, it is in fact transmitted by several hematophagous triatomine species (Añez, 1982).

The recent molecular tools with their great discrimination power that have been developed in the last decades have resulted in extensive revisions and descriptions of new genera and species. In fact, classifications up to 3 decades ago were made based on parasite morphology combined with the host species in which trypanosomatids were found. Another important point is the attention that was classically given to trypanosomatids with enzootic potential or potential to affect animals of economic interest. The current awareness of the importance of biodiversity (and parasites as important components of this) has widened the focus of attention in order to increase the interest in parasites not necessarily of humans or of domestic animals (Gardner and Campbell, 1992; Poulin, 2014; Jenkins et al., 2015).

Section Salivaria

Trypanosomes included in this section are highly prevalent in sub-Saharan Africa in the so termed Tsetse-Belt region and represent important health threats for humans and other animals with concomitant economic impacts in the areas of occurrence. Several species of the section Salivaria occur and can be found being transmitted in several countries beyond the African continent and the possibility of even greater geographic dispersion should not be neglected (Osório et al., 2008; Aregawi et al., 2019). Transmission occurs through inoculation of infective metacyclic forms as the insect vector feeds on blood of an infected mammal. For most species of salivarian trypanosomes but one, the only proven insect-vectors are various species of the hematophagous flies in the genus *Glossina* (the infamous and notorious tsetse flies of Africa). The only species that can be transmitted mechanically by other dipetera is *T. vivax*. *Glossina* spp. (Diptera: Glossinidae) (Figures 3 and 4) are the biological vectors of all salivarian trypanosomes and an individual fly may harbor

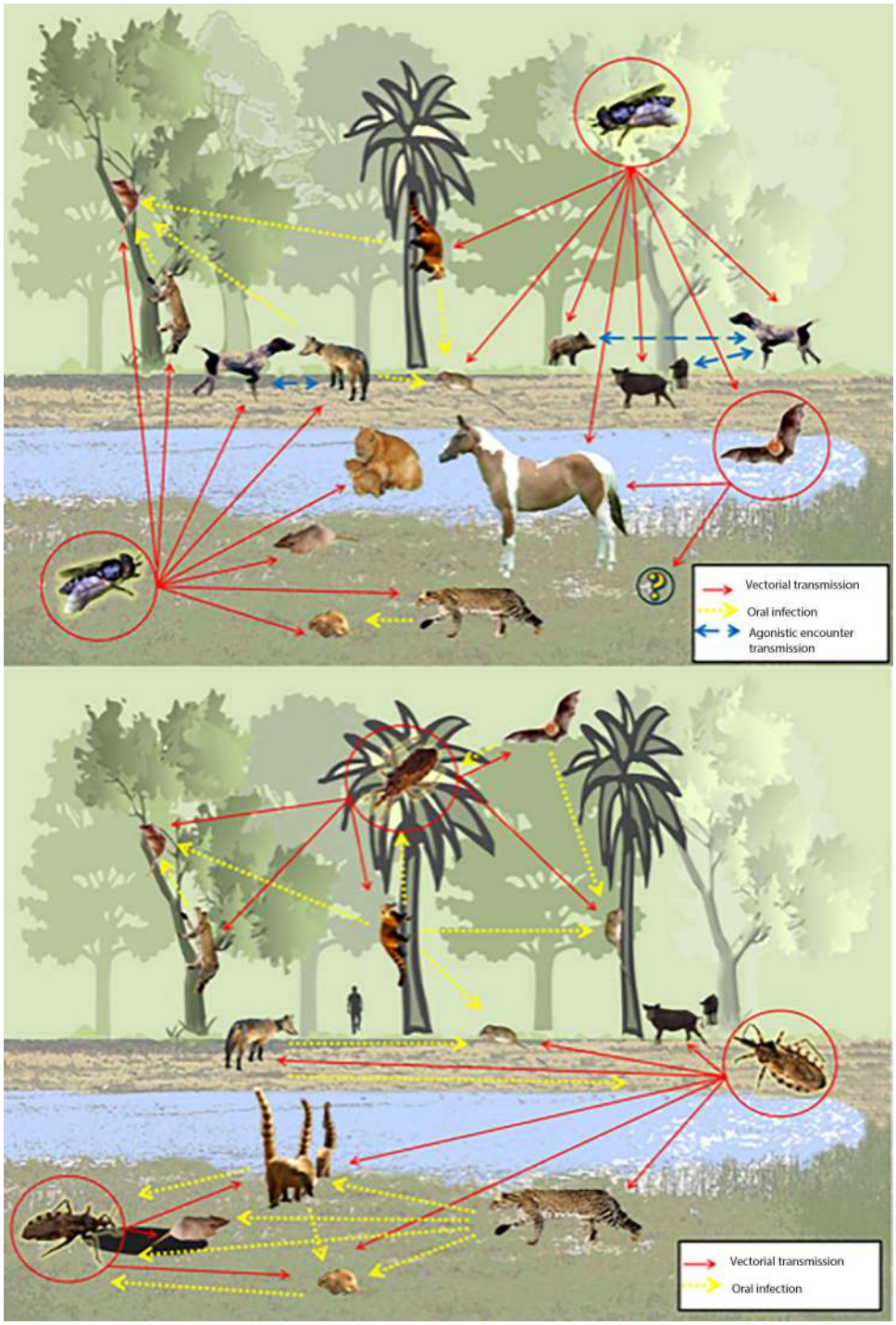

Figure 2. The wild and synanthropic reservoirs of *Leishmania* species represented graphically. Source: Rodrigues Roque and Jansen, 2014. License: CC BY-NC-SA 4.0 International.

more than one trypanosome species (Van den Bossche et al., 2004). All of the approximately 33 known species of *Glossina* that have been tested are able to act as vectors of salivarian trypanosomes; However, in contrast to many species of dipteran vectors, such as the blood-feeding Tabanidae, in which only females take blood meals, both sexes of *Glossina* are hematophagous and are capable of transmitting trypanosomes via saliva during blood feeding. Moreover, *Trypanosoma vivax* (another species of Salivarian trypanosome that lives in African mammals) can be transmitted by *Glossina* as well as by other hematophagous diptera (Fetene et al., 2021). Interestingly, *Trypanosoma evansi* does not appear to

Figure 3. A female tsetse fly (*Glossina morsitans morsitans*) from the Serap Aksoy Lab colony at Yale University School of Public Health. Tsteste flies transmit African trypanosomiasis. (Note: Taken by Geoffrey M. Attardo, this photo was a winner of the 2010 Fogarty Grantee Photo Contest, Fogarty International Center, United States, National Institutes of Health.) Source: G. M. Attardo, 2010. License: CC BY-NC-SA 4.0.

be transmitted by *Glossina* spp. and is mechanically transmitted from ungulate to ungulate by tabanids (order Diptera: family Tabanidae) and other hematophagous vector insects such as species of *Stomoxys*, *Atylotus*, *Lyperosia* (see Brun et al., 1998), and other slash and bite blood feeders such as *Desmodus rotundus* (common vampire bat) or *Diaemus youngi* the white-winged vampire bat in the Americas. *Trypanosoma equiperdum* the causative agent of Dourine in horses is a venereally-transmitted trypanosome and is generally considered to have a cosmopolitan (worldwide) distribution with the parasite generally absent in North America (north of Mexico), Australia, and western Europe (Brun et al., 1998; Gizaw et al., 2017).

The development of salivarian trypanosomes in the tsetse fly may be generally complex among *Trypanosoma* species and may be influenced by the insect immune response modulated by the fly's gut and symbiotic microbiota. Sexual reproduction in trypaonsomes has been reported, but only when the protozoans are actively reproducing within the insect vector-hosts (Gibson, 2015). Three interaction patterns of trypanosomes in the tsetse fly are recognized:

1) *Trypanosoma vivax* group (*Dutonella* subgenus) includes the trypanosomes with the lowest degree of interaction with the insect vector. This species is found across the Tsetse-Belt in Africa as well as in several countries in Latin America, occurring in wild and domestic animals. Recorded hosts for *T. vivax* include water buffalo, cattle, dogs, dromedary camels, horses, suids, and small ruminants. As noted, this species also occurs in wild animals that can serve as reserviors of infection for domestic animals. In this species development of the flagellates in the insect is restricted to the proboscis and cibarium where the parasite passes through 2 forms including both the **epimastigote** and **trypomastigote** stage. The entire life cycle of this species in the tsetse fly-vector is completed in as little as 3 days after initial infection. Forms of *T. vivax* in the blood stream of mammals are only of the monomorphic **trypomastigote** type. Representatives of this subgenus have evolved independently of tsetse flies and have adapted to mechanical transmission as noted earlier.
2) *Trypanosoma congolense* group *(Nannomonas* subgenus). Species included in this subgenus use a larger area of the digestive tract of the tsetse fly relative to species in the *Dutonnela* subgenus: In the gut, the ingested blood trypomastigotes differentiate into long trypomastigotes that migrate to cibarium and the proboscis of the flies where they differentiate into epimastigotes and, then, into metacyclic forms. Like *T. vivax*, mammalian blood stream trypomastigotes are also monomorphic.
3) Except for *T. evansi* and *T. equiperdum*, the representative of *T. brucei* group (*Trypanozoon* subgenus) are pleomorphic and go through a much more complex cycle in the vector. There are 2 proliferative forms in the fly, procyclic trypomastigotes in the gut and epimastigotes in the salivary gland and the entire life cycle can be completed in as little as 3 weeks (Sharma et al., 2009).

Salivarian trypanosomes may be highly pathogenic and lethal for their mammalian hosts, but a wide range of host species are tolerant to several of these trypanosome species. This is the case of native cattle breeds N'Dama and the West African shorthorn (WASH) (Ganyo et al., 2018). The basis of

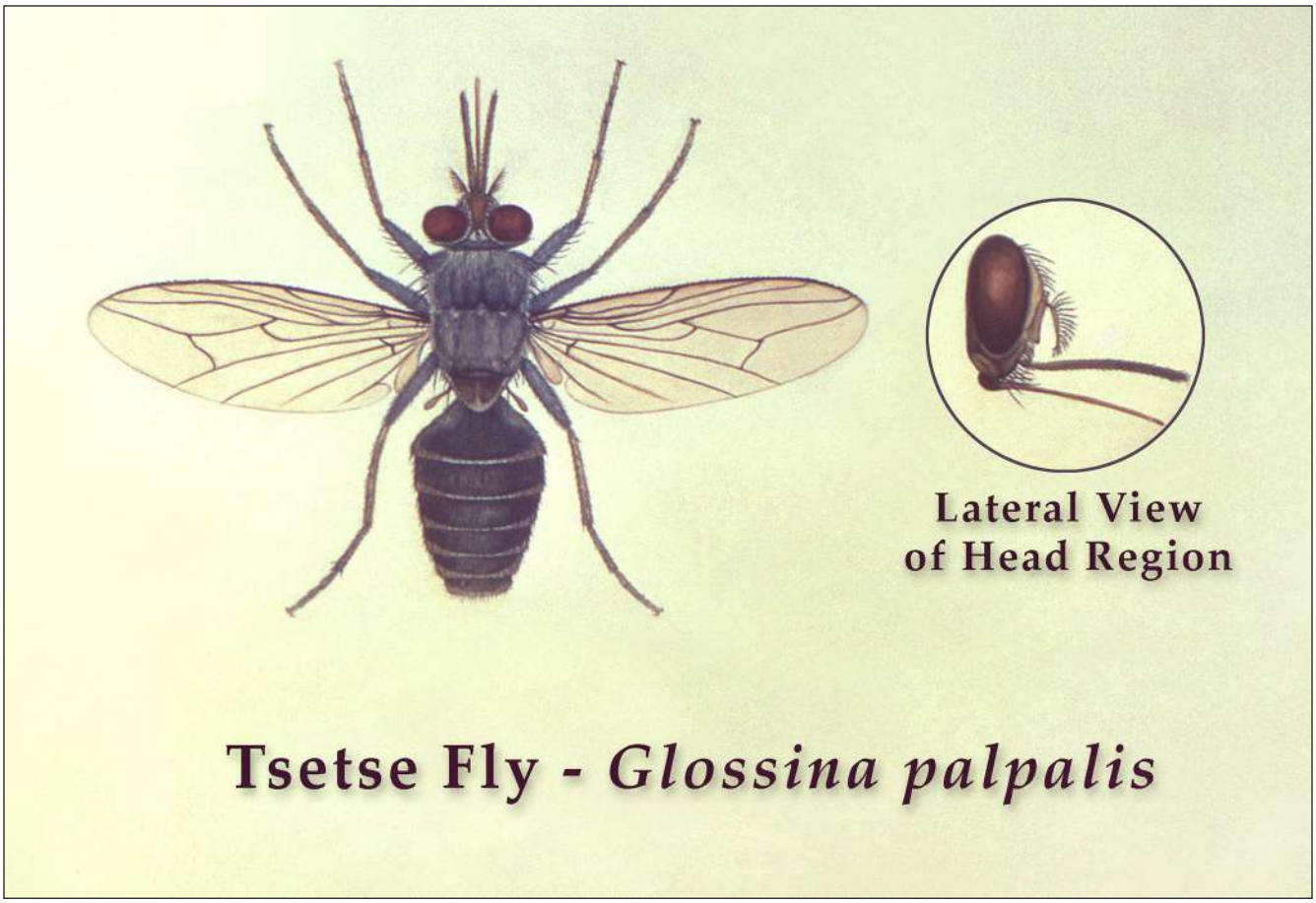

Figure 4. Tsetse fly, *Glossina palpalis*, top view with lateral view of head region. Source: United States Public Health Image library, image 17638; R. Darsie, 1976. Public domain.

this tolerance, although much studied with several hypotheses already formulated, is still a controversial subject. One unquestionable point is that whether the animals are trypanotolerant and trypanosusceptible is defined by their capacity to control anemia, which is the major outcome of the infection, and that determines whether or not host-animals remain competitive and productive.

The salivarian trypanosomes are not able to invade cells, and only extracellular trypomastigote and epimastigote forms are recognized. Trypomastigote forms are maintained in blood and body fluids of the infected mammal host. In the trypomastigote forms, 3 characteristics can be highlighted:

1) Two main types of blood trypomastigotes can be observed in most of the parasites from this section, including: a) Thin or slender forms are the dividing stage that are completely adapted to the mammal host, and b) broad or stumpy forms do not divide and possess well-developed mitochondria throughout the cell, and are adapted to the cycle in the vector dipterous host.
2) In the mammal host, where access to glucose is unrestricted, the mitochondrion is reduced in size and complexity and the parasite performs compartmentalized glycolysis, that is, within glycosomes. In this organelle, glucose is broken down into pyruvate and ATP, which is very effective whereas in the vector, where glucose is much more scarce, the now active mitochondria carries out an oxidative catabolism cycle generating CO_2, H_2O, and ATP oxidative cycle that results in CO_2 and H_2O; which is a more efficient mode of metabolizing glucose. Recently, it was shown that blood forms of *Trypanosoma brucei* are able to perform gluconeogenesis using glycerol as substrate for ATP production. The implications of this metabolic pathway are still unknown, but it demonstrates an important physiological plasticity. This pathway was suggested as being related to the passage of the parasite through tissues of its multiple host species (Kovařová et al., 2018). In the same way, gluconeogenesis was proposed as an important ATP source and is used by the procyclic forms in the midgut of the tsetse fly vector. In this environment, the parasite uses proline as substrate (Wargnies et al., 2018).

3) The ability of trypanosomes to sequentially modify their surface glycoproteins is an efficient escape mechanism from the mammalian host immune system. This variation in the surface coat of the protozoan are known as **VSGs** (**Variable Surface Glycoproteins**) initially described as **VATs (Variable Antigen Types)** (Barry et al., 1979). Salivarian trypanosomes live an extracellular existence in the blood and lymphatic system of the mammalian host and, are therefore, completely exposed to the humoral immune response, these parasites evolved to periodically alter all of their surface glycoproteins, as if changing a coat, in an efficient programmed mechanism where only one VSG is expressed at a time and is never repeated. It is estimated that there are about 10 million molecules anchored on the surface of these trypanosomatids being periodically exchanged, and about 2,000 vsg encoding genes regulating this process (Mugnier et al., 2016; Romero-Meza and Mugnier, 2020). This phenomenon is responsible for the parasitemia waves characteristic of mammals infected by salivarian trypanosomes. Also, their high motility, capacity of supporting non-immune defense mechanisms, and the mechanical forces inherent to blood circulation are important to survival in the mammal host (Stijlemans et al., 2016). The metacyclic forms of *T. brucei* develop in the salivary glands of the tsetse flies and also synthesize VSGs that; however, differ from the VSGs from the blood trypomastigotes (Kolev et al., 2017; Romero-Meza and Mugnier, 2020).

Tsetse flies live in moist savannah and woodlands, regions of more than 500 mm of rain a year, which include more than 30 countries across Africa (Cecchi et al., 2015). In those areas, human sleeping sickness is well-known and is caused by *Trypanosoma brucei rhodensiense* while *T. b. gambiense* causes Nagana, the non-human disease that is generally fatal in livestock and cycles in wild mammals (Büscher et al., 2017).

Humans are protected from infection by most of the African trypanosomes because of their production of a trypanolytic protein named Apolipoprotein 1 (APOL1), which is secreted by 2 protein complexes (TLF-1 and TLF-2) leading the formation of pores in the parasite membrane, resulting in its lysis. However, the 2 subspecies of *Trypanosoma brucei* associated with human sleeping sickness produce substances that are capable of lysing APOL1, the RAS factor in *T. b. rhodesiense* and TgsGP in *T. b. gambiense* (Capewell et al., 2015). Interestingly, baboons and one African human population named G1/G2 present a mutation in APOL1 that confers resistance to RAS and, therefore, to the infection by *T. b. rhodesiense*. The rare cases of humans that become infected with *T. b. brucei* and other species of trypanosomes are usually associated with people with some mutations that result in the absence of APOL1 production.

The genetic exchange that occurs solely in the insect vector of the salivarian trypanosomes, besides their huge repertoire of surface antigens, implies that new genotypes of salivarian trypanosomes may emerge to infect humans, and domestic and wild animals, and pose an important and worldwide health risk that would be magnified due to the absence of vaccines (Gibson, 2015).

Trypanosoma (Nanomonnas) congolense

Nannomonas trypanosomes include species that infect wild and domestic suidae (*Trypanosoma simiae* and *T. godfreyi)*, and *T. congolense*, a parasite that infects a broad spectrum of domestic and wild mammalian species and is the main cause of Nagana in Africa (Hamill et al., 2013; Morrison et al., 2016). These parasites are restricted to areas of occurrence of tsetse flies in sub-Saharan Africa and are described as extremely pathogenic for mammals (Cecchi et al., 2015). Some African breeds (Djallonke sheep, N'Dama cattle, and West African dwarf goats) are trypanotolerant, meaning that they are able to support the infection without anti-therapy and still maintain good health. The mechanisms underlying this tolerance probably depend on a genetic basis but this is still under debate (Yaro et al., 2016).

The trypomastigote forms are characterized by a flagellum that runs through the body of the parasite with a very short free end. The infection often starts with a skin lesion, a canker, where the parasites multiply, before reaching the bloodstream and lymphatic vessels of the host. In the vector, the multiplication takes place throughout the digestive tract before migration to the salivary gland, where the differentiation to metacyclic forms occurs (Dyer et al., 2013).

Human infection by *Trypanosoma congolense* has been only rarely reported (Truc, 1996). Compared to other African trypanosomes that infect livestock, *T. congolense* is considered as the most pathogenic, most prevalent, and most widely distributed trypanosomatid within the area of occurrence of *Glossina* spp. Three different genotypes of *T. congolense* are recognized: 1) The genotype Savannah, which is the most pathogenic and can affect a greater diversity of hosts, including carnivores; 2) the genotype Forest, described as poorly pathogenic and more often observed in cattle, goats, pigs, and dogs; and 3) the genotype Kilifi, considered as non-pathogenic and found infecting domestic ruminants (Rodrigues et al., 2014).

Trypanosoma (Trypanozoon) brucei

Trypanosoma brucei is the etiological agent of sleeping sickness in Africa and one of the few parasites able to cross the human blood-brain barrier, resulting in the nervous symptomatology observed in human disease. This parasite displays 3 subspecies: 1) *T. b. gambiense*, present in West Africa, associated with the more chronic form of human sleeping sickness, reported as less pathogenic; 2) *T. b. rhodensiense*, present in East Africa, associated with the acute form, the most pathogenic form of human disease; and 3) *T. b. brucei*, which infects wild and livestock animals, is associated with Nagana and is only rarely associated with human disease (Büscher et al., 2017).

The initial and recurrent symptoms of sleeping sickness are fever, tremors, muscle and joint pain, lymphadenopathy, malaise, weight loss, anemia, and thrombocytopenia. Later, neurological symptoms and meningoencephalitis can be present associated with mental retardation, convulsions, somnolence, and apathy that can progress to coma and death. This serious human disease is endemic in 36 African countries, including some epidemic areas in Angola, Democratic Republic of the Congo, and Sudan, and affects mainly people living in rural areas where tsetse fly is present. About 95% of human infections are caused by *Trypanosoma brucei gambiense* and commonly associated with *Glossina palpalis*. Those cases have a slower evolution of the disease and the infection can be present by months and even years without clinical signs. However, when the first signs appear, the disease is usually already in an advanced state and with the central nervous system compromised. The other 5% of the infections are caused by the *T. b. rhodensiense* and usually transmitted by *G. morsitans*. In these latter cases, the infection course is much faster and in a few months or even in a few weeks the disease progresses to the central nervous system presenting the characteristic neurological symptoms (Büscher et al., 2017).

In mammals, *Trypanosoma brucei* is always present in the extracellular trypomastigote forms and 2 morphotypes are recognized: 1) The slender forms, the divisionary forms that perform glycolysis in glycosomes and cannot survive in the insect vector; and 2) the stumpy forms, that do not divide in blood vessels, and display well developed mitochondrial ridges that are essential for survival in the vector.

Inside vectors, *Trypanosoma brucei* can colonize the whole digestive tract where both procyclic trypomastigotes and epimastigotes are present and can replicate themselves. The epimastigote forms that reach the salivary glands adhere to the microvilli of glands and perform cell division with subsequent differentiation to pre-metacyclic trypomastigotes, still attached to the salivary gland, but with fewer adhesion plaques. These pre-metacyclic trypomastigotes start to reacquire the coat of glycoproteins that are important in the blood phase of the infection. This form, named nascent metacyclic form, begins to detach itself from the glandular epithelium and will be totally free in the salivary gland when this differentiation is complete. At this point, the glycosomes are reverted to their spherical shape and the mitochondria are reduced to a small structure, which will no longer be functional while this parasite is in its mammalian host. The transmission will take place through the inoculation of these metacyclic trypomastigotes in the following blood meal (Vickerman et al., 1988; Dyer et al., 2013).

Trypanosoma (Dutonella) vivax

Trypanosoma vivax has a wide diversity of ungulate hosts; especially ruminants (order Artiodactyla). The infection occurs in Africa, Asia, Central America, and South America. Both wild and domestic ungulates (including buffaloes and antelopes); as well as equines and camels, are their most common hosts. Infection in laboratory animals has never been established (Osório et al., 2008). This parasite species can be transmitted both in cyclic and mechanical transmission. Cyclic transmission is reported in Africa, in areas where tsetse flies are present, and usually results in a more severe form of the disease. Mechanical transmission occurs in other parts of Africa (apart from the tsetse geographical area), in addition to Asia and the Americas, where the disease tends to be milder in cattle. In Brazil for example, outbreaks have always been associated with low mortality and few economic losses (Silva et al., 1996; Batista et al., 2007; Bastos et al., 2017).

In the mammalian host the trypomastigote forms are found exclusively in the blood. In *Glossina* spp., where the transmission is cyclic, this parasite differs in the replicative epimastigote forms that are restricted to earlier parts of the digestive tract. After the differentiation to trypomastigote forms, parasites migrate from the hypopharynx to the salivary gland where they differentiate into metacyclic trypomastigotes, which are the infective forms inoculated by the tsetse. Once in the salivary gland, *Trypanosoma vivax* can be maintained throughout the whole life of the vector. In mechanical transmission, there is no differentiation and the trypomastigote forms are carried from one mammal host to another by other hematophagous flies, especially those from the *Stomoxys* and *Tabanus* genera.

The first symptoms of *Trypanosoma vivax* infection are usually unspecific, such as anemia, fever, apathy, weight loss, and diarrhea. Diverse reproductive problems are associated with the infection, including transplacental transmission and abortion. When present, the neurological symptoms observed

are incoordination, muscle tremors, transient and/or permanent blindness, meningoencephalitis, and malacia (Batista et al., 2007; 2009).

Trypanosoma vivax was introduced into the Americas, probably with cattle brought from the European colonies in Africa. On this new continent, the parasite adapted to the mechanical transmission by several hematophagous insects, circulating among recently introduced domestic cattle and, perhaps, has spread among wild ungulates such as the cervids, never before exposed to this parasite. In Brazil, the first report of the parasite was in a buffalo in the swampy regions of Marajó Island, in the Brazilian Amazon (Shaw and Lainson, 1972). Later, in the late 1970s, this parasite was reported in sheep and cattle in the State of Amapá, also in Amazon region, and only a decade after this reported outside the northern region of the country, in cattle from the Brazilian Pantanal biome (Silva et al., 1996).

Possibly, the first outbreaks in the Pantanal were due to the increase of the displacement of animals from the north to the center-west of Brazil. In this biome, *Trypanosoma vivax* epidemiology is directly associated with drought and flood periods. In flooding periods, the reduction of pasture area increases the animal density per area, resulting in nutritional problems and resulting in higher susceptibility to infections, including trypanosomiasis (Silva et al., 1996).

Trypanosoma vivax can also be pathogenic for horses (da Silva et al., 2011). In both natural and experimental conditions, asinins were demonstrated to present high infection rates in subpatent and asymptomatic infections (Rodrigues et al., 2015). The infection of domestic ruminants by *T. vivax* results in severe economic losses, especially in South America. In spite of this, *T. vivax* remains poorly studied as a consequence of the inability to grow this trypanosome species in mice or in culture media.

Trypanosoma (Trypanozoon) evansi

The species *Trypanosoma evansi* masterfully exemplifies the genetic plasticity and consequent evolutionary success of salivarian trypanosomes. Together with *T. equiperdum*, *T. evansi* seems to have branched off from *T. brucei* due to the profound alterations of its kinetoplast DNA and, as a consequence, to have gained independence from a biological vector and to be transmitted mechanically, which has resulted in its enormous dispersion throughout Asia, the Americas, and Africa. The distinct kDNA alteration patterns observed in *T. evansi* samples suggest that *T. evansi* arose multiple times from a different *T. brucei* ancestor. These findings point to the necessity of revisiting the nomenclature of the members of the subgenus *Trypanozoon* (Radwanska et al., 2018).

Among all trypanosomes, *Trypanosoma evansi* is the one that is able to infect the largest variety of mammalian hosts, being dispersed in all continents (Desquesnes et al., 2013a). This parasite is the etiological agent of one of the major diseases affecting horses, called **Surra** (in the Old World) or **Mal de Caderas**, **Quebra Bunda**, or **Derrengadera** (in South America). The transmission is mechanical, being carried out normally by hematophagous flies from *Tabanus* spp. and *Stomoxys* spp. (Desquesnes et al., 2013b). The *Trypanosoma evansi* infection has been recently considered by OIE (World Organization for Animal Health) as a mandatory disease (Jaimes-Dueñez et al. 2017). Human cases are rare, but have been reported in Africa and Asia, usually associated with an extremely rare condition termed Tangier disease (Tomlinson et al., 1995).

Trypanosoma evansi belongs to the same *Trypanozoon* subgenus of the *T. brucei* species but is different from *T. brucei* whose transmission is totally dependent on cyclic transmission. *Trypanosoma evansi* is dependent only on mechanical transmission, even by tsetse flies. That is because, at some point on the evolutionary path of these parasites, *T. evansi* differentiated from *T. brucei* and lost the kDNA maxi-circles where most of the genes responsible for the oxidative metabolism and multiplication are located in *Glossina* spp., making *T. evansi* incapable of multiplying in the biological vector. Currently, some researchers suggest that this species comprises total or partial diskynetoplastic *T. brucei* strains. Although still considered to be a different species, some authors propose that *T. evansi* (and *T. equiperdum*) be classified as subspecies of *T. brucei,* or even variant strains of *T. b. brucei* (Carnes et al. 2015; Wen et al. 2016).

Trypanosoma evansi is a monomorphic parasite, and the trypomastigote form is the only recognized morphotype. Its trypomastigotes are the replicative forms observed exclusively in the blood of infected mammals and its morphology is exactly identical to the stumpy forms of *T. brucei*. Infection in mammals usually results in very high parasitemias, favoring mechanical transmission. Besides the mechanical transmission by hematophagous flies and the iatrogenic form through sharing of contaminated fomites, hematophagous bats display a differentiated feature, being able to act both as reservoir and vector of this parasite. That is because, in addition to being infected as all other mammalian hosts (which means that the parasite multiplies in the blood of these animals), it can still transmit the parasite that is easily present in its saliva during a blood meal (Desquesnes et al., 2013b). The oral route and agonistic encounters are also proposed as transmission routes in the wild (Herrera et al., 2011).

The estimated date of entry into South America by *Trypanosoma evansi* is still controversial. Although it is

hypothesized that *T. evansi* was introduced by infected horses of European colonizers (Desquesnes et al., 2013a), other authors propose that their introduction was much earlier, perhaps carried by the first primates and caviomorph rodents that came directly from Africa to South America, about 35–40 Ma (= million years ago). Caviomorph rodents, including capybaras, are considered to be important *T. evansi* reservoirs in the Pantanal region (Herrera et al., 2004) and infected rodents may have entered the Americas through the well described island-hopping or even sweepstakes dispersal processes (Lavocat, 1974; Raven and Axelrod, 1975; Flynn and Wyss, 1998).

In South America, trypanosomiasis caused by *Trypanosoma evansi* is economically very important in the flooded areas of the Brazilian Pantanal and Argentinean Chaco regions. These regions have a great concentration of livestock, and horses are essential for cattle handling. Outbreaks occur sporadically, though more common after a flood period, and result in a diversity of clinical features, from mild to severe fulminant forms (Herrera et al., 2004). In most severe cases, horses may present with anemia, emaciation, and subcutaneous edema of the lower body regions, with reports of abortion in pregnant females, and various types of neurological manifestations. The characteristic symptoms that inspired the name of the horse disease are the atrophy of the great muscular masses of the pelvic limbs, devolving to incoordination and ataxia (Desquesnes et al., 2013a).

The main wild reservoirs in the Pantanal region are the coatis and capybaras because they: 1) Are quite abundant in the region; 2) present high infection rates with long-lasting patent parasitemia; and 3) may remain infected for a long time. Capybaras support infection by *Trypanosoma evansi* without anemia and while maintaining good general health. Coatis, in contrast, when infected by *T. evansi* display anemia (Herrera et al., 2002; 2004). Infected horses may also act as reservoirs because the persistence of the parasite in asymptomatic animals after treatment is not rare (Herrera et al., 2004).

Usually reported in domestic animals around the world, studies on *Trypanosoma evansi* transmission in the wild are generally restricted to the Brazilian Pantanal. In this region, *T. evansi* can be considered a typical enzooty, with several infected wild mammals already found and occurring in sympatry with other trypanosomes, such as *T. cruzi* and *T. rangeli*. Interestingly, most infections observed in the wild seem to be subpatent and anemia is not commonly observed (Rademaker et al., 2009).

Trypanosoma (Trypanozoon) equiperdum

The other species within the *Trypanozoon* subgenus, also considered as a subspecies of *Trypanosoma brucei* or a mutant strain of *T. b. brucei* by some authors (Carnes et al., 2015; Wen et al., 2016), is *Trypanosoma equiperdum.* This species is the causative agent of **equine Dourine**, a disease that affects horses and other equidae. The transmission is exclusively venereal and the parasite is found only in genitals and their secretions. Asinines are usually reported as asymptomatic carriers of the parasite (Gizaw et al., 2017). Although as widespread as *Trypanosoma evansi* in the world, the reports of infection are quite intermittent and most studies are only case reports. The symptoms are similar to other trypanosomiases, such as fever, anemia, and emaciation, besides symptoms more specific to the genital organs, such as edema of the genitalia and mammary glands. More severe forms may progress to incoordination, facial paralysis, and death (Gizaw et al., 2017).

The difficulty in studying *Trypanosoma equiperdum* is that there are almost no available isolates of this parasite, and most isolates are from the beginning of the last century and lack essential information such as isolation site, year, and even host. After a long time without description of new isolates, in 2015, a group from Venezuela obtained the first 2 isolates of this parasite in Latin America (Sánchez et al., 2015). In 2016, another group obtained a new isolate from the urogenital tract of a horse in Mongolia (Suganuma et al., 2016). The lack of knowledge about *T. equiperdum* reinforces the question about the validity of this species (or subspecies).

Trypanosoma (Tejeraia) rangeli

One trypanosome that can be classified as neither Stercoraria nor as Salivaria**,** is *Trypanosoma rangeli* (see Grisard, 2002). Actually, *T. rangeli* is a parasite species transmitted by the saliva of its insect vector but shares numerous characteristics with other species of the Stercoraria section, as it is easily cultivated in axenic media (which is not observed in the Salivarian trypanosomes). It also shares mammalian hosts and vectors with *T. cruzi*. Currently, this parasite species is classified in the subgenus *Tejeraia* that was created in 1982 only to classify *Trypanosoma rangeli*, until then classified within the *Herpetosoma* subgenus, within the Stercoraria section (Añez, 1982).

Trypanosoma rangeli is a multi-host (mammal) parasite found exclusively in the Americas and is capable of infecting humans. The main vector species are triatomines from the *Rhodnius* genus, in which *T. rangeli* differentiates to the infective metacyclic forms in the salivary gland of the insect (Guhl and Vallejo, 2003). Moreover, other triatomine species may also maintain *T. rangeli*, as in the case of *Triatoma vitticeps* (see Dario et al., 2017a). The genetic diversity within *T. rangeli* was first observed based on the difference of a nucleotide sequence from its mini-circles, resulting in

the recognition of 2 separate populations, KP1(+) and KP1(−) (Vallejo et al., 2002). Further studies have shown that this genetic polymorphism is even more extensive and 5 lineages could be described, from A to E (Maia da Silva et al., 2009). It was first proposed that the divergence of these lineages was related to different *Rhodnius* species involved in the transmission, but it is now accepted that this separation of lineages is not strict. In fact, the characterization in distinct lineages is recent and we do not have enough sampling to propose associations between *T. rangeli* lineages and mammal hosts and/or ecotypes (Urrea et al., 2011; Dario et al., 2017a).

In the mammal host, only the blood trypomastigote form is observed and the current consensus in the scientific community is that *Trypanosoma rangeli* does not differentiate in amastigote forms, as occurs in *T. cruzi*. In the *Rhodnius* vector, the predominant and replicative form is the epimastigote that colonizes the vector's gut. Some of these parasites differentiate into trypomastigotes in the final portion of the intestine and are eliminated with the feces, but these forms are not infective for mammals. Most of the epimastigote forms invade the vector's hemocoel, where they can multiply both inside and outside hemocytes. From the hemocoel, some of these parasites reach the salivary gland and differentiate into metacyclic trypomastigotes that are transmitted through the saliva during a blood meal (Guhl and Vallejo, 2003).

Some points of this transmission cycle still need to be clarified. First, only the blood trypomastigote form is described in mammal hosts, which is considered to be non-replicative. Otherwise, this parasite is possibly able to multiply in the mammalian host because there are numerous cases of persistence of parasitism even after long periods after exposure. For instance, *Trypanosoma rangeli* was isolated from Brazilian patients who had been out of risk areas for many years and were being treated as having Chagas disease (de Sousa et al., 2008). These cases suggest that this parasite species can multiply in the mammal host, in a mechanism still not identified.

Another intriguing aspect is the niche occupied by *Trypanosoma rangeli* in mammal hosts. This parasite is always diagnosed in blood, but it has been isolated from bone marrow from an anteater in the Amazon region. The presence of *T. rangeli* in this unorthodox niche probably occurred through blood or lymph circulation, as described for other trypanosomes, and could had been influenced by the coinfection of *T. cruzi* and *Leishmania infantum* that were also diagnosed in the same host (De Araújo et al., 2013).

The *Trypanosoma rangeli* infection in mammals are considered innocuous or non-pathogenic, although due to the unknown mechanism of parasite multiplication in mammals, the discovery of *T. rangeli* in bone marrow and the isolation in Chagas disease patients are aspects that have to be considered in the endorsement of this assumption. On the other hand, the pathogenicity of *T. rangeli* infection in invertebrate hosts is well described and reports of the influence of the parasite load on insect molt and destruction of intestinal epithelium during parasite invasion to the hemocoel are some of the damaging effects usually observed in infected bugs (Ferreira et al., 2010; García et al., 2012).

Section Stercoraria

Trypanosomes of this section include numerous species of *Trypanosoma* that live in the intercellular spaces and/or in the blood of their mammalian hosts. Most of these do not display mechanisms of colonizing the intracellular environment or disguising immune response by using variable surface antigenic variation as observed in some salivarian trypanosomes. This is the case for several species of *Trypanosoma* from this section. Stercorarians include heteroxenous parasites that are transmitted between species belonging to all vertebrate classes and hematophagous invertebrates on all continents. Three main evolutionary stages are recognized in this section, including: 1) Extracellular trypomastigote forms, 2) extracellular epimastigote forms, and 3) intracellular amastigote forms. Interestingly, in the stercorarian trypanosomes, replication by cellular division/binary fission of these trypanosomes in mammals occurs only in either the amastigote or the epimastigote stages; fission of the trypomastigote forms in this group is unknown (Hoare, 1972).

All species from this section possess trypomastigote forms with flagella protruding anteriad of the cell with a large and non-terminal kinetoplast. In the vector insects, the final development of the parasites, which corresponds to the formation of metacyclic trypomastigotes, occurs in the posterior portion of the digestive tract of the insect vector host. The main characteristic, which gives the name to the section (stercoraria or posterior station), is their transmission method which is always via insects' feces by rubbing metacyclic trypomastigotes into a wound, eye mucus membranes, or oral mucus membranes (Hoare, 1972).

Trypanosoma (Herpetosoma) lewisi

The subgenus *Herpetosoma* comprises all the species described in the *Trypanosoma lewisi* group, which is the type species of the subgenus. Species from this subgenus include trypanosomes from many species of rodents, in addition to a lagomorph trypanosome called *T. nabiasi*. Most known vectors are fleas, but for many *Trypanosoma* (*Herpetosoma*) species, the transmission cycle is still completely unknown.

Trypanosoma lewisi is a cosmopolitan parasite of *Rattus* spp. This trypanosomatid species was probably introduced to the different continents and countries due to its association

with *Rattus rattus*, a synanthropic rodent that in turn has accompanied humans since their first voyages. *Trypanosoma lewisi* was previously considered specific to *Rattus* spp. and not capable of infecting the other cosmopolitan rodents (*Mus musculus*) in experimental conditions. Currently, it is recorded in some wild rodent species (Mafie et al., 2019), besides primates, including humans (de Sousa, 2014). The phylogenetic analysis of *T. lewisi* isolates from *Rattus* spp. and primates proposed that *T. lewisi* underwent a process of host switching between rodents and primates through accidental contamination of primates with infected fleas from these rodents (Maia da Silva et al., 2010). Some cases have been reported in Asia, associated with fever, anemia, and immunosuppression (Verma et al., 2011). Some authors consider *T. lewisi* a neglected re-emerging human pathogen (Lin et al., 2015) in spite of the rarity of cases of human infection by *T. lewisi* worldwide and because it always presents in immune incompetent individuals or people living in close contact with rats (Verma et al., 2011; Shah et al., 2011).

The recognized vectors of *Trypanosoma lewisi* are *Xenopsylla cheopis* and *Nosopsyllus* sp., but it is believed that other fleas, such as *Ctenocephalides canis*, *Leptopsylla segnis*, and *Pulex irritans*, can also act as vectors (Hoare, 1972). No cell invasion is reported in vertebrates, and the infection is considered innocuous in immunocompetent hosts, although it can be lethal in newborn rats or increase susceptibility to other parasites when presenting as a co-infection, as with *Toxoplasma gondii* (Ríos Carrera et al., 2009) or *Cryptococcus neoformans* (Gross et al., 2006).

Two stages of the parasite can be observed in a vertebrate's blood, the replicative epimastigote forms, and the trypomastigote forms. Inside the digestive tract of fleas, the trypomastigote forms invade the epithelial cells of the stomach and differentiate into amastigote forms, which are replicative, and differentiate again to trypomastigote forms before returning to the digestive lumen. After reaching the flea's midgut, the trypomastigote forms differentiate into replicative epimastigotes that will differentiate into metacyclic trypomastigotes at the end of the digestive tract. Besides the contaminative route, oral transmission through accidental ingestion of fleas has been demonstrated for *Trypanosoma lewisi* and other species from the same subgenus, namely, *T. microti*, *T. evotomys*, and *T. grosi* (see Maraghi et al., 1995).

In mammals, infection by *Trypanosoma* (*Herpetosoma*) species is characterized by intense and short parasitemias. The infection is easily controlled by the host in about 3 weeks due to a humoral immune response because the parasite has limited capacity for antigenic variation and does not invade mammal cells. A characteristic observed in *T. lewisi*, and also believed to occur in other species from the same subgenus, is the production of an IgG immunoglobulin named ablastin that inactivates the replicative forms of the parasite leading to the abrupt remission of the parasitemia. After this, rodents become resistant to new infections (Drew and Jenkin, 1982). The abrupt remission of parasitemia occurs only when preceded by an initial phase of infection establishment where, most probably, other factors are involved. The passive transfer of ablastin from one infected rodent to another was observed, which resulted in a partial control of the infection, but not as abruptly as observed in natural infections (Drew and Jenkin, 1982).

An intriguing aspect of the transmission cycle has been observed in *Trypanosoma musculi*, a parasite considered restricted to *Mus musculus*. After the phenomenon of ablastin, rodents infected by *T. musculi* become resistant to new infections and parasitemia is no longer observed, but throughout their life, the infected rodents still maintain some parasite forms, including replicative forms, in the vasa recta of the kidneys. These forms are biochemically and molecularly different from blood forms and appear to represent a new evolutionary stage of the parasite that are not inactivated by the host's immune system, due to the high concentration of urea (Monroy and Dusanic, 2000). The consequences of the existence of this distinct stage in the life cycle, the occurrence of this phenomenon in other *Herpetosoma* species, and the evolutionary impact of this parasite persistence are still unknown aspects.

Besides *Trypanosoma lewisi* and *T. musculi,* some other related species described in the same *Herpetosoma* subgenus are: *T. microti,* described in *Microtus* spp. and also directly transmitted through agonistic contact in the reproductive season; *T. evotomys* and *T. grosi* from Old World rodents; *T. kuseli* and *T. otospermophili* from squirrels; and *T. nabiasi,* a parasite species specific of the lagomorph *Oryctolagus cuniculus* present in the New World and Australia, the latter as a result of the human introduction of infected fleas in an attempt to control the rabbits (introduced one century before) with the Myxomatosis virus (Hamilton et al., 2005).

Trypanosoma (Megatrypanum) theileri

The morphology of parasites from the subgenus *Megatrypanum* is very typical: they have very large cells with a visible undulating flagellum that adheres to the entire body of the parasite. Trypanosomes from this subgenus are transmitted by a diversity of vectors, including hematophagous flies, fleas, ticks, and *Pseudolynchia* sp. This subgenus comprises species that infect a wide variety of hosts, including marsupials, rodents, primates, and, mainly, varieties of bovinae cattle (Kingston, 1991; Kelly et al., 2017). The species *Trypanosoma* (*Megatrypanum*) *theileri* is a hemoparasite found in

Artiodactyla species worldwide and is the type species of the subgenus *Megatrypanum*. Recently, a second escape mechanism of modeling the trypanosome cell surface, distinct from the VSG's coat of the salivarian trypanosomes, has been described. Actually, the surface of *Trypanosoma theileri* cells can be modeled by the expression of a mixture of proteins (Kelly et al., 2017).

The transmission cycle of *Trypanosoma theileri* is not completely elucidated yet. Mammals are proposed to have a replicative epimastigote form, but this parasite form is very rarely observed in field studies, probably because these forms quickly differentiate into trypomastigotes, which is the predominant morphological type detected in mammals' blood. In mammals, those trypomastigote forms are capable of invading all body fluids, in addition to some tissues, such as lymph nodes, kidney, spleen, and brain. For long time, it was accepted that *T. theileri* did not differentiate into amastigotes, but the existence of these stage forms was recently demonstrated in vitro, including the ability to differentiate and invade other cells in a process similar to that observed in *T. cruzi* (see Lee et al., 2013). In vectors, the epimastigote forms of the parasite replicate in the gut before differentiating into metacyclic trypomastigotes that are eliminated in the feces. The tabanids are the vectors of *T. theileri,* but a tick species (*Hyalomma anatolicum*) was proposed before as an alternative vector (Latif et al., 2004). As observed for other Stercorarian trypanosomes, oral transmission through accidental ingestion of infected vectors is proposed as an important infection route (Kelly et al., 2017).

Trypanosoma theileri is a cosmopolitan and opportunistic parasite. The parasitemia in bovines is usually associated with some unspecific clinical signs. Infection may remain subpatent and asymptomatic for several years but may result in high parasitaemia and pathogenicity when associated with other factors, such as stress, malnutrition, or pregnancy. This parasite species is recognized in bovines and buffaloes from South America, and at least 10 genotypes are recognized, most of them specific in terms of mammal species (García et al., 2011).

Besides *Trypanosoma theileri,* some other species from the same *Megatrypanum* subgenus are: *T. minasense* from New World primates; *T. melophagium* from sheep*; T. theodori* from goats; *T. cervi* and *T. mazamarum* from cervids; *T. freitasi* that colonize scent glands of *Didelphis* spp.; *T. samueli* and *T. saloboense*, from *Monodelphis* sp. from the Amazon region; *T. peba* from the 6-banded armadillo *Euphractus sexcinctus; T. legeri* from the anteater *Tamandua tetradactyla; Trypanosoma pestanai* isolated from European badgers; and *T. caimani* from alligators from the Brazilian Pantanal; as well as a high diversity of bird trypanosomes. For the less studied *Megatrypanum* species, other vectors (apart from tabanids) are involved in the transmission: keds (*Melophagus ovinus*) are involved in *T. melophagium* transmission (Gibson et al., 2010), *T. pestanai* is transmitted by fleas (Peirce and Neal, 1974; Lizundia et al., 2011), while *T. cervi* are also spread by keds (Böse and Petersen, 1991). It is worth mentioning that many of these parasite species were described based on morphology, before the employment of molecular techniques for parasite identification. As a result, some species may not be valid, as in the case of *T. saimirii* and *T. leeuwenhoeki,* currently recognized as synonyms of *T. rangeli* (See Stevens et al., 1999a; Ziccardi et al., 2005).

Trypanosoma (Schizotrypanum) spp. and Other Species Related to *T. cruzi*

Almost all the *Trypanosoma* (*Schizotrypanum*) species are parasites exclusive of bats. The exceptions are *T. cruzi,* a multihost parasite that may also infect bats, and *T. dionisii,* a parasite species considered specific to bats, but recently observed also in human cardiac tissue and in the marsupial *Monodelphis americana* (See Lima et al., 2015a; 2015b; Dario et al., 2016; 2017a; 2017b). Parasites from this subgenus are morphologically identical and all of them are able to differentiate into metacyclic forms and invade cells, where they differentiate into amastigotes.

Until the 1990s, most *Trypanosoma* infections in bats were described as *T. cruzi*-like because of the difficulties differentiating among *Schizotrypanum* species. Currently, due to advances of molecular techniques, especially since it is easier now to analyze DNA sequences, the variety of *Trypanosoma* species infecting bats has been shown to be enormous, and this diversity has been increasing annually with descriptions of new *Trypanosoma* species infecting bats. Some *Trypanosoma* species reported in bats are: *T. cruzi marinkellei* and *T. wauwau,* described in bats from Central America and South America; *T. vesperitilionis* and *T. dionisii,* described in bats from both the Old World and the New World; *T. pipistrelli* from Old World bats; *T. pteropi*, *T. hipposideri*, and *T. teixeirae* from Australian bats, including the enormous flying foxes; *T. hedricki* and *T. myoti* from bats surveyed in North America; *T. erneyi* and *T. livingstonei* from Africa; and yet others (Lima et al., 2012; 2013; 2015a; 2015b).

The majority of the descriptions of *Trypanosoma* infection derived from insectivorous bats suggest that, besides the vectorial contaminative route, oral infections must also occur (Lima et al., 2015a; 2015b; Dos Santos et al., 2018). The vectors involved in the majority of bat trypanosome transmission are unknown. *Trypanosoma cruzi marinkellei* is currently recognized as a subspecies of *T. cruzi* and reported to be transmitted only by triatomines of the genus *Cavernicola*,

associated with caves (Marinkelle, 1982). Vectors of *T. dionisii* and *T. vespertilionis* are reported to be cimicids, but both *T. c. marinkellei* and *T. dionisii* were recently found infecting *Triatoma vitticeps,* a triatomine bug very common in the Atlantic rainforest (Dario et al., 2017a). The close association of *Schizotrypanum* parasites and bats suggests a long coevolutionary process. However, the association with different vectors points to an independent evolution between species within this subgenus.

Other *Trypanosoma* species more recently described were not classified in any subgenus but are phylogenetically closer to *T. cruzi* and known as trypanosomes of the *T. cruzi* clade. Besides all the above mentioned trypanosomes of bats, this clade includes *T. noyesi,* a trypanosome described from the Australian endangered woylie (*Bettongia pencillata*); 2 isolates from African terrestrial mammals (1 feline and 1 primate); *T. conorhini*, a species taxonomically classified in the subgenus *Megatrypanum*, but phylogenetically in the *T. cruzi* clade; and *T. janseni,* a species recently described in Brazilian opossums that, along with *T. wauwau,* forms a sister group of the trypanosomes found in Australian marsupials (Hamilton et al., 2009; Botero et al., 2016; Lopes et al., 2018).

From these, one of the most studied is *Trypanosoma conorhini.* This species infects *Rattus rattus* a synanthropic rodent and the kissing bug *Triatoma rubrofasciatta* all over the world, and only experimentally was demonstrated to be able to infect *Mus musculus* and *Macaca mulatta* (Deane et al., 1986). The only parasite form observed in the rodent's blood is the trypomastigote, without evidence of cell invasion and differentiation to amastigote forms. The biological cycle in the vector includes the replication of the epimastigote forms in the bug's gut and differentiation in metacyclic trypomastigotes that are eliminated in the feces (Hoare, 1972). The fact that this trio–*Rattus rattus*, *Triatoma rubrofasciatta*, and *Trypanosoma conorhini*–are found together in different parts of the world indicate that their dispersal process occurred together, probably from Asia, which represents a unique joint migration process within the heteroxenous protozoa (Deane et al., 1986).

In general, the vectors involved in the transmission of most of the *Trypanosoma* species from the *T. cruzi* clade are unknown, but all of the vectors described up to now are hematophagous hemipterans. In fact, the knowledge of the diversity of *Trypanosoma* species has been a growing subject of study, especially in the past decade when DNA sequencing tools became more accessible. These studies have shown a much greater diversity than was previously known and that the evolutionary paths within this clade are still not fully understood.

Trypanosoma cruzi

Trypanosoma cruzi is the etiological agent of one of the most important and neglected parasitic disease, Chagas disease (Chagas, 1909). However, the human infection is only a minor trait of this parasite ecology that is a wild animal parasite maintained among dozens of species of mammals and triatomines (family Reduviidae, subfamily Triatomiane). In fact, *T. cruzi* is a multi-host parasite, able to infect virtually any mammal species and, within them, any nucleated cell (Jansen et al., 2015). In spite of presenting a population structure that is basically clonal, mitochondrial introgression events are not unusual and evidence of genetic exchange has already been described in *T. cruzi*. Also, recombinant strains have been described (Lewis et al., 2011).

There are 2 non-mutually exclusive hypotheses that explain the origin of *Trypanosoma cruzi*. The first, called **Southern Supercontinent**, places the origin of this parasite from an ancestral trypanosome of marsupials, which represented the dominant local fauna, in a supercontinent formed by South America, Antarctica, and Australia. This hypothesis is supported by: 1) The presence of a trypanosome related to *T. cruzi*, named *T. noyesi* (Botero et al., 2016), isolated from an Australian woylie (besides other related isolates from Australian marsupials); 2) the estimated divergence between *T. cruzi* and *T. brucei* (approximately 100 million years) that is the approximate time of separation of this supercontinent from Africa (Stevens et al., 1999b); and 3) the recent description of *T. janseni*, which was described in Brazilian opossums and clusters with *T. wauwau* in a well-supported clade, representing a sister group of the trypanosomes found in Australian marsupials (Lopes et al., 2018).

The second hypothesis points to the origin of *Trypanosoma cruzi* from ancestral *Trypanosoma* species of bats and is called the **Bat Seeding Hypothesis** (Hamilton et al., 2012). This hypothesis is supported mainly by: 1) The increasing evidence of the diversity and polyphyly of bat trypanosomes; and 2) the description of infection of trypanosomes from the *T. cruzi* clade in African monkeys and palm civets (which does not represent a single lineage that could have been introduced more recently; that is, post-separation of the continents) (Hamilton et al., 2009). Several processes of trypanosome spillover from bats to terrestrial mammals must have occurred until one of them was successfully established and the evolution of this parasite resulted in a new species currently known as *T. cruzi* (Hamilton et al., 2012).

The contaminative transmission of the parasite, described as the classic form of transmission, occurs when the insect vector eliminates metacyclic trypomastigote forms with feces during a blood meal. These parasites penetrate through lesioned skin or mucous membranes or when the person

scratches the location of the insect bite. In the mammal host, these parasites invade the nucleated cells, in which they differentiate into the replicative amastigote forms. A novel differentiation into blood trypomastigotes occurs before the cellular disruption that results in the release of these forms that can invade other cells or circulate in blood vessels and be ingested by other Triatominae in a new blood meal. In invertebrate hosts, *Trypanosoma cruzi* differentiates into the replicative epimastigote forms and only in the final portion of the insect gut, the parasites differentiate into the metacyclic trypomastigote forms that are the infective forms eliminated in the feces.

This classical route of transmission, however, does not reflect all possible routes of infection in mammals, including humans. From the 4 evolutionary stages of the parasite, only epimastigotes may not be infective, mainly when they are in the exponential growth phase. Moreover, epimastigotes of the stationary phase are already resistant to the complement system and already infective (Kessler et al., 2017). Nutrient impoverishment and starvation of the parasite acts as a trigger for metacyclogenesis (Barisón et al., 2017). Human infections can also occur during blood transfusion and organ transplantation, and these are the most important infection routes in non-endemic countries such as the United States, Spain, and Japan (Gascon et al., 2010). The congenital form, well characterized in humans, seems to have a more regionalized importance, especially in the southern region of South America and non-endemic areas. The oral route has emerged as a very important transmission route in the wild and has been responsible for an increasing number of human infections in the past few decades (Coura, 2015). In the South American Amazonian area, especially in Brazil, this transmission route represents more than 90% of new cases and is usually associated with intake of food contaminated by feces of infected triatomines or infected vectors accidentally crushed along with food, such as sugar cane and açaí juice (Figure 5) (PAHO, 2009). This transmission route requires specific surveillance measures whose operation is still not completely known (Xavier et al., 2014).

Figure 5. The oral route has emerged as a very important transmission route in the wild and has been responsible for an increasing number of human infections in the past few decades. In Amazonia, especially Brazil, this transmission route represents more than 90% of new cases and is usually associated with intake of food contaminated by feces of infected triatomines or infected vectors accidentally crushed along with food. Source: Pan-American Health Organization, 2009. Permissions: Reproduction is permitted with attribution.

In nature, the oral route is probably the oldest and most efficient route for parasite transmission and occurs mainly in 2 situations: 1) Ingestion of triatomine feces when the animal scratches the site of an insect bite with its mouth; or 2) predation of infected bugs or mammals. Other possible routes include fights between mammals (when the oral mucosa of a mammal comes in contact with the infected blood of the other mammal) and fomites contaminated by scent gland material of infected *Didelphis* sp. (Deane et al., 1984; PAHO, 2009), although these are still not empirically confirmed.

This biological plasticity of *Trypanosoma cruzi* results in a parasite widely dispersed in nature and immersed in transmission cycles that can be characterized as multivariable, complex, and peculiar to each locality (Jansen et al., 2015). Being found in the most diverse ecological niches from the southern parts of Argentina to southern United States, its transmission cycles are quite complex as it includes hundreds of mammalian species and vectors in scenarios of transmission that can be independent and overlapping with each other (Jansen et al., 2018).

As pointed out before, *Trypanosoma cruzi* has been circulating among wild mammalian fauna for at least 20 million years (depending on which hypothesis for the parasite's origin is considered). Humans are believed to have existed for no more than 500,000 years. Humans are estimated to have entered into the Americas (and therefore, exposed within this time to *T. cruzi* transmission cycles) only 15,000 to 24,000 years ago (Bourgeon et al., 2017). As such, there is evidence of human infections in mummies long before the arrival of Europeans in the Americas. Reports in paleoparasitological studies demonstrated the infection in 40% of the mummies examined in Chile, including at least one of them dating to 9,000 years. Almost half of the mummies had signs of megasyndromes (especially megacolon) and infections were seen among mummies from different cultural groups (Aufderheide et al., 2004). It is reported in many South American Aboriginal cultures the habit of feeding on raw or undercooked meat or even drinking the fresh blood of hunted mammals, which would result in infection by *T. cruzi* (Guhl et al., 2014). The contaminate vectorial transmission probably also occurred: 1) Inside caves used as human dwellings, due to the presence of kissing bugs associated with rocky environments, as in the case of *Triatoma brasiliensis* (Araújo et al., 2003); and 2) in peridomiciliary environments, as a consequence of the presence of small mammals such as guinea pigs that provided an abundant blood source for bugs in this environment (Aufderheide et al., 2004). These ancient scenarios demonstrate that there are no new or old ways of *T. cruzi* transmission. Humans have been exposed to the infection by both the contaminative and oral routes since they reached the Americas.

The species *Trypanosoma cruzi* is a monophyletic and extremely heterogeneous taxon that presents a multiclonal population structure influenced by mechanisms of gene exchange, epigenetic factors, introgression, and others (Stevens et al., 2001; Leonard et al., 2011). The heterogeneity of the parasite has been observed since its discovery, and thin and broad parasite forms observed in patients' blood were first associated with male and female gametocytes (Chagas, 1909). The first attempts to group *T. cruzi* isolates with similar characteristics dated from the 1970s: the biodemes, based on a distinct infection pattern in laboratory animals (Andrade et al., 1970) and the zymodemes, based on biochemical patterns of isoenzymes (Miles et al., 1977). The advent of molecular techniques and the proposal of distinct molecular targets in the 1980s and 1990s have revealed that this heterogeneity was much higher than previously thought. In the last decade, however, with the availability of gene sequence analysis in research labs, an even higher diversity of *T. cruzi* populations (and other related species) has emerged. In this scenario, the most employed gene targets are the small subunit (18S) ribosomal RNA gene and the nuclear glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) (Lima et al., 2015a; Lopes et al., 2018). These are the ones with the highest discriminatory power and those targets with the highest number of sequences deposited for comparison. Moreover, more easy accessibility to Next Generation Sequence (NGS) will probably uncover even higher trypanosomatid diversity, especially due the capacity to identify and characterize mixed infections directly on blood samples (Dario et al., 2017b; Pronovost et al., 2020).

Independent of the different molecular targets employed, 2 distinct and phylogenetically distant *Trypanosoma cruzi* populations have been recognized and were the basis for the first nomenclature consensus for *T. cruzi* (Luquetti et al., 1999). One year later, Brisse and colleagues (2000) proposed the grouping of *T. cruzi* isolates into 6 genotypes, or 6 **Discrete Typing Units** (**DTU**), named TcI to TcVI (Tc stands for *T. cruzi*). This is the basis of the current consensus of nomenclature: *T. cruzi* is a single species composed of 6 distinct DTUs (Zingales et al., 2009).

In addition to these DTUs, in 2009, a new *Trypanosoma cruzi* genotype, first associated with bats, was described: Tcbat (see Marcili et al., 2009). This genotype is phylogenetically close to TcI but represents a well separated clade. As a *T. cruzi* parasite, Tcbat is able to infect mice in experimental conditions. Although, its development in triatomines is more efficient in cave triatomines, which is the same pattern observed for another trypanosome associated with bats, *T. c. marinkellei* (See Marinkelle, 1982). Although first associated with bats, human infections by Tcbat have been reported in a Chilean mummy (Guhl et al., 2014) and a Colombian patient (Ramírez et al., 2014).

The currently most-accepted hypothesis to explain the establishment of the 6 DTUs points to TcI and TcII as the 2 ancestor lineages, and the emergence of the DTUs TcIII and TcIV as a result of a first hybridization process between them. A second and more recent hybridization process involving TcII and TcIII would have given rise to the current hybrid lineages TcV and TcVI (Westenberger et al., 2005).

The geographic distribution of *Trypanosoma cruzi* DTUs is not completely known and further knowledge of both the geographical and host-expanding data for each DTU will certainly result in distinct distribution maps (Jansen et al., 2015; 2018). However, some aspects can be highlighted: 1) TcI is the most dispersed DTU throughout the Americas; 2) outside South America, mainly TcI and TcIV are detected; 3) TcII occurs in the wild, but in restricted transmission foci and is the genotype associated with human infections in the former endemic areas of the disease (Jansen et al., 2015; 2018); 4) TcIII and TcIV are commonly found in wild mammals and the latter has been associated with oral outbreaks in the Amazon region (Santana et al., 2019); 5) TcV and TcVI are rarer than the others, both in humans and wild mammals; and 6) the subdividing genotypes of the *T. cruzi* population, previously known as zymodeme 2 (TCII in the first nomenclature consensus), are more recent. Because of that, ancient descriptions of TCI are still considered TcI, but TCII was subdivided into the other 5 subpopulations. This means that, except for TcI, all other *T. cruzi* DTUs are actually underestimated on the current distribution maps.

The widespread transmission of *Trypanosoma cruzi* to humans, which led to hundreds of thousands cases per year in the last century, was directly associated with the presence of the domiciliary bug *Triatoma infestans* (See Figure 6). This bug species originates from the Bolivian valleys of the eastern Andes, where it is found both in domiciles and in wild environments (Panzera et al., 2014). The domiciliary process of *Tri. infestans* occurred concurrently with the beginning of human settlements near forest environments. At the same time, the process of domestication of local guinea pigs near residences attracted the insects to the home environment (Aufderheide et al., 2004). After the colonization of the Americas by Europeans, the greater movement of people and materials favored the establishment of the intradomiciliary colonies of *Tri. infestans*, first in Bolivia and subsequently in other countries from South America, especially in those regions south of the Amazon basin. The presence of infected domiciliary bugs in stick houses was what Carlos Chagas found when he described the parasite and the associated vector (Chagas, 1909).

This classical scenario of transmission started to change in the 1970s, with insecticide campaigns to eliminate domiciliary colonies of this kissing bug species (Dias, 2007). In 1991, the governments of Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay created the Southern Cone Initiative aiming to eliminate the intradomiciliary transmission of *Trypanosoma cruzi* by *Triatoma infestans,* which was achieved in 2006 (Dias, 2007). The certified interruption of this method of transmission, however, did not represent the end of contaminative vectorial transmission. This manner of transmission continues to occur, but now in the extradomiciliar environment, when humans expose themselves to wild transmission cycles, or in the intradomiciliar environment, usually associated with the invasion of wild vectors and not to domiciliary colonies (Dias et al., 2016). In both cases, control measures adopted against *Tri. infestans* are not effective. With the virtual elimination of *Tri. infestans* in some countries, including Brazil, the majority of the new reported cases are concentrated in the Amazon region, a previously considered non-endemic area due to the absence of *Tri. infestans* in the area. Only in Brazil, approximately 150–200 new cases of Chagas disease are reported per year (Dias et al., 2016). These cases are always associated with infected sylvatic kissing bugs that came in contact with humans.

These sylvatic bugs are immersed in a huge net of *Trypanosoma cruzi* transmission along with mammals from distinct orders. This transmission net resembles the trophic energy characteristic of food webs (Herrera et al., 2011). Both the oral route, through the predation of mammals and insects, and contaminative transmission are involved in the dispersion of this parasite in nature. Triatomines can be preyed upon by smaller mammals, and these by medium or top-chain carnivores (Herrera et al., 2011; Rocha et al., 2013). Moreover, the different mammal hosts are distributed in distinct forest strata and enable the parasite transmission in the most diverse habitats. This is especially important for mammals with generalist habits, like coatis, opossums, and felids, which frequent different forest strata and, thus, can connect parasite transmission cycles from different environments (Jansen et al., 2018).

Trypanosoma cruzi transmission can occur at all levels of the food web, and each group of mammals also presents varying importance as a reservoir of this parasite. In the pyramid base of consumers are the small terrestrial mammals. These mammals are excellent models of study because they comprise large populations and are usually the group of mammals with the highest biomass in any ecotope (Mills and Childs, 1998). In addition, they have a short lifespan and fast generational turnover, which allows the early identification of environmental changes. In the second level of the pyramid are the bats and medium-sized carnivores. They are classified as mesopredators, since they may be exposed to *T. cruzi* infection by predation of both infected vectors and small mammals. They are usually generalists and capable of exploiting

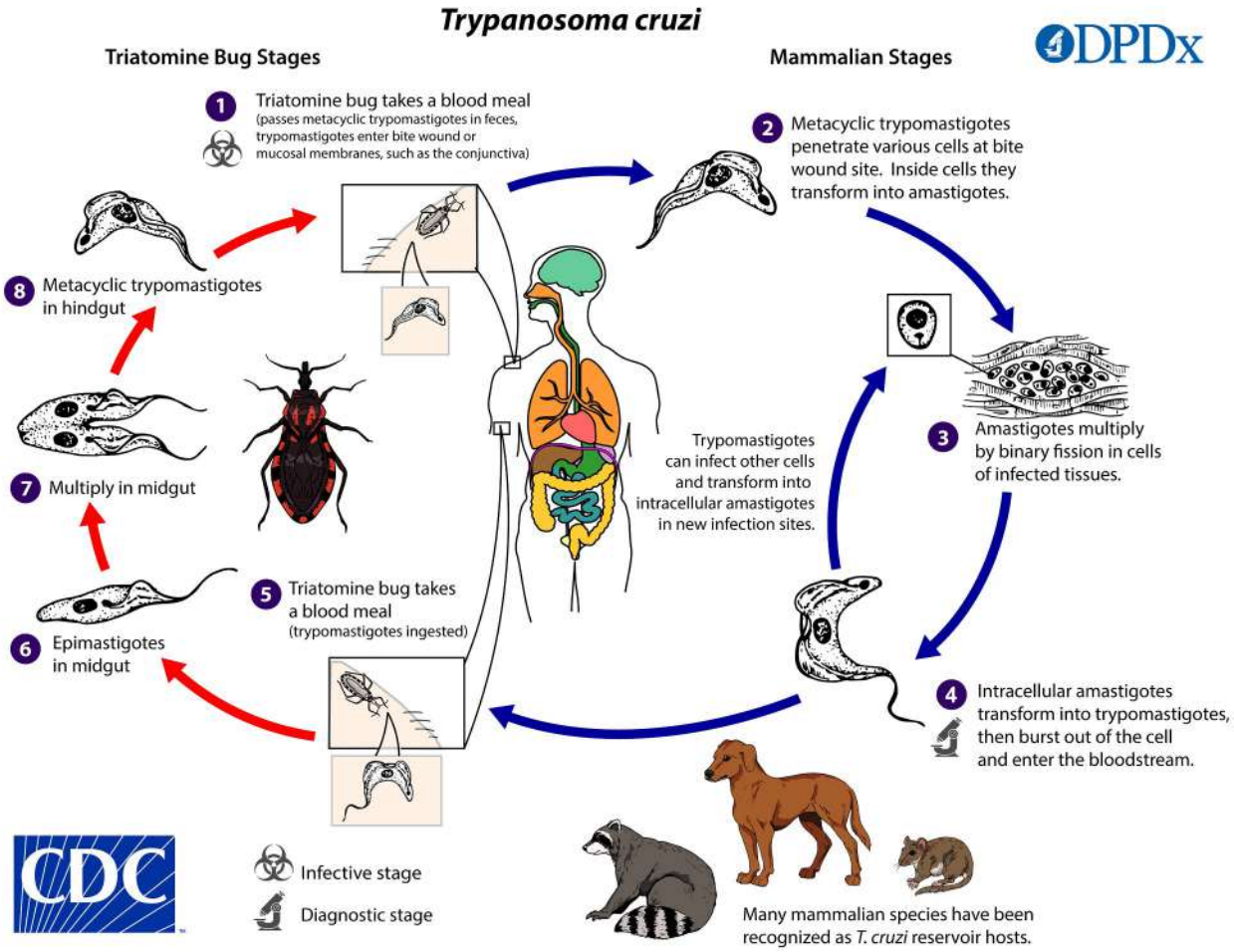

Figure 6. American Trypanosomiasis. *Trypanosoma cruzi*, is a parasitic protozoan that is the causative agent of Chagas disease (American trypanosomiasis). Currently, 6 distinct lineages of *T. cruzi* are classified into discrete typing units (TcI–VI), which vary in their geographic occurrence, host specificity, and pathogenicity. **Life cycle diagram:** An infected triatomine insect vector (or kissing bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the bite wound or intact mucosal membranes, such as the conjunctiva (1). Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes (2). The amastigotes multiply by binary fission (3) and differentiate into trypomastigotes and then are released into the circulation as bloodstream trypomastigotes (4). Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The kissing bug becomes infected by feeding on human or animal blood that contains circulating parasites (5). The ingested trypomastigotes transform into epimastigotes in the vector's midgut (6). The parasites multiply and differentiate in the midgut (7) and differentiate into infective metacyclic trypomastigotes in the hindgut (8). Other less common routes of transmission include blood transfusions, organ transplantation, transplacental transmission, and foodborne transmission (via food or drink contaminated with the vector and/or its feces). Source: United States Centers for Disease Control and Prevention, Global Health, Division of Parasitic Diseases and Malaria, 2021. Public domain.

distinct forest strata. At the top of the pyramid are those animals at the top of the food chain. They are mammals characterized by a wide geographic range and capacity for displacement, which are important conditions for parasite dispersion. In addition, they are considered bioaccumulators of orally transmitted parasites, as is the case for *T. cruzi* (see Herrera et al., 2011; Rocha et al., 2013).

Small mammals are excellent models of study for identifying spatial and temporal variation in *Trypanosoma cruzi* transmission. Concerning the spatial differences, the effect of habitat fragmentation on *T. cruzi* transmission among small mammals has been demonstrated by Vaz et al. (2007). Fragments of forest patches of different sizes (small, medium, and large) were investigated, in addition to a national park, as a

preserved control area. Vaz et al. (2007) observed that the greater the fragmentation of the wild environment, the lower the diversity of small placental mammals and the greater the abundance of marsupials. This different faunal composition was reflected in distinct infection prevalences, especially in the medium and large fragments (Vaz et al., 2007). Temporal differences were observed in the evaluation of infection prevalences in small mammals from the same locality (Jaguaruana, Ceará State, Brazil) during a 4-year follow-up. The anthropogenic devastation of the area resulted in lower species richness of placentals and a higher abundance of opossums. The consequence for *T. cruzi* transmission was an increase from an initial rate of 10% of the mammals infected to a rate higher than 50% after the fourth year (Jansen, unpublished data).

An example of a mesopredator that, throughout several years of study, demonstrated to be an important *Trypanosoma cruzi* reservoir is the coati (*Nasua nasua*) from the Pantanal region of Brazil. Distinct *T. cruzi* DTUs (TcI–TcIV) have been found in single mixed infections and long-term follow-up showed that coatis may present high and long-lasting parasitemias that are influenced by seasonality and gender (Herrera et al., 2008; 2011; Alves et al., 2016; Jansen et al., 2018). Coatis nest in very tall trees and their nests serve as microhabitats for several other mammals and insects, including kissing bugs that have been found to be infected by *T. cruzi* (see De Lima et al., 2015).

Golden lion tamarins—GLT (*Leonthopitecus rosalia*)—are also demonstrated to be competent *Trypanosoma cruzi* reservoirs (Lisboa et al., 2015). This callitrichid is an endangered species restricted to the Atlantic rainforest of Rio de Janeiro State, Brazil. It was during the GLT survey for *T. cruzi* that the presence of TcII (the DTU until then related only to human infections), was observed for the first time in the wild (Lisboa et al., 2000). An 11-year follow-up demonstrated that individuals of *L. rosalia* are able to maintain long-lasting infections and infectivity potential when infected by *T. cruzi* from both DTUs TcI and TcII (Lisboa et al., 2015).

Top-predator mammals are still poorly studied, mainly due to the difficulty in trapping and handling them, but are known as important bioaccumulators of *Trypanosoma cruzi*. In this sense, a positive correlation between the proportion of insects or other mammals in the diet and the infection rates by *T. cruzi* has been demonstrated (Rocha et al., 2013).

The composition of the local fauna may indicate which *Trypanosoma cruzi* DTUs will predominate in an area. *Didelphis* spp., for example, are known to maintain high and long-lasting parasitemias (as expressed by positive blood cultures), but only when infected by DTU TcI (Legey et al., 2003), while GLTs can maintain stable infections also by TcII (Lisboa et al., 2015). In contrast, wild and domestic canids usually exhibit a short period of high parasitemia, exactly as observed for humans (Jansen et al., 2018). This diversity explains the differences in the enzootic cycles of the parasite in different areas. In fact, each area is unique and has its own particularities (Jansen et al., 2015).

Currently, what will impact in the infection rates that have emerged in recent outbreaks of Chagas disease in the Americas include: 1) Control of domiciliary transmission of *Trypanosoma cruzi* by *Triatoma infestans*; 2) control of the parasite in the wild fauna of vectors and mammals; and 3) environmental disturbances that result in faunal modifications. With these in mind, and aiming to identify common risk factors that could be used in surveillance programs, domestic dogs were proposed as sentinels of imminent risk of transmission to humans (Roque and Jansen, 2008). In these outbreak areas, where a well-established wild transmission cycle of *Try. cruzi* was observed, dogs showed high prevalence of infection as diagnosed by serology, but did not contribute to the amplification of the parasite population, as attested by negative hemocultures (Roque et al., 2008; 2013). This proposal was further confirmed by spatial analysis using interpolation and map algebra tools, which also considered the environmental factors that interfered with the transmission. In this study, the authors confirmed the hypothesis that loss of wildlife richness was associated with higher parasitemias in wild fauna and higher serological prevalence in the associated canine population (Xavier et al., 2012).

In fact, the study of a multi-host zoonotic parasite such as *Trypanosoma cruzi* encourages analysis of the transmission to humans using a One Health approach (Thompson, 2013). Additionaly, implementation of the DAMA protocol is urgently needed in order to understand and take action in these and other parasites (Brooks et al., 2014; 2019). Parasite spillover or more properly known as ecological fitting (Janzen, 1985) is a phenomenon frequently amplified by changes in the landscape that: 1) Result in selection of individuals; 2) change the dynamics of transmission; and 3) in the case of zoonosis, will ultimately result in higher or lesser risk of emergence of human cases. Multidisciplinary studies are essential to better understand *T. cruzi* control and transmission, which are key conditions for successful surveillance programs. As a primarily enzootic parasite, *T. cruzi* transmission will never be eliminated, but prediction of new human cases and outbreaks is possible and urgently needed.

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12

Protozoa

Trypanosomatidae

Leishmania (Genus) and Leishmaniasis

Mary Ann McDowell and Jennifer Robichaud

Phylum Euglenozoa

Class Kinetoplastea

Order Trypanosomatida

Family Trypanosomatidae

Genus *Leishmania*

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Chapter 12

Leishmania (Genus) and Leishmaniasis

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Introduction

Leishmaniasis comprises a group of diseases caused by protozoans of the genus *Leishmania* (Ross, 1903b; Gibson, 1983) that are transmitted by the bites of phlebotomine sand flies. Of the 53 *Leishmania* species described, approximately 20 are known to be human pathogens (Table 1) (Akhoundi et al., 2016). The clinical manifestations of *Leishmania* infections range from lesions of the skin and mucous membranes to lethality (Herwaldt, 1999). Cutaneous leishmaniasis (CL), the most common form of the disease, comes in many forms including localized cutaneous leishmaniasis (LCL), characterized by a single, self-healing ulcer, diffuse cutaneous leishmaniasis (DCL) that presents as non-ulcerating lesions that are widespread on the body, disseminated cutaneous leishmaniasis (DCL), characterized by more than 10 lesions of mixed-types, mucocutaneous leishmaniasis (MCL), associated with destruction of the nasopharyngeal mucus membranes, visceral leishmaniasis (VL) where there is no initial cutaneous pathology and parasites spread to the visceral organs, and a complication of VL termed post kala-azar dermal leishmaniasis that is characterized by a erythmatous maculopapular rash that can extend to the entire body.

Leishmania species cause morbidity and mortality throughout large areas of the Old and New World and leishmaniasis is considered an emerging disease with an annual incidence of 0.9–1.7 million cases (Alvar et al., 2012). Leishmaniasis is found on all continents except Australia and Antarctica and is endemic in 98 countries, with 350 million people at risk of infection and causing 20,000 to 40,000 deaths per year (Alvar et al., 2012). An increased prevalence of *Leishmania*-HIV co-infection is responsible for the recent emergence of leishmaniasis in the Western world (Alvar et al., 2012; Desjeux and Alvar, 2003). Morbidity and mortality caused by leishmaniasis amount to an estimated 2.4 million disability-adjusted life-years (DALYs) (Desjeux, 2004) and the disease has recently been declared by the World Health Organization (WHO) as a category I Neglected Tropical Disease (NTD).

Historical Evidence

Evidence of *Leishmania*-like organisms from the blood of reptiles exists in fossil ambers of an extinct sand fly from Burma estimated to be approximately 100 million-years-old (Poinar et al., 2004a; 2004b) and from 20–30-million-year-old ambers from the Dominican Republic, although the vertebrate host is unknown in this case (Poinar, 2008). Human lesions, similar to those known as an ailment termed Oriental Sore, were first described in tablets from the Assyrian King Ashurbanipal in the 7th century BCE (= before current era), however, the information is thought to be derived from texts dating as old as 1500–2500 BCE (Steverding, 2017). In addition, Ancient Egyptian medical reports from 1500 BCE describe a condition known as Nile Pimple that is thought to refer to cutaneous leishmaniasis (Maspero, 1910). Physical evidence of *Le*. *donovani* DNA has been documented in Egyptian mummies dating as far back as 2050–1650 BCE (Zink et al., 2006) and immunological technique have been used to demonstrate *Leishmania* in a Peruvian mummy from 800 BCE (Frias et al., 2013).

In the Middle Ages, Arabic scientists made many references to descriptions of lesions, reminiscent of cutaneous leishmaniasis; the first being in 930 from the Baghdad region in Iraq (Edrissian et al., 2016) and a dermal condition known as Balkh Sore from Afghanistan by the Persian philosopher and physician Avicenna (980–1037) (Severding, 2017). In the New World, disfiguring facial lesions are depicted on pre-Columbian ceramics from the 5th century (Tuon et al., 2008) and skulls dating back to the 11th century discovered in northern Chile have morphological and molecular evidence of leishmaniasis in the New World (Costa et al., 2009).

Table 1. Clinical and Epidemiological Characteristics of *Leishmania* Species

***Leishmania* species**	**Subgenus**	**Old World and/or New World**	**Proven vector species**	**Clinical manifestation**	**Primary reservoir hosts**	**Distribution**	**Estimated global incidence**
Le. donovani	*Leishmania*	OW	*P. alexandri* *P. argentipes* *P. martini* *P. orientalis*	VL, PKDL	Dogs, foxes, opossums, rodents	Central Africa, South Asia, Middle East, India, China	50,000–90,000 VL cases; unknown number of PKDL cases
Le. tropica	*Leishmania*	OW	*P. arabicus* *P. guggisbergi* *P. rossi* *P. saevus* *P. sergenti*	LCL, RCL, rarely VL	Rock hyraxes	Central Africa, North Africa, Middle East, Central Asia, India	200,000–400,000 CL; unknown number of viscerotropic or RCL
Le. aethiopica	*Leishmania*	OW	*P. longipes* *P. pedifer* *P. sergenti*	LCL, DCL, DsCL, MCL	Rock hyraxes	East Africa	20,000–40,000 CL; breakdown of LCL, DCL, DsCL, MCL unknown
Le. major	*Leishmania*	OW	*P. duboscqui* *P. papatas,* *P. salehi*	CL	Gerbils, other rodents	Central Africa, North Africa, Middle East, Central Asia	230,000–420,000 LCL
Le. infantum	*Leishmania*	OW/NW	*Lu. almerio,* *Lu. cruzi* *Lu. evansi* *Lu. longipalpis* *Lu. migonei* *P. ariasi* *P. balcanicus* *P. brevis* *P. chineesis* *P. kandelakii* *P. langeroni* *P. longiductus* *P. perfillewi* s.l. *P. perniciosus* *P. sichuanensis* *P. smimovi* *P. tobbi* *P. turanicus* *P. wui*	LCL, VL	Dogs	North Africa, Mediterranean basin, Middle East, Central Asia, North America, Central America, South America	6,200–12,000 VL in Old World; 4,500–6,800 VL in New World; Unknown number of CL cases
Le. mexicana	*Leishmania*	NW	*Lu. ayacuchensis* *Lu. olmeca olmeca,* *Lu. ovallesi* *Lu. anthaphora*	LCL, DCL, DsCL	Forest rodents	North America (including the United States), Central America, South America	†Included in the 187,200–300,000 total cases of New World CL
Le. amazonensis	*Leishmania*	NW	*Lu. faviscutellata* *Lu. longipalpis* *Lu. nuneztovari anglesi* *Lu. omeca novice* *Lu. olmeca reducta*	LCL, DCL, DsCL	Rain forest rodents, marsupials, foxes, bats	South America	†Included in the 187,200–300,000 total cases of New World CL
Le. venezuelensis	*Leishmania*	NW	*Lutzomyia* spp. implicated but not proven		Unknown	Northern South America	†Included in the 187,200–300,000 total cases of New World CL

Table 1. Clinical and Epidemiological Characteristics of *Leishmania* Species (continued)

***Leishmania* species**	**Subgenus**	**Old World and/or New World**	**Proven vector species**	**Clinical manifestation**	**Primary reservoir hosts**	**Distribution**	**Estimated global incidence**
Le. braziliensis	*Viannia*	NW	*Lu. carrerai* *Lu. complexa* *Lu. fischeri*	LCL, MCL, DCL, RCL	Opossums, rain forest rodents	Western Amazon basin, South America, Central America	†Included in the 187,200–300,000 total cases of New World CL
Le. guyanensis	*Viannia*	NW	*Lu. anduzei* *Lu. ayacuchensis* *Lu. shawi* *Lu. umbratilis,* *Lu. whitmani*	LCL, DsCl, MCL	Sloths	Northern South America	†Included in the 187,200–300,000 total cases of New World CL
Le. lainsoni	*Viannia*	NW	*Lu. nuneztovari anglesi* *Lu. ubiquitalis*	LCL	Forest rodents	Brazil, Bolivia, Peru	†Included in the 187,200–300,000 total cases of New World CL
Le. lindenbergi	*Viannia*	NW	*Lu. atunesi* implicated	LCL		Brazil	†Included in the 187,200–300,000 total cases of New World CL
Le. naiffi	*Viannia*	NW	*Lu. ayrozai* *Lu. squamiventris*	LCL	Armadillos, rodents	Brazil, French Guiana	†Included in the 187,200–300,000 total cases of New World CL
Le. panamensis	*Viannia*	NW	*Lu. gomezi* *Lu. harmanni* *Lu. panamensis* *Lu. trapidol* *Lu. yulli*	LCL, MCL	Sloths	Central America, South America	†Included in the 187,200–300,000 total cases of New World CL
Le. peruviana	*Viannia*	NW	*Lu. ayacuchensis* *Lu. peruensis*	LCL, MCL	Opossums, dogs	Peru, Bolivia	†Included in the 187,200–300,000 total cases of New World CL
Le. shawi	*Viannia*	NW	*Lu. whitmani*	LCL	Sloths, rodents	Brazil	†Included in the 187,200–300,000 total cases of New World CL
Le. martiniquensis	*Mundinia*	OW/NW	Unknown	LCL, VL	Horses, cattle	Martinique, Thailand, Central Europe, United States	Unknown
Le. orientalis	*Mundinia*	OW	Unknown	LCL	Unknown	Thailand	Unknown
Le. colombiensis	*Mundinia*	NW	*Lu. hartmanni*	LCL, VL	Sloths	Colombia	†Included in the 187,200–300,000 total cases of New World CL

VL = visceral leishmaniasis, PKDL = post kala-azar dermal leishmaniasis, DCL = diffuse cutaneous leishmaniasis, DsCL = disseminated cutaneous leishmanniasis, LCL = localized cutaneous leishmaniasis, MCL = mucocutaneous leishmaniasis, RCL = recidivans cutaneous leishmaniasis, *P.* = *Phlebotomus*, *Lu.* = *Lutzomyia*. † Accounting of CL cases in the New World is complex as there are multiple *Leishmania* species circulating in the same geographical area, variable clinical manifestations associated with each species and species identification is rarely reported. Table compiled from multiple authoritative sources.

In modern times (16th–19th century) there are many reports of cutaneous leishmaniasis (CL), generally conditions named for the location they were acquired (for example, Aleppo boil, Baghdad boil, Jericho boil); interestingly, many of these terms are still used today (Severding, 2017). The earliest report of a disease likely to be visceral leishmaniasis did not occur until the 19th century with a description of an outbreak of a disease in 1824–1827 that caused emaciation, enlarged spleens, acute anemia, intermittent fever, and a dried, scaly appearance of the skin (Gibson, 1983; Twining, 1827). The Hindi term kala-azar that roughly translates to black fever and referring to the grayish discoloration of the skin was coined late in the 19th century to describe VL (Steverding, 2014). In the 20th century the Scottish pathologist William Boog Leishmana discovered ovoid bodies from the spleen of a soldier who died from a disease of emaciation while serving at Dum Dum, a town in India, and termed the disease Dum Dum Fever (Leishman, 1903); at the same time Charles Donovan identified similar bodies from splenic aspirates of Indian patients (Donovan, 1903), but the 2 scientists could not agree if the parasites were trypanosomes or a new species. Ronald Ross, investigating kala-azar in India, found similar parasites from spleens of patients with chronic splenomegaly (Ross, 1903a) and settled the controversy, declaring the parasites a new species named *Leishmania donovani* (Ross, 1903b). Leishmaniasis was not reported in the new world until the 20th century; CL reported from Brazil in 1909, referred to as Baurú ulcers (Carini and Paranhos, 1909; Lindenberg, 1909), and VL, also from Brazil, in the 1930s (da Cunha et al., 1937).

Leishmaniasis continues to be a major global health threat and, although endemic in Europe, Africa, Asia, and America, 90% of cases occur in just 13 countries (Afghanistan, Algeria, Bangladesh, Bolivia, Brazil, Columbia, Ethiopia, India, Iran, Peru, South Sudan, Sudan, and Syria). The burden of leishmaniasis is largely underestimated, however, due to misdiagnosis and lacking surveillance systems. Human migration, political instability, climate change, and warfare is expanding *Leishmania*-endemic regions and increasing the propensity for epidemics worldwide. As an example, cutaneous leishmaniasis is currently spreading as refugees move from Syria through Turkey into Europe and other regions throughout the world (Hayani et al., 2015; Nimer, 2018). As a consequence, there is a significant risk that cutaneous leishmaniasis (CL) will reemerge in southern Europe, where the natural sand fly vectors for *Leishmania tropica* and *Le. major* are already endemic, and travelers, not only refugees, may be affected (Di Muccio et al., 2015).

Nomenclature and Morphology

Leishmania species are flagellated, single cell, protozoans in the order Kinetoplastida and the family Trypanosomatidae. As with other members of this group, *Leishmania* spp. are characterized by a unique mitochondrian that contains a kinetoplast at the base of the flagellum. The kinetoplast contains DNA (kDNA) that represents 10–20% of the total cellular DNA (Simpson, 1987) and is organized as an interlocked network containing dozens of maxi- and thousands of mini-circles. Mini-circles encode for guide RNAs (gRNAs) that function in a unique RNA editing mechanism (Read et al., 2016) and maxi-circles are analogous to mitochondrial DNA in other eukaryotes. Due to the high copy number of mini-circles, kDNA can be utilized for diagnostic purposes via polymerase chain reaction (PCR) (Van der Auwera and Dujardin, 2015; Galluzzi et al., 2018).

Historically, *Leishmania* species were classified based on the clinical symptoms they generated, those causing CL considered as *Le. tropica* and those causing VL as *Le. donovani*. As more parasites from around the world were examined and molecular techniques became available, it became clear that there were many different subgroups within the genus *Leishmania* (Lainson and Shaw, 1987). Today, at least 4 subgenera exist for the genus *Leishmania*: *Sauroleishmania, Leishmania, Mundinia,* and *Viannia* (Espinosa et al., 2018), the latter 3 subgenera contain species known to infect humans. Species of the subgenera *Sauroleishmania* infect lizards do not cause human disease. The vast majority of species that cause human disease belong to the *Leishmania* and *Viannia* subgenera; those belonging to *Leishmania* are found in both the Old and New World and those belonging to *Viannia* are exclusively found in the neotropics. These 2 subgenera can be distinguished by the location the parasites grow within the sand fly gut, *Leishmania* (*Leishmania*) spp. found anterior to the pylorus and *Leishmania* (*Viannia*) in the mid and hindgut.

Life Cycle

Leishmania are digenic parasites, completing their life cycle within 2 hosts. These parasites develop within the alimentary track of phlebotomine sand flies (order Diptera: family Psychodidae, subfamily Phlebotominae) and are transmitted to humans when female sand flies blood feed (Figure 1). Once deposited in the vertebrate host the parasites are quickly phagocytosed by cells of the mononuclear phagocyte system, where they establish their niche. *Leishmania* have 2 primary morphological forms, long (5–15 μm), extracellular promastigotes with long flagella in sand flies and small (3–5 μm), intracellular amastigotes with rudimentary flagella within vertebrate hosts.

Figure 1. A *Phlebotomus papatasi* sand fly, which landed atop the skin surface of the photographer who had volunteered himself as host for this specimen's blood meal. The sand flies are members of the Dipteran family Psychodidae and the subfamily Phlebotominae. This specimen was still in the process of ingesting its blood meal, which is visible through its distended transparent abdomen. Source: United States Public Health Image Library, image 10275; J. Gathany, 2006. Informed consent granted by human subject: J. Gathany, 2006. Public domain.

Leishmania are obligate intracellular parasites that survive and multiply within the mature phagolysosome compartment of mononuclear phagocytes. Metacyclic promastigotes that enter the skin are quickly phagocytosed either directly by macrophages or dendritic cells or indirectly through apoptotic infected neutrophils that are rapidly recruited to the bite site (van Zandbergen et al., 2004). *Leishmania* do not actively invade host cells, but rely on the phagocytic capacity of these cells to gain entry. This process is receptor-mediated (Alexander and Russell, 1992; Chang and Dwyer, 1978), eventually resulting in the formation of a phagolysosome, and is known to induce host-cell signal transduction pathways (Guy and Belosevic, 1993). Many receptors are known to mediate entry of *Leishmania* parasites including, complement receptors 1 and 3, FcReceptors, mannose receptors, scavenger receptors, and fibronectin receptors (Ueno and Wilson, 2012). Recently, Toll-like receptors also have been implicated during *Leishmania* infection (Chauhan et al., 2017). These receptors are part of the innate system of pattern recognition, a system used by host cells to discriminate between infectious non-self and self. Upon interaction with ligand, these receptors initiate signal transduction pathways that ultimately lead to the modulation of phagocyte functions. The repetitive structure and glycan modifications associated with many *Leishmania* cell surface molecules serve as pathogen associated molecular patterns (PAMPs) that are recognized by the pattern recognition receptors (PRRs) (Podinovskaia and Descoteaux, 2015).

Once phagocytosed, *Leishmania* parasites are delivered to an intracellular vacuole termed a phagosome. Phagocytosis of a foreign body typically results in phagosome fusion with lysosomes allowing for degradation of phagosomal contents within 30 minutes. This *Leishmania* delays the phagosome maturation process, taking approximately 5 hours, the delay theoretically allowing enough time for the parasites to start differentiating toward the more resistant amastigote stage (Desjardins and Descoteaux, 1997; Scianimanico et al., 1999); full differentiation into amastigotes occurs between 24 and 48 hours post engulfment. *Leishmania* amastigotes multiply within the phagolysosome and eventually escape from the cell by a poorly defined mechanism and re-invade other phagocytes. Different species of *Leishmania* induce morphologically distinct phagolysosomes. *Leishmania*. *mexicana* resides in spacious vacuoles that contain many amastigotes, whereas *Le*. *major* and *Le*. *donovani* induce tight fitting phagosomes that contain only 1 amastigote (McConville et al., 2007).

When a sand fly ingests blood, it is retained within a peritrophic matrix in the abdominal midgut until digestion is completed. *Leishmania* amastigotes within infective blood transform into procyclic promastigotes within 12–18 hours within the peritrophic matrix. Procyclic promastigotes rapidly divide within the digesting blood meal and differentiate into longer, more motile nectomonads. When blood digestion is completed and excreted (~ 3–5 days), nectomonads are found in the gut attached to the epithelial cell microvilli via their flagella (Sacks and Kamhawi, 2001). Approximately 7 to 10 days following the initial blood meal the parasites transform into short, actively dividing forms called leptomonads and migrate to the thoracic midgut and stomodeal valve (an invagination of the foregut into the midgut). Leptomonads produce a substance, promastigote secretory gel that imbeds the parasites (Gossage et al., 2003). The end-point of parasite development in the sand fly is differentiation into the infectious stage, metacyclic promastigotes. These infectious forms are short, slender forms with a flagella twice as long as the body and are highly motile (Sacks and Perkins, 1984). Different than *Plasmodium* sporozoites that reside in mosquito salivary glands and are injected during blood-feeding, *Leishmania* metacyclic promastigotes do not invade the salivary glands and are regurgitated. The stomodeal valve is physically obstructed by the promastigote secretory gel, interfering with blood-feeding and leading to regurgitation and persistent but intermittent feeding (Rogers et al., 2004).

Clinical Manifestations

Leishmaniasis is a group of diseases characterized by a range of clinical symptoms that fall under 2 primary

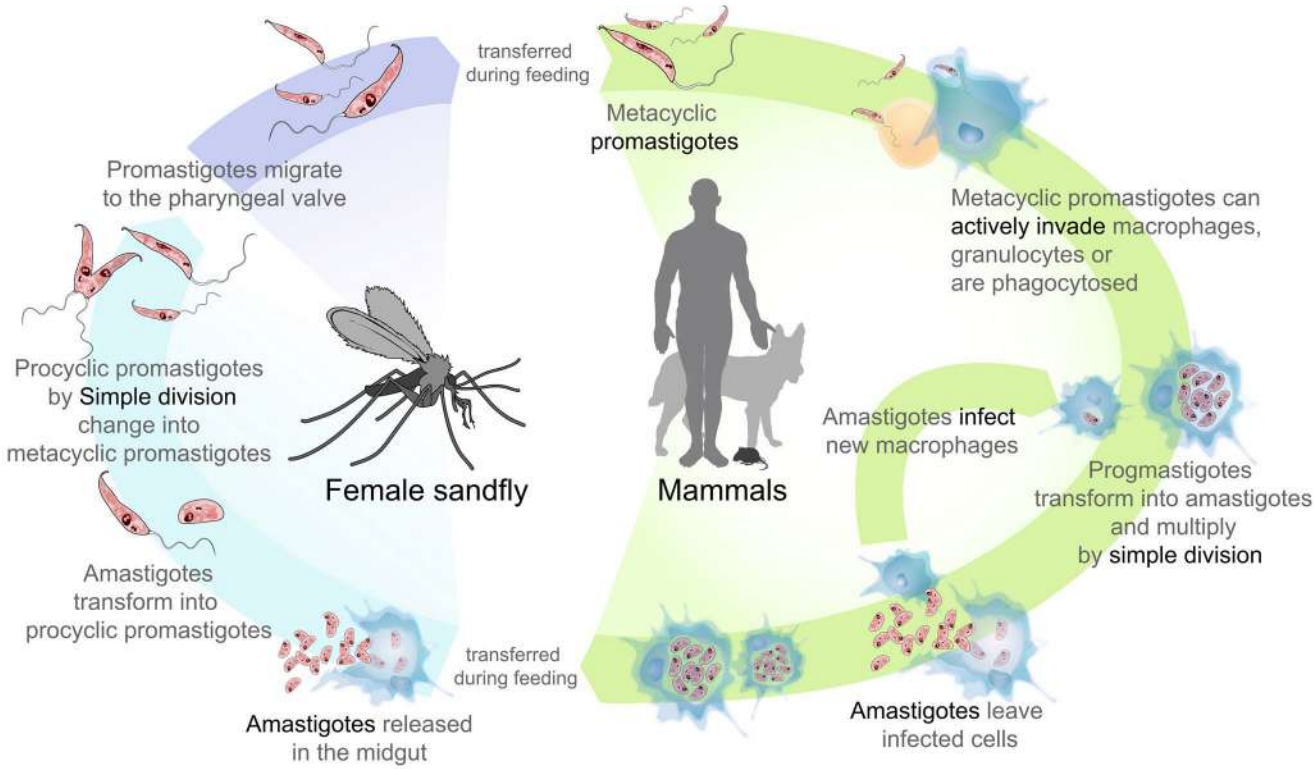

Figure 2. Life cycle of the parasites from the genus *Leishmania*. Source: Wikimedia Commons; Ruiz Villarreal, 2008. Public domain.

categories, **cutaneous leishmaniasis** (CL) and **visceral leishmaniasis** (VL). Although this is an ever-changing number as new species are described and taxonomic phylogenies revised, there currently are 22 *Leishmania* species known to be pathogenic to humans (Table 1; PAHO, 2024). The different pathological manifestations that present are primarily associated with the *Leishmania* spp. initiating the infection.

Cutaneous Leishmaniasis (CL)

The World Health Organization (WHO) estimates that 1.2 million annual cases of CL worldwide with 70–75% of the cases occurring in just 10 countries, Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, North Sudan, Peru, and Syria. However, these statistics are largely underestimated due to misdiagnosis and lack of surveillance systems (see Figures 3 and 4).

There has been a recent increase in the incidence of CL in the United States due to several factors, including travel to endemic regions, immigration, and military operations. Twenty cases of CL were identified in United States military personnel deployed during the 1990–1991 Gulf War in the Middle East. More recently, CL has had a profound effect on United States troops; 1,287 deployed United States military personnel contracted leishmaniasis during campaigns in Iraq and Afghanistan during 2001–2006 and 522 cases in personnel who served in southwest and central Asia (Pavli and Maltezou, 2010). In addition, CL is found in the United States, being endemic in southern Texas and may be spreading north to Oklahoma (Clarke et al., 2013; Kipp and Hergert, 2019).

The most common form of this CL is **localized cutaneous leishmaniasis (LCL)**, characterized by localized, self-limiting cutaneous ulcers and powerful lifelong immunity upon healing. The lesions most often occur on exposed areas of skin where the sand fly vector can take a blood meal and begin as papules that eventually ulcerate. The patients are generally well and slight pain may or may not be associated with the lesions. Multiple lesions of this condition usually correspond to multiple sand fly bites. Although the lesions eventually heal (3–18 months), even without treatment, they are associated with substantial scarring and often social stigm (Bennis et al., 2018). Ancient civilizations noted that individuals who had healed from Oriental sores were protected from further disease (Steverding, 2017). Leishmanization, the practice of innoculating individuals with exudates from active lesions into the buttocks of young children, particularly girls, has been used in the Middle East and Central Asia for centuries to prevent the development of facial scars

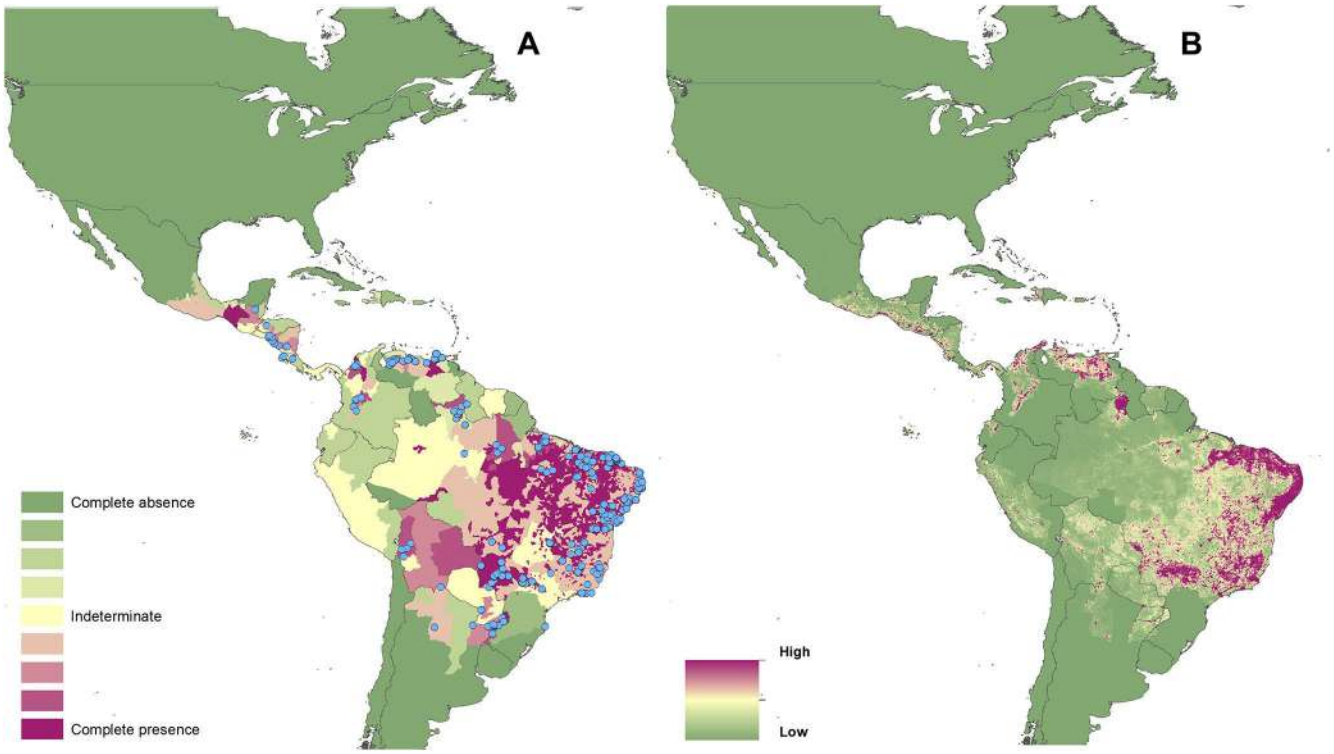

Figure 3. Reported and predicted distribution of cutaneous leishmaniasis in the New World. A) Evidence consensus for presence of the disease ranging from green (complete consensus on the absence: −100%) to purple (complete consensus on the presence of disease: +100%). The blue spots indicate occurrence points or centroids of occurrences within small polygons. B) Predicted risk of cutaneous leishmaniasis from green (low probability of presence) to purple (high probability of presence). Source: Pigott et al., 2014. License: CC0 1.0 Universal.

(Steverding, 2017). Today, leishmanization is still the only effective leishmaniasis vaccine that leads to lifelong protection (Nagill and Kaur, 2011).

Diffuse cutaneous leishmaniasis (DCL) is less common and characterized by multiple slow growing, non-tender nodules that disseminate all over the body. The nodules do not ulcerate and typically contain large numbers of parasites. DCL is a chronic disease that can persist for 20 years or more (Hashiguchi et al., 2016) and is often misdiagnosed as lepromatous leprosy. The condition is thought to reflect an underlying lack of a cellular immune response as evidenced by poor ability to respond to *Leishmania* antigen (Scott and Novais, 2016).

Disseminated cutaneous leishmaniasis (DsCL) is a non-chronic condition where there are multiple (≥ 10) lesions of different types, often ulcerating on more than 2 parts of the body. Patients are strongly positive for the Leishmanin skin test, indicating strong cellular immunity (Hashiguchi et al., 2016).

Recidivans cutaneous leishmaniasis (RCL) is a reactivation after a lesion is healed, usually within 2 years. Reactivated lesions typically encircle the previous scar and can be difficult to treat (Gitari et al., 2018). This condition is primarily associated with *Leishmania tropica* infection.

Mucocutaneous leishmaniasis (MCL) occurs after an initial local cutaneous lesion has healed. The parasites disseminate to the nasopharyngeal mucus membranes. The disease is characterized by destruction of the nasal septum, lips, palate, and sometimes larynx. Patients with MCL are at risk of death due to aspiration pneumonia.

Leishmania major.

Cutaneous leishmaniasis in the Old World accounts for the majority of global CL incidence in the world. The majority of these cases are caused by either *Leishmania major* or *Le. tropica* (Table 1). *Leishmania major* is endemic in the Middle East and North, West, and East Africa, and Central Asia. Confirmed vector species include *Phlebotomus duboscqi* in western and eastern Africa*, P. papatasi* and *P. bergeroti* in the Middle East, North Africa, and Europe, and *P. salehi* in India, Iran, and Pakistan. *Leishmania major* is a zoonosis with several rodent species implicated as reservoirs, differing geographically.

Leishmania major infection predominately manifests as LCL with lesions starting as a small papule, occasionally with nodules, at the site of the sand fly bite. Lesions generally

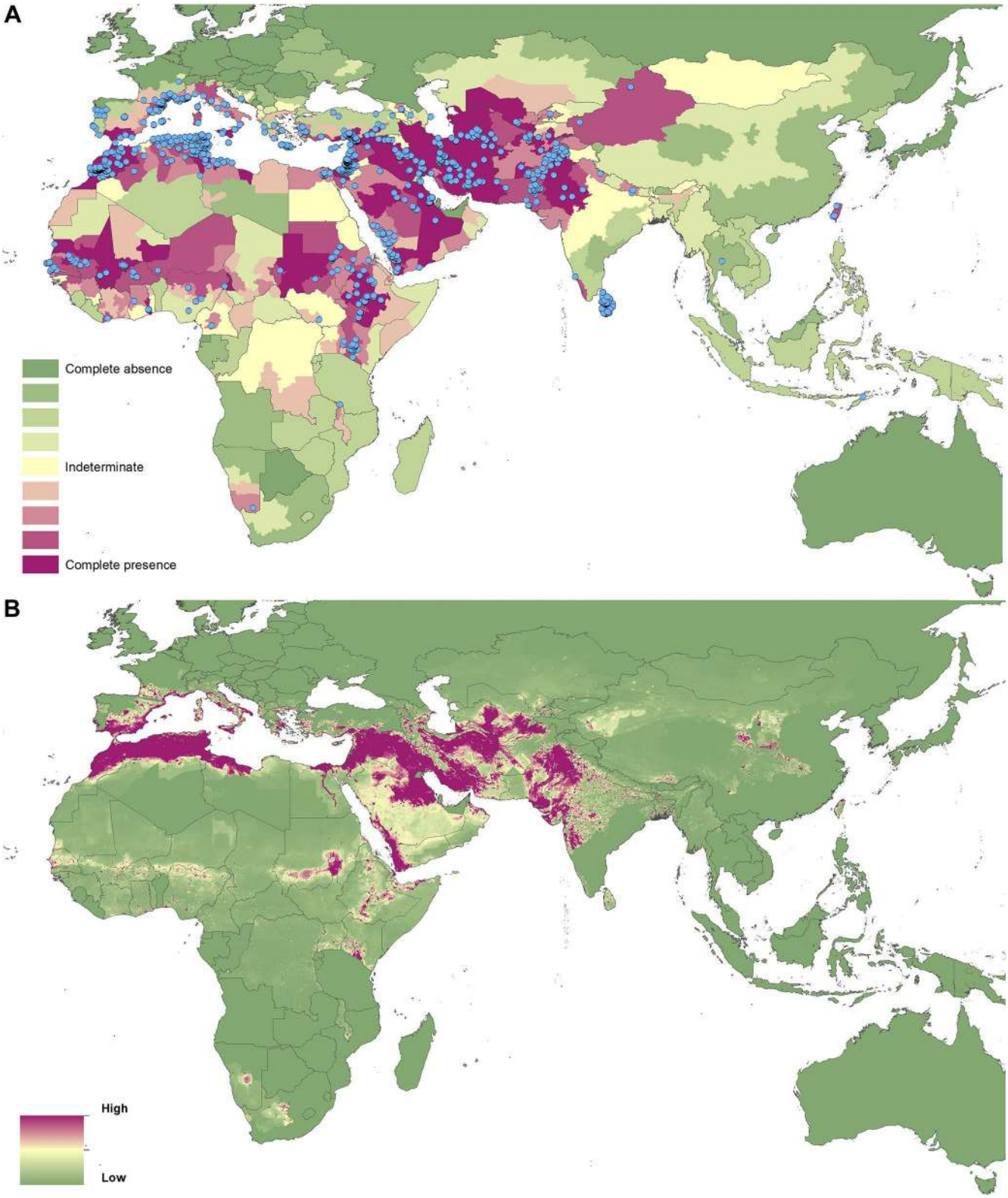

Figure 4. Reported and predicted distribution of cutaneous leishmaniasis in the Old World. A) Evidence consensus for presence of the disease ranging from green (complete consensus on the absence: −100%) to purple (complete consensus on the presence of disease: +100%). The blue spots indicate occurrence points or centroids of occurrences within small polygons. B) Predicted risk of cutaneous leishmaniasis from green (low probability of presence) to purple (high probability of presence). Source: Pigott et al., 2014. License: CC0 1.0 Universal.

occur on the head, neck, or extremities where sand flies have access to the skin. The incubation period is generally 2–4 weeks but may range from days to years (Goto and Lindoso, 2010). The papules enlarge and develop into large painless ulcers with a raised darker pigmented border (Darmstadt et al., 1993; Morris-Jones and Weber, 2004). The lesions are typically wet and associated with severe inflammation (Burza et al., 2018). Rarely is there more than 1 lesion present and self-healing usually occurs within 1 year (Melby et al., 1992); however, there is severe scarring due to the necrosis and inflammation associated with the lesions (Burza et al., 2018). Those that heal become immune to further infection; this natural occurring immunity provides the rational for vaccine development.

Leishmania tropica.

The geographic range of *Leishmania tropica* includes Central and North Africa, the Middle East, and Central Asia. *Le. tropica* infection has generally thought of as an anthroponotic infection, however, in some locations it appears to be a zoonosis (Kamhawi et al., 1995; Saliba et al., 1997; Talmi-Frank et al., 2010), with the rock hyrax as a reservoir host (Talmi-Frank et al., 2010). Vector species include *Phlebotomus guggisbergi*, *P. rossi*, *P. saevus*, and *P. arabicus*, and *P. sergenti*, with the latter 2 being the most common.

Similar to *Leishmania major* infections, the majority (76%) of lesions occur on the head and neck, followed by 30–36% on the extremities and trunk (Solomon et al., 2014). Most patients present with 1 LCL lesion and 95% have fewer than 3 lesions (Solomon et al, 2014). Lesions due to *Le. tropica* infection typically take longer to heal than those caused by *Le. major*, with the majority healing within 2 years (Handler et al., 2015). The lesions ulcerate but are dry in nature (Burza et al., 2018). In some cases, lesions can develop into hyperkeratotic plaques that resemble large warts (Burza et al., 2018). Individuals that have healed from a previous cutaneous ulceration due to *Le. tropica* can relapse, causing RCL (Burza et al., 2018). This chronic relapsing form of the disease generally begins with nodules, with lesions forming at the periphery of the old lesion scar.

Of the 32 cases of leishmaniasis identified in United States soldiers during the 1990–1991 Desert Storm Campaign, 12 were characterized as viscerotropic caused by *Leishmania tropica* (Hyams et al, 1995). Viscerotropic leishmaniasis is a syndrome where parasites spread to visceral organs and is associated with a prolonged systemic illness that includes fever, malaise, abdominal pain, and intermittent diarrhea but does not progress to fatal VL (Magill et al., 1993).

Most recently, the civil war in Syria has been associated with a large epidemic of *Leishmania tropica* CL (Rehman et al., 2018). Before the onset of the civil war, WHO statistics indicate approximately 14,000 new CL cases per year in Syria with incidence increasing to 27,825 in 2010 (Rehman et al. 2018) and to 89,357 in 2019 (WHO, 2024). The governmental surveillance system for leishmaniasis has lost access to some provinces in Syria so only sparse reliable data have been published since 2011. A recent humanitarian organization reported nearly 65,000 cases from a few provinces in northern Syria, with the majority associated with *Le. tropica* infection, although *Le. major, Le. infantum,* and *Le. donovani* are also present (Rehman et al, 2018). Syrian refugees are migrating to nearby areas where suitable vectors are endemic, expanding the epidemic.

Leishmania aethiopica.

Leishmania aethiopica is a zoonotic disease, primarily occurring in the highland areas of Ethiopia with the rock hyrax as a reservoir. The annual burden of CL in Ethiopia is approximately 20,000–40,000 cases per year (Alvar et al., 2012) with 99% with *Le. aethiopica* as the etiological agent (van Griensven et al., 2016). While LCL is the most frequent manifestation, clinical symptoms are diverse with MCL and DCL also being relatively common (Padovese et al., 2009). The reasons for the diverse symptomology are unclear and may involve many factors, including the high level of genetic polymorphism exhibited by this species (Pratlong et al., 2009).

Leishmania aethiopica LCL lesions are slower to develop, typically do not ulcerate, and are more chronic compared to other LCL lesions, requiring 2–5 years to heal (Handler et al., 2015). Both MCL and DCL caused by *Le. aethiopica* are reportedly less responsive to common antileishmanial drugs (Padovese et al., 2009). Treatment for DCL has been notoriously difficult and even if lesions regress, relapse is common upon cessation of chemotherapy (van Griensven et al., 2016).

Leishmania mexicana.

Cutaneous leishmaniasis epidemiology in the Americas is extremely complex with multiple *Leishmania* species with overlapping geographical distributions, a variety of sand fly vectors, and many different reservoir hosts. Due to this complexity and that reporting structures do not require species identification, the exact incident levels of each species is difficult to discern. *Leishmania mexicana* is endemic in North America, Central America, and South America and is the species endemic to the United States.

Forest rodents serve as reservoir hosts in most of the Americas, with 3 species of woodrats serving as reservoir hosts in the United States, including *Neotoma micropus, N. albigula,* and *N. floridana*. Several confirmed vectors have

been identified (Table 1); *Lutzomyia anthaphora* is the only confirmed vector in the United States (Endris et al., 1987; McHugh et al., 1993).

Leishmania mexicana lesions resemble those caused by *Le. major* being generally less severe and healing quickly. However, they can be slow to develop, sometimes taking up to 6 months, and can persist for 20 years (Handler et al., 2015). Lesions occur roughly 50% of the time on the ear, a manifestation referred to as chiclero's ulcer; the term is used because LCL on the ear is common among men that visit the forests to collect chicle (natural form of gum). DCL rarely presents with infection with *Le. mexicana*.

Leishmania braziliensis.

Leishmania braziliensis, endemic in South America and Central America (Grimaldi et al., 1987) is known in some places locally as espundia. Infection with *Le. braziliensis* results in severe cutaneous lesions and is associated with satellite subcutaneous nodules and lymph node involvement (Melby et al. 1992). LCL lesions caused by *Le. braziliensis* generally ulcerate and may heal within 6 months; however, 2–5% of cases develop into MCL and these require treatment (Burza et al., 2018). Although several species have been implicated in MCL, *Le. braziliensis* accounts for the majority of cases in the New World (Strazzulla et al., 2013).

MCL usually presents after the healing of a primary skin lesion but can begin to develop prior to lesion resolution (Daneshbod et al., 2011). Mucosal involvement normally appears within 2 years of LCL but has been reported to take up to 30 years (Samady et al., 1996). Although general subsequent mucosal involvement generally occurs in < 5% of cases, it may be as high as 20% in certain regions (David et al., 1993).

It is unknown why some patients are more susceptible to MCL. *Leishmania* RNA virus (LRV)-1 was identified in both *Le. braziliensis* and *Le. guyanensis*, both associated with MCL, leading to the hypothesis that MCL is actually virally mediated (Scheffter et al., 1995). It is hypothesized that virally infected *Leishmania* are recognized by host PRR that induce killing of the parasite and allowing dispersal of the virus. This dispersal in turn triggers a metastatic hyperinflammatory reaction, resulting in tissue damage (Weinkopff et al., 2013; Zangger et al., 2013).

Additional New World *Leishmania* Species.

Leishmania amazonensis is restricted to South America and is associated with LCL, DCL, and DsCL. *Leishmania peruviana* causes LCL, a disease known as uta in preschool age children in the Peruvian Andes (Davies et al., 1997). Ulcera de Bejuco is CL caused by *Le. panamensis*, characterized by shallow ulcers that metastasize along lymphatic vessels. There is no spontaneous healing of the lesions and 2–5% develop MCL (Koff and Rosen, 1994). *Leishmania guyanensis* infection also is associated with multiple ulcers that can spread along the lymphatics and is known as pianbois. These lesions generally require treatment and often reoccur (Burza et al., 2018).

Visceral Leishmaniasis

Visceral leishmaniasis (VL) affects the spleen, liver, bone-marrow, and other visceral organs. There is no cutaneous pathology associated with initial presentation and clinical manifestations include persistent fever, hepatosplenomegaly, and weight loss. The disease can be either acute or gradual and is generally fatal within 2 years without treatment as a result of secondary bacterial infections or severe anemia. Acute malnutrition and high parasite burdens are present in young children with VL (Harhay et al., 2011). In the Indian subcontinent, hyperpigmentation of the skin is associated with VL, so is often referred as kala-azar, meaning black fever. Although endemic in 97 countries and territories, nearly 90% of the global burden of VL occurs in just 6 countries: Brazil, Ethiopia, India, Somalia, South Sudan, and Sudan (WHO, 2017).

Post-kala-azar dermal leishmaniasis (PKDL) is a late manifestation of VL caused by *Leishmania donovani* following treatment. PKDL presents as a hypopigmented macular or erythematous maculopapular rash on the face that can, in some instances, extend to the entire body. In PKDL, the parasites seem to persist in the skin after treatment. The syndrome can be mistaken for lepromatous leprosy but can be distinguished by the preservation of sensation (Burza et al., 2018).

Figure 5. Distribution of hunt clubs with confirmed cases of visceral leishmaniasis, United States and Canada. States in which hunt clubs or kennels had ≥ 1 dog infected with *Leishmania infantum* are shaded. *Leishmania*-positive foxhounds were also found in Nova Scotia and Ontario, Canada. Source: Duprey et al., 2006. Public domain.

Leishmania donovani.

The WHO estimates that over 70% of global VL caused by *Leishmania donovani* cases occurs in the Indian subcontinent and eastern Africa (WHO, 2016). Currently, East Africa has the highest burden of VL due to ongoing success with elimination efforts in Southeast Asia (Alves et al., 2018). VL caused by *Le. donovani* is considered anthroponotic because humans are the primary reservoir, although domestic dogs have been implicated as a possible minor reservoir host (Jambulingam et al., 2017).

The primary sand fly vector for VL in India and Bangladesh is *Phlebotomus arentipes*, and *P. orientalis* and *P. martini* for East Africa. In India the disease is associated with poor housing conditions where houses typically are made of mud walls and livestock and humans live under the same roof, creating an excellent ecological niche for the vector. VL in East Africa occurs primarily in arid and semi-arid lowland areas and is associated with migrant agricultural workers that typically sleep outdoors (Argaw et al., 2013).

Asymptomatic *Leishmania donovani* infections are common in endemic areas with seroprevalance in healthy individuals ranging between 7–63% in India (Srivastava et al., 2013) and 7–46% in Ethiopia (Ayehu et al., 2018; Abbasi et al., 2013). The underlying mechanisms that lead to clinical disease are not elucidated although malnutrition is thought to play a role and immunosuppression, particularly HIV coinfection in Ethiopia, is a major contributor. Commonly the incubation period is between 2 and 6 months and between 2–23% of asymptomatic individuals will present with VL symptoms within a year (Burza et al., 2018). VL caused by *Le. donovani* is almost always fatal unless treated and viable parasites can persist even after successful treatment, reactivating to cause disease if the individual becomes immunosuppressed (Diro et al., 2015).

Even without immunosuppression, PKDL can develop after apparently successful treatment. PKDL occurs in 25–50% of treated patients in Sudan within 6 months but is less common (5–10%) and occurs much longer after treatment (2–3 years) in India (Zijlstra et al., 2003). Interestingly, 5% of Indian PKDL patients report no previous VL episode (Zijlstra et al., 2016). In Asia, 90% of PKDL cases are of the macular type and African PKDL cases are primarily papular rash (Burza et al., 2018). Up to 85% of PKDL cases in East Africa are self-curing within 12 months and primarily pose aesthetic problems, although a small number will develop severely debilitating forms (Zijlstra et al., 2016). Important, however, is that PKDL lesions remain infectious for sand flies, serving as a reservoir of infection (Molina et al., 2017).

Leishmania infantum.

Leishmania infantum has a wide geographic distribution being endemic in the Americas, North Africa, the Mediterranean Basin, the Middle East, and Central Asia. Over 90% of VL cases due to *Le. infantum* occur in Brazil. VL was first discovered in the new world in 1937 and the parasite isolates were thought to be a new species and named *Le. chagasi* (Da Cunha et al., 1937). The following year, the discoverers realized that the parasites behaved like *Le. infantum* and concluded that the parasites that cause VL in the New World was identical to *Le. infantum* (Da Cunha, 1938), however the name *Le. chagasi* continued to be utilized in the literature. Modern molecular tools also are not able to distinguish *Le. infantum* from *Le. chagasi* (Mauricio et al., 1999), leading to the general agreement that the isolates from different geographical and host origins are, indeed, the same species. However, minor phenotypic and genotypic differences have led some authors to separate them into 2 species or, alternatively, 2 subspecies named *Le. infantum infantum* and *Le. i. chagasi* (Lainson and Rangel, 2005).

Infection with *Leishmania infantum* is primarily zoonotic, with the domestic dog as the reservoir host. Transmission occurs through the bite of sand flies of the genus *Lutyzomia* in the New World and *Phlebotomus* in the Old World. VL in Brazil used to be primarily restricted to rural areas in northeastern Brazil (De Melo and Fortaleza, 2013). Deforestation and associated changes in ecological habitats for the vector, urbanization, human migration, and the spread of HIV are changing the epidemiological profile to include urban epicenters of disease and a southward expansion (Arias et al., 1996). This changing epidemiological picture complicates prevention as measures directed at controlling the disease through vector control (insecticide spraying, use of repellents, or environmental management) or through management of canine leishmaniasis (dog culling or vaccination) are more difficult in urban settings (De Melo and Fortaleza, 2013). Moreover, control methods directed at controlling canine leishmaniasis have had varied outcomes (Romero and Boelaert, 2010).

As with *Leishmania donovani,* asymptomatic infections are common (9–24%) with individuals infected with *Le. infantum*. Conversely, there is little PKDL associated with *Le. infantum* infection except in cases of immunosuppression (Stark et al., 2006). *Leishmania infantum* can also cause LCL associated with single nodules and minimal inflammation that self-heal and induce immunity (Burza et al., 2018).

Visceral leishmaniasis (VL) caused by *Leishmania infantum* in the Old World continues to be primarily a rural disease, however a recent outbreak in Spain occurred in an urban area and was linked to a wild hare reservoir host (Arce et

al., 2013). Direct transmission without a sand fly vector also has been documented in Spain between intravenous drug users co-infected with HIV through needle sharing (Alvar et al., 1997).

During 2001–2016, 25 VL diagnoses were reported from United States soldiers deployed in the Middle East (Stahlman et al., 2017). Over the past decade, it has begun to be appreciated that asymptomatic VL is common in endemic regions, however the role of asymptomatic individuals in disease transmission and how many may progress to fulminant VL is less clear. Recently, higher risk United States military personnel were assessed 11 years after deployment in the Iraq War (2002–2011) to determine the rate of asymptomatic individuals; nearly 20% of these individuals were positive for *Leishmania infantum* infection (Modý et al., 2019). The risk of reactivation to VL for United States military veterans or to blood safety in the United States blood supply remains to be determined.

Canine Leishmaniasis

Zoonotic leishmaniasis can be found in all forms: Visceral, cutaneous, and mucocutaneous. However, canines act as the major reservoir of infection for *Leishmania infantum* which makes zoonotic visceral leishmaniasis (ZVL) the most pervasive of all forms of zoonotic leishmaniasis diseases (Gramiccia and Gradoni, 2005). Canine leishmaniasis (CanL) traditionally affects dogs in the same geographic regions as human visceral leishmaniasis such as the Middle East, South Asia, Central America, South America, North Africa, and East Africa (Tuon et al., 2008). Although dog ownership is not strictly necessary to place a person at higher risk of infection with leishmaniasis, regions with higher rates of CanL observe higher rates of human disease as well. In recent years, cases of CanL have been seen in non-endemic areas (Duprey et al., 2006).

Figure 6. Canine visceral leishmaniasis. Source: Dantas-Torres, 2008. License: CC BY 2.0.

In the United States, it was previously thought that CanL cases seen were strictly due to foreign travel. However, in the late 1990s, infections began to appear in the foxhound kennels (over 40% presented with disease) where foxhounds had no history of travel. A survey of the United States and Canada in the early 2000s found that 18 states and 2 provinces were enzootic for canine leishmaniasis (Duprey et al., 2006; see Figure 5). With no seropositive cases of *Leishmania* found in wild canines or in humans with close contact to the kenneled dogs, dog-to-dog transmission was considered to be the main route of infection. Risk factors associated with dog-to-dog transmission were thought to be the large number of animals housed together at a time, travel of foxhounds internationally and across state lines for breeding and club practices, and the inherent nature of the breed (Duprey et al., 2006).

Transmission.

As with human infections, *Leishmania* transmission to dogs principally occurs through the bite of phlebotomine sand flies through most of the world. Old World transmission occurs through the bite of *Phlebotomus* spp. sand flies, and *Lutzomyia* spp. sand flies in the New World (Killick-Kendrick, 1999). The sand fly vector appears to have preferential feeding on short-haired canines as higher rates of infection have been observed in short hair breeds (Dantas-Torres, 2008; França-Silva et al., 2003). Dogs receive bites in hairless areas such as the ear pinna, nose, and inguinal and perianal areas (Alvar et al., 2004). Although any dog is at risk for infection, dog breeds such as cocker spaniel, boxer, rottweiler, and German shepherd appear to be the most susceptible to infection (Killick-Kendrick, 1999). Thus far, very few breeds, like the Ibizian Hound, have shown resistance to developing clinical signs (Solano-Gallego et al., 2009).

Experimental studies have demonstrated that phlebotomine sand flies from non-endemic areas may become infected with *Leishmania* after feeding on an infected animal (Travi et al., 2002; van Griensven and Diro, 2019; Aronson and Joya, 2019). PCR analysis can deliver results with a quicker turnaround time (under 24 hours) and is able to detect low levels of parasitemia compared to the lengthier traditional methods

of diagnosis (Srivastava et al., 2011; Aronson et al., 2017; Akhoundi et al., 2017). However, PCR-based assays can still be disadvantageous for many endemic regions as the techniques are generally more expensive and complex than microscopy, require well trained staff, and require more financial resources to maintain equipment and purchase reagents (Burza et al., 2018; Vink et al., 2018).

Loop mediated isothermal amplification (LAMP) assays may be the answer to keeping all the advantages of molecular techniques while also reducing the associated disadvantages. In LAMP assays, there is no longer a need for expensive equipment as DNA amplification occurs at a constant temperature (~ 60 °C), reagents are lyophilized, and the product is easily visualized with simple methods (Mukhtar et al., 2018). Currently, the Loopamp *Leishmania* Detection Kit™ (Eiken Chemical Company, Japan) is available on the market and has shown promise for point-of-care testing with multiple sample types for several *Leishmania* species and assessment of cure for VL and PKDL (Vink et al., 2018; Mukhtar et al., 2018; Verma et al., 2017).

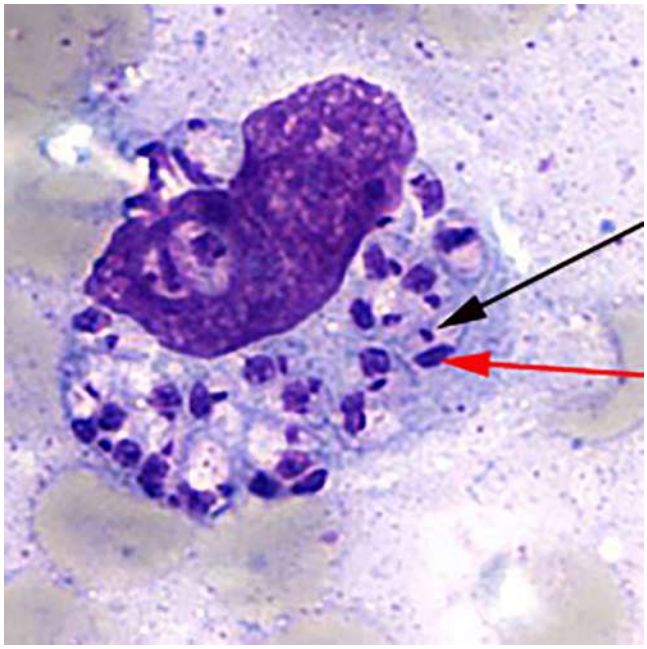

Figure 7. Light-microscopic examination of a stained bone marrow specimen from a patient with visceral leishmaniasis—showing a macrophage (a special type of white blood cell) containing multiple *Leishmania* **amastigotes** (the tissue stage of the parasite). Note that each amastigote has a **nucleus** (red arrow) and a rod-shaped **kinetoplast** (black arrow). Visualization of the kinetoplast is important for diagnostic purposes, to be confident the patient has leishmaniasis. Source: United States Centers for Disease Control and Prevention, DPDx. Public domain.

Treatment

Visceral Leishmaniasis

Many of the treatments for VL are toxic and/or expensive. The use of the different drugs, dosage, and treatment regimens differs depending on the *Leishmania* species initiating the infection, the geographical location, associated drug resistance in the region, and HIV co-infection. For current guidelines consult Aronson et al. (2017) and Burza et al. (2018).

The heavy metal antimony was first introduced as a therapy for leishmaniasis in the 1940s and for decades pentavalent antimonials were used as a monotherapy. Currently there are 2 antimony formulations in use: Sodium stibogluconate marketed as Pentostam® from Glaxo-Smith Kline and megalumine antimoniate marketed as Glocontime® from Rhone-Poulenc. These drugs have poor oral bioavailability so are delivered either by intramuscular injection or intravenously at a dose of 20 mg/kg per day for 16–28 days. The treatment is painful to administer and there are many adverse symptoms associated with the treatment, including pancreatitis, hepatotoxicity, cardiotoxicity, and induction of arrhythmias (Sundar and Chakravarty, 2010). Drug resistance is emerging to sodium stibogluconate on the Indian subcontinent so is no longer recommended as a therapy in this area (WHO, 2010; Sundar et al., 2000).

Miltefosine (hexadecylphosphocholine) was introduced as a chemotherapeutic to treat visceral leishmaniasis in 2002 as a result of a special public-private program initiated by the WHO with the pharmaceutical company Asta Medica to repurpose the anti-cancer compound (Sunyoto et al., 2018). Miltefosine is a broad spectrum anti-microbial, originally developed as an anti-cancer agent in the 1980s but adverse events in several phase I and II clinical studies resulted in the discontinuation of the drug's development as an oral anti-cancer drug (Planting et al., 1993; Verweij et al., 1993). As the only oral drug available to treat leishmaniasis, its introduction was seen as a breakthrough treatment. Unfortunately, this compound, marketed as Impavido® never reached its potential in controlling leishmaniasis due to gastrointestinal side effects, emergence of drug resistance, and cost (Dorlo et al., 2014; Ostyn et al., 2014; Rijal et al., 2013; Sundar et al., 2012). An economic analysis concluded that to be an effective public health tool, the drug should cost no more than US$ 50–60 per treatment (den Boer et al., 2011); currently the price for the public or non-for-profit sector in developing countries is US$ 117–160 and set at a market price of US$ 33,000–51,000 in the United States (Sunyoto et al., 2018).

Injectable paromomycin was introduced to combat leishmaniasis in 2006 and is used in combination treatment regimens with both pentavalent antimonials and miltefosine (Burza et al., 2018). The low cost of this drug (~ US$ 10–15)

is of great benefit to the public health sector. Amphotericin B deoxycholate, an anti-fungal drug, and its liposomal formulation are also used as monotherapy and in combination with other drugs.

Cutaneous Leishmaniasis

Most CL lesions will spontaneously heal within 2–18 months (David and Craft, 2009). For LCL caused by *Leishmania major* and *Le. mexicana* where 70% and 88%, respectively, of cases heal within 4 months, no treatment may be warranted. Treatment may be conducted to accelerate healing to reduce scarring, to reduce the risk of dissemination, or if the lesions are on the face or joints (Hodiamont et al., 2014; Weina et al., 2004). The Infectious Diseases Society of America and American Society of Tropical Medicine and Hygiene Clinical Practice Guidelines (Aronson et al., 2017) recommend local/topical therapy for non-healing simple lesions and systemic therapy for complex (MCL, RCL, DCL, DsCL, multiple lesions, lesions on face) and suggest that if resolution is apparent, management can occur without treatment if the patient concurs.

Treatment of CL has traditionally been administered by intralesional injection of the aforementioned drugs or topical application of antimicrobials such as paromomycin with or without methylbenzethonium chloride or gentamycin. Cryotherapy, a stimulus that decreases the lesion tissue temperature and results in cryonecrosis, has also been utilized (López-Carvajal et al., 2016). Recently, thermotherapy has been reintroduced as a therapy as amastigotes are heat sensitive and devices that deliver the radiofrequencies that deliver a temperature of 40–42 °C are relatively inexpensive (Burza et al., 2018). Systemic treatment is generally reserved for immunocompromised patients, individuals with multiple or refractory lesions, or patients at a risk for developing MCL (Burza et al., 2018; Aronson et al., 2017).

Table 2: Clinical Staging of Canine Leishmaniasis, Therapy and Prognosis

Clinical stage	Serology[a]	Clinical signs	Laboratory tests	Therapy	Prognosis
Stage I mild disease	Negative to low antibody levels	Mild clinical signs such as peripheral lymphadenopathy or papular dermatitis	Usually no clinicophathological abnormalities observed; normal renal profile	Allopurinol alone or with meglumine animoniate or miltefosine	Good
Stage II moderate disease	Low to high[b] antibody levels	Stage I signs plus diffuse or symmetrical cutaneous lesions such as exfoliative dermatitis/ onychogryphosis ulcerations (planum nasale, footpads, boy prominences, mucocutaneous junctions), anorexia, weight loss, fever, and epistaxis	Clinicopathological abnormalities such as mild non-regenerative anemia, hypergammaglobulinemia, hypoalbuminemia, serum hyperviscosity syndrome; normal renal profile [blood creatinine levels < 1.4 mg/dl] and non-proteinuric [urine protein to creatinine ratio (UPC) < 0.5] or UPC = 0.5–1	Allopurinol with meglumine animoniate or miltefosine	Good to guarded
Stage III severe disease	Medium to high antibody levels	Stage I & II signs plus may present vasculitis, arthritis, uveitis, or glomerulonephritis	Clinicopathological abnormalities from Stage II and chronic kidney disease (CKD) with UPC>1 or creatinine 1.4–2.8 mg/dl	Allopurinol with meglumine animoniate or miltefosine and follow guidelines for CKD	Guarded to poor
Stage IV extreme disease	Medium to high antibody levels	Stage I, II, & III signs plus pulmonary thromboembolism or nephrotic syndrome and end stage renal disease	Clinicopathological abnormalities from Stage II and creatinine 2–5 mg/dl or > 5 mg/dl. Nephrotic syndrome (UPC > 5)	Allopurinol alone and follow guidelines for CKD	Poor

[a] Dogs with negative to low antibody levels are confirmed as infected with additional diagnostic techniques. [b]High antibody levels are a conclusive diagnosis and are detected through immunofluorescence antibody test (IFAT) or enzyme-linked immunosorbent assay (ELISA). Table modified from Solano-Gallego et al., 2017.

Animal Models and Immunology

Susceptibility and resistance to *Leishmania* infection are regulated by genetic determinants and animal models have been instrumental in deciphering these mechanisms (Blackwell et al., 2009). In addition, many immunological aspects of the disease have been elucidated through the use of animal models, including mice, hamsters, dogs, and non-human primates (Loría-Cervera and Andrade-Narváez, 2014).

Mouse Model

Due to the existence of multiple strains of inbred mice, the simplicity of maintaining large numbers, and the vast number of reagents available for murine systems, experimental leishmaniasis in mice has been the primary animal model utilized for leishmaniasis research. For VL in mice, infection is primarily introduced via intravenous injection of large numbers of parasites. Two primary genetic loci, *Slc11a1* (also known as *Lsh/Bcg/XXX*) and *H2* [Major Histocompatibility Complex (MHC)], are associated with resistance to *Leishmania donovani* in mice. *Slc*11, the gene that encodes the transporter Nramp is responsible for resistance to *Le. donovani*, *Mycobacteria*, and *Salmonella* (Bellamy, 1999). In mouse strains that express the wild-type Nramp (for example, CBA mice), *Le*. *donovani* proliferation in the liver is inhibited. In strains that express a mutant Nramp (for example, BALB/c and C57Bl/6), parasite growth is unrestrained (Bellamy, 1999). The resistance mechanism only manifests in the initial stages of infection and MHC alleles that ultimately dictate adaptive immune responses can override Nramp susceptibility by inducing cure associated with reduced parasitic load in the liver. The non-cure mice progress to a chronic phase without clearing of the parasites.

The immune response to experimental VL and ultimate outcome of infection depends on the organ (liver or spleen) that is being assessed, the inoculation route and dose, and the parasite genotype (Loría-Cervera and Andrade-Narváez, 2014). Importantly, mice do not present the pathological features of human disease so are best used to determine infection susceptibility or resistance, rather than for assessing disease.

Rodents are a natural host for many cutaneous leishmaniasis causing species and so provide a good model for elucidating both immunological and genetic mechanisms of infection and pathology. Experimental infections with *Leishmania major* in particular have been instrumental in dissecting the immunological mechanisms of resistance (primarily C57Bl/6 strain) and susceptibility (primarily BALB/c strain) to infection and disease.

Resolution of cutaneous lesions in C57Bl/6 mice has been associated with a T-helper 1 (Th1) adaptive immune response that involves the production of interferon-gamma (IFN-γ) by CD4+ T-helper cells and stimulation of nitric oxide that is involved in destruction of amastigotes (Sacks and Noben-Trauth, 2002). Susceptibility in Balb/c mice correlates with a T-helper 2 (Th2) response characterized by the production of interleukin-4, interleukin-10, and transforming growth factor-beta (TGF-β) (Sacks and Noben-Trauth, 2002). The susceptible mice develop non-healing lesions and progressive disease, but not the clinical manifestations associated with VL. Genetic mapping assessing healing and non-healing phenotypes of progeny from crosses between resistant and susceptible mice revealed multiple genetic loci that influence both immune responses and wound healing (Sakthianandeswaren et al., 2009).

There are profound differences in the mechanisms that mediate susceptibility and resistance to infection and pathology associated with different *Leishmania* species. For example, C57Bl/6 and C3H mice that heal *Le. major* infections develop chronic disease when infected with either *Le*. *mexicana* or *Le*. *amazonensis* (Loría-Cervera and Andrade-Narváez, 2014). Chronic lesions due to *Le*. *amazonensis* are not dependent on a Th2 phenotype (Afonso and Scott, 1993) and parasite burden and pathology is exacerbated by Th1 cells (Soong et al., 1997). On the other hand, the non-healing phenotype of *Le*. *mexicana* infections in C57Bl/6 mice is associated with a Th2 response (Satoskar et al., 1995). There is no mouse model that recapitulates MCL caused by any *Leishmania* species so this system has had limited utility in understanding the pathology associated with MCL.

Hamster Model

The Syrian golden hamster (*Mesocricetus auratus*) is considered the best experimental model to study visceralizing *Leishmania* species (*Le*. *donovani* and *Le*. *infantum*) because this species is highly susceptible and reproduces the clinical pathology associated with visceral disease in humans (Melby et al., 2001). However, the dearth of immunological reagents (for example, antibodies for cell markers and cytokines) has hindered full understanding of the mechanisms of immunity.

Most studies in hamsters involve injection of large numbers of parasites via intravenous, intracardial or intraperitoneal injection that does not mimic the natural route of infection. Progressive disease involves uncontrolled parasite replication in the liver, spleen, and bone marrow despite the induction of T-helper 1 cytokines. Failure to control VL is associated with an immune suppression response associated with the production of TGF-β that triggers lymphocyte apoptosis (Banerjee et al., 2011), a lack of nitric oxide, the cytotoxin known to be required for killing of parasites (Pérez et al., 2006) and an inability of macrophages to process and present antigens to T-cells (Rodrigues et al., 1992).

Dog Model

Wild canines and domestic dogs serve as the primary reservoirs of zoonotic *Leishmania infantum* infection so the use of dogs as a model has recently gained momentum both to understand human VL and to identify mechanisms to treat canine VL. The primary mechanisms of protective immunity to *Le. infantum* in dogs is the activation of macrophages by a Th1 immune response (Vouldoukis et al., 1996). Canine VL is a multisystemic disease with variable clinical manifestations. Studies indicate a mixed cytokine response in CanL (Loría-Cervera and Andrade-Narváez, 2014). However, studies on experimentally infected dogs have shown that asymptomatic or resistant dogs produce a cell-mediated immune response to parasite antigens and more T-helper 1 associated cytokines than symptomatic dogs (Pinelli et al., 1994). The continued use of the dog as a model to study VL has the possibility of better understanding this complicated disease (see Table 2).

Non-Human Primate Models

Non-human primates are valuable models of human disease because of their similarities to human physiology and immunity. However, they are expensive and difficult to obtain and maintain, thus limited studies employ this model.

The Asian rhesus macaque (*Macaca mulatta*) is quite susceptible to *Leishmania* infection and the progression of CL and immune responses mimic CL in humans (Loría-Cervera and Andrade-Narváez, 2014). New World owl monkeys (*Aotus trivirgatus*) develop VL when infected with *Le. donovani* and exhibit weight loss, anemia, and hepatosplenomegaly similar to humans (Broderson et al., 1986). In contrast, squirrel monkeys (*Saimiri sciureus*) develop VL when infected with *Le. donovani* but recover and are resistant to reinfection (Dennis et al., 1986). Both symptomatic and asymptomatic *Le. donovani* infections are detectable in vervet monkeys (*Chlorocebus pygerythrus*) (see Gicheru et al., 1995) and Indian langurs have been used in vaccination studies (Misra et al., 2001).

Although the development of a non-human primate model that mimics human VL would certainly help elucidate the mechanisms of pathogenesis and immunity in humans, due to financial and ethical reasons, these models are typically only used when another model is not sufficient to answer a particular research question or as a final test for vaccines and drugs developed using other animal models.

Sand Flies

There has been partial success utilizing animal models for vaccine development against leishmaniasis; however, there still has been no licensure of an efficacious vaccine for humans. This general failure has led scientists to posit that the animal models should more closely mimic a natural infection (Reed et al., 2016). Sand flies are not simply syringes that inoculate parasites; rather they are a sort of pharmacy, capable of dispensing a plethora of pharmacologically active compounds into the skin of their hosts. These inoculated molecules have profound effects on the host immune system, exhibiting anti-haemostatic, anti-inflammatory, and immunosuppressive activities that facilitate blood feeding, while enhancing the establishment of the pathogens they transmit (McDowell, 2015). Moreover, *Leishmania*-infected sand flies also deposit a parasite-derived molecule, promastigote secretory gel (PSG), that accelerates wound healing while simultaneously enhancing survival and growth of *Leishmania* parasites (Giraud et al., 2018). To mimic a natural route of infection, many studies now incorporate sand fly saliva in the challenge inoculum and it has been posited that utilization of sand flies to inoculate parasites in the laboratory setting would advance vaccine development (Reed et al., 2016).

Inoculation of *Leishmania* parasites in the presence of sand fly saliva leads to enhancement of disease in animal models (Belkaid et al., 1998; Bezerra and Teixeira, 2001; Norsworthy et al., 2004; Samuelson et al., 1991; Theodos et al., 1991; Titus and Ribeiro, 1988). On the other hand, sand fly saliva has powerful immunogenic molecules that elicit strong hypersensitivity reactions in individuals that are repeatedly bitten. Repeated exposure to sand fly bites causes a delayed-type hypersensitivity (DTH) response recognized by local human inhabitants as a painful skin disease called harara (Adler and Theodor, 1957). Elicitation of this response has been suggested to be an evolutionary advantage for sand flies, by increasing blood-flow at the bite site and, therefore, decreasing the amount of time it takes for a sand fly to take a full blood meal (Belkaid et al., 2000). Although advantageous for sand flies, the DTH elicited by repeated exposure to sand fly bites (Kamhawi et al., 2000) or salivary gland homogenate (Belkaid et al., 1998) inhibits *Leishmania* infection in animal models. Thus, the inflammatory processes induced by the bite influence immunity to *Leishmania* parasites that are co-delivered with salivary components, effectively limit the ability of the parasite to cause devastating disease.

The prevalence of *Leishmania* infected sand flies in the field is quite low. For example, assessment of the sand fly vectors in specific areas in Iraq revealed that the highest infection rate was 2.3% (Aronson et al., 2003). Individuals in these areas can receive from 100 to 1,000 bites in a single night; therefore, the amount of sand fly saliva that is injected far outweighs any *Leishmania* antigen that may be inoculated, suggesting that sand fly saliva could be utilized as a part of a multi-component anti-leishmanial vaccine (Reed et al., 2016).

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MYXOZOA

13

Myxozoa

Myxozoa (Subphylum)

Terrence L. Miller

Phylum Cnidaria

Subphylum Myxozoa

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Chapter 13

Myxozoa (Subphylum)

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Introduction

Images that often come to mind upon hearing or seeing the word Cnidaria are swarms of jellyfish following the sun around lakes in Palau or the colorful tropical coral reef ecosystems with their vast diversity of hard and soft corals, and anemones housing charismatic clownfish. Rarely do images of parasites come to mind. However, recent phylogenetic and protein expression analyses have revealed the diverse group of obligate endoparasites of the subphylum Myxozoa (Grassé, 1970) (once considered a phylum in its own right) are in fact morphologically simplified, although highly specialized, cnidarians (Atkinson et al., 2018; Collins, 2009; Shpirer et al., 2018; Zrzavy and Hypsa, 2003). The primary uniting morphological feature of this group is the presence of a nematocyst-like structure termed the polar capsule in the myxozoan descriptive literature, which contains the polar filament that fires off in the presence of a suitable host, similar to the firing of nematocysts in free-living cnidarians used to capture prey (Figure 1).

The Cnidaria contained only 10 parasitic species previously, all of which had free-living stages at some point in their life cycle. The impacts of this seemingly innocuous change in classification now result in the once relatively parasite-free Cnidaria, now consisting of approximately 20% obligate parasites of a wide range of vertebrate and invertebrate hosts (Atkinson et al., 2018). This newly recognized and unique adaptive diversification of endoparasitic Cnidaria reveals that they are incredibly diverse in their specializations and ecologies, and greatly affect aquatic animal health in wild and cultured animal production systems.

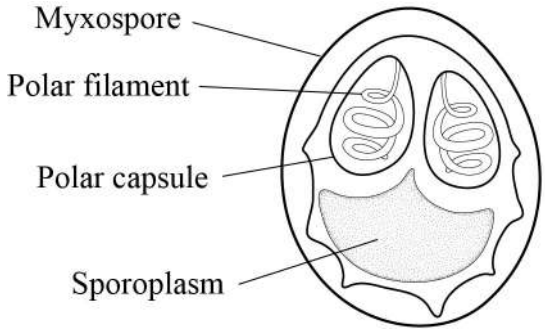

Figure 1. An uncharacterized species of *Myxobolus* (subphylum Myxozoa: class Myxosporea) found in *Acanthopagrus australis* from Moreton Bay, Australia illustrating the coiled nematocyst-like polar filament structure and general morphological characters. Source: T. Miller. License: CC BY-NC-SA 4.0.

Classification and Host Associations

The subphylum Myxozoa currently contains over 2,400 species in 2 disparately populated classes, the Malacosporea Canning et al., 2000, which currently consists of 2 genera (*Buddenbrockia* Schröder, 1910 and *Tetracapsuloides* Canning et al., 2002), and the Myxosporea Bütschli, 1881, which has over 60 genera (Atkinson et al., 2018). Generally, species within both classes require 2 hosts (an invertebrate as the definitive host and a vertebrate as the intermediate host) to complete their life cycle. The primary biological characteristics that distinguish the 2 groups are that malacosporeans use freshwater bryozoans and myxosporeans use marine or freshwater annelids as their invertebrate hosts (Patra et al., 2017). Species of both classes predominantly infect fish as their vertebrate host (> 95% of all reported species), however, a number of myxosporean taxa have now been reported from mammals, waterfowl, amphibians, and reptiles (Bartholomew et al., 2008; Hallett et al., 2015).

The fluid nature of taxonomic classification, especially above the level of the family, is demonstrated with respect to the Myxozoa due to the many hypotheses regarding the origin of these species and the nature of the evidence used to classify the group over the last couple of centuries. Their microscopic size and simple spore morphology led to the initial classification of myxozoans that were discovered in the early to mid-1800s as belonging to the Sporozoa, a group of unicellular spore-forming organisms in early literature (Okamura and Gruhl, 2015). Multicellularity of these organisms was identified in the late 19th century, but it wasn't until 1970 that the Myxozoa was formally considered a phylum in the Metazoa (Grassé, 1970; Štolc, 1899). Subsequent DNA sequence analyses of the nuclear small subunit ribosomal DNA

region (SSU rDNA) of myxozoans revealed their close phylogenetic relationships with the Cnidaria (Kent et al., 2001; Siddall et al., 1995).

Prior to 1994, classifications of the Myxozoa (that is, when it was then considered a phylum) contained 2 subclasses, the Myxosporea and Actinosporea, which were characterized based on distinctly different spore morphology and host associations (fish and annelids, respectively). It wasn't until experimental work investigating the transmission of *Myxobolus cerebralis* (the causative agent of whirling disease in salmonids) revealed that the markedly dissimilar spore morphologies observed in the annelid and fish hosts were actually just different developmental spore stages of the same organism (Markiw and Wolf, 1983; Wolf et al., 1986). Actinosporea was subsequently suppressed as a taxon (Kent et al., 2001; Kent and Margolis, 1994). Currently, DNA markers are primarily used (experimental infection trials less commonly) to determine conspecificity of actinospore and myxospore stages recovered from infected intermediate or definitive hosts (Hallett et al., 2002).

Life Cycle Stages and Infection Dynamics

Reproduction within the Myxozoa is as diverse as that observed within the free-living Cnidaria. Asexual and sexual reproduction in the Myxozoa are complex and detailed aspects of haploid and diploid cell formation within the group remains unknown. Evidence to date suggests meiosis generally occurs in the annelid or bryozoan host, which indicates these are the definitive hosts for these taxa (Okamura et al., 2015b). Transmission of myxosporean infections occurs through the production and release of actinospores from the annelid host and myxospores from the vertebrate host (Eszterbauer et al., 2015). Malacosporean transmission is similar in that infectious malacospores are released by the bryozoan host and the spores released from the fish host are characterized as fish malacospores (Hartikainen and Okamura, 2015; Patra et al., 2017).

Research into the causative agent of proliferative kidney disease (PKD), a significant disease impacting wild and cultured salmonids infected with *Tetracapsuloides bryosalmonae*, resulted in the original implication of bryozoans as hosts involved in the life cycle of this malacosporean pathogen (Hartikainen and Okamura, 2015). This and subsequent research into species of *Buddenbrockia*, which infects cypriniform and perciform fish, has contributed to the bulk of knowledge on the transmission and developmental stages of malacosporeans in their bryozoan and fish hosts (Hartikainen and Okamura, 2015; Patra et al., 2017). Malacospores in fish develop and mature in the kidney tubules and are released into the environment through urine excretion and infect the bryozoan host through ingestion of infectious spores or direct contact (Patra et al., 2017). Development within the bryozoan host results in the formation of multiple sac- or vermiform-like (termed myxoworm) stages containing many spores. These stages then burst inside of the bryozoan and spores are released into the water column via the vestibular pore (Hartikainen and Okamura, 2015; McGurk et al., 2006; Patra et al., 2017). Fish become infected when malacospores contact the gills or epidermis, the nematocyst-like polar filament everts to facilitate attachment, and the amoeboid sporoplasm invades the adjacent tissue (Hartikainen and Okamura, 2015).

Waterborne stages of individual myxosporean species released from the invertebrate and vertebrate hosts are often so morphologically dissimilar in appearance that, as discussed above, their original taxonomic classifications were unclear. The annelid hosts of myxosporeans vary depending on their occurrence in marine or freshwater environments. In freshwater ecosystems worldwide, oligochaetes are much more species rich than polychaetes (≈ 1,000 versus ≈ 170 species, respectively); whereas in marine ecosystems, oligochaetes are vastly outnumbered in species diversity by polychaetes (≈ 200 versus ≈12,000 species, respectively). Consequently, and possibly as a byproduct of coevolutionary radiation, the known annelid hosts of myxosporeans are primarily oligochaetes in freshwater and polychaetes in marine ecosystems (Alexander et al., 2015), although it should be noted that the life cycles are known for only very few species overall.

Infection of the annelid host is initiated via contact with infectious myxospores released from the vertebrate host (predominately fish). The site of infection in the fish host plays an important role in how myxospores are released into the environment. For example, species that form cyst-like pseudoplasmodia containing many infectious myxospores in various fish tissues (for example, in skeletal muscle, organs, or viscera), generally rely on the death of the host and post-mortem changes (decomposition or digestion) to facilitate spore release. For taxa present in marine ecosystems, direct predation or scavenging of fish harboring tissue-dwelling myxosporeans may be an important mode of viable spore release and spread in the environment through fecal material which settles in the benthos for exposure to their suitable annelid host. Species which develop mature spores in the biliary or urinary tracts of their fish hosts (for example, *Ceratomyxa* spp. or *Myxidium* spp.) are released directly into the environment through excretion of feces and urine.

Infection of the annelid host is considered to be primarily via ingestion of myxospores, although infection by direct contact with spores and amoeboid sporoplasms invading through the epidermis is likely (Alexander et al., 2015).

Once within the annelid host, the parasite then migrates to the preferred site of infection (for example, intestinal epithelium or body wall) and proceeds to undergo proliferative phases including schizogony, sporogony, and gamogony to produce numerous actinospores (Alexander et al., 2015; Eszterbauer et al., 2015; Hallett et al., 1999). Actinospores are then released into the water through the anus or epithelium of the annelid host by mechanisms which are still unclear (Alexander et al., 2015). Actinospore stages often have large caudal processes that presumably facilitate buoyancy and dispersal within the water column in order to come into contact with their suitable fish host. Infection of the fish host is through, 1) the anchoring of the actinospore to the gills or epithelium via the discharged polar filaments, and 2) the spore valves releasing the infectious sporoplasm, which 3) invades the adjacent epidermis and 4) subsequently migrates to the preferred site of infection (Kallert et al., 2015).

Diagnostics and Characterization

Diagnostic identification and taxonomic characterization of myxozoans via morphology only are complicated by the relatively few distinct morphological characters that can be used to discriminate between taxa. Most formal species descriptions of malacosporeans and myxosporeans are of the stages present in their vertebrate hosts, with taxa causing disease receiving particular attention. Descriptions of taxa generally incorporate characters such as **spore** size, shape, **pseudoplasmodia** dimensions and numbers of **spore valves**, **polar capsules** (including location and orientation), and coils of the **polar filaments** contained within being particularly important (Lom and Dyková, 2006; Patra et al., 2017). Other biological or ecological characteristics that are often important information accompanying species descriptions and aiding discrimination between taxa are the host species infected, tissue tropism, or site of infection. One of the most useful reviews and pictorial overviews for identification of myxozoan genera for someone new to the field is that of Lom and Dyková (2006; see Figure 2).

Currently, the inclusion of DNA sequence data as accompanying characters used to discriminate taxa are becoming critical for revealing taxonomic, ecological, and evolutionary relationships that were previously unresolved. The most common genetic markers used for comparative phylogenetic analyses and species-level distinction are the nuclear large and small subunit ribosomal DNA regions (LSU rDNA and SSU rDNA), which account for the majority of genetic data available on the publicly accessible databases GenBank and EMBL (Atkinson et al., 2015; Fiala, 2006). These ribosomal sequences contain a combination of highly conserved and variable regions, which correspond to the stem and loop motifs of the folded ribosome involved in the protein production machinery of eukaryotic cells. This combination allows for the robust alignment of conserved regions for phylogenetic or primer design purposes, and enough variability to reliably distinguish different sequence variants (Atkinson et al., 2015).

The consensus to date is that new species descriptions or revisions of taxonomic affinities within the Myxozoa should attempt to incorporate a combination of morphological characters (that is, examined via traditional microscopic and ultrastructural techniques), tissue tropism, host associations (including life cycle and host specificity data if possible), and DNA sequence data (Atkinson et al., 2015). The usefulness of these robust taxonomic treatments has been demonstrated through recent advances in knowledge of the biodiversity and ecology of myxozoans in aquatic ecosystems. Extraordinary species richness of myxozoans in aquatic environments is being revealed through recent biodiversity surveys, with some estimates suggesting the species diversity of myxozoans exceeds that of the number of fishes present in these ecosystems (Gunter and Adlard, 2008; 2009; Heiniger et al., 2011).

Aquatic Animal Health Implications

Much understanding of the biology, life cycles, and transmission dynamics of myxozoans has been prompted by investigations into the severe pathology and disease elicited by some myxozoan taxa, which cause significant negative economic and population-level impacts in aquatic wildlife and aquaculture. A few of the major diseases or production issues due to myxozoan infections are mentioned here to illustrate these impacts.

Proliferative Kidney Disease Caused by the Malacosporean *Tetracapsuloides bryosalmonae*

Proliferative kidney disease (PKD) is a condition which results in significantly high mortality rates in salmonid fish in Europe and North America caused by infections with the malacosporean *Tetracapsuloides bryosalmonae* (Canning et al., 1999). This disease is characterized by marked immunosuppression of the host, the proliferation of the parasite in kidney interstitia resulting in chronic hyperplasia and granulomatous reactions that cause distinct splenomegaly, renomegaly, and pathology in affected renal tissues (Sitjà-Bobadilla et al., 2015). Freshwater bryozoans of the class Phylactolaemata have been identified as hosts of this species. Infections in both the bryozoan and fish hosts appear to be temperature dependent, with higher prevalence and intensities observed in warmer months (Jones et al., 2015). The combination of habitat loss and degradation, warming climate, and impacts of PKD have been implicated in the decline of vulnerable trout populations throughout their native ranges.

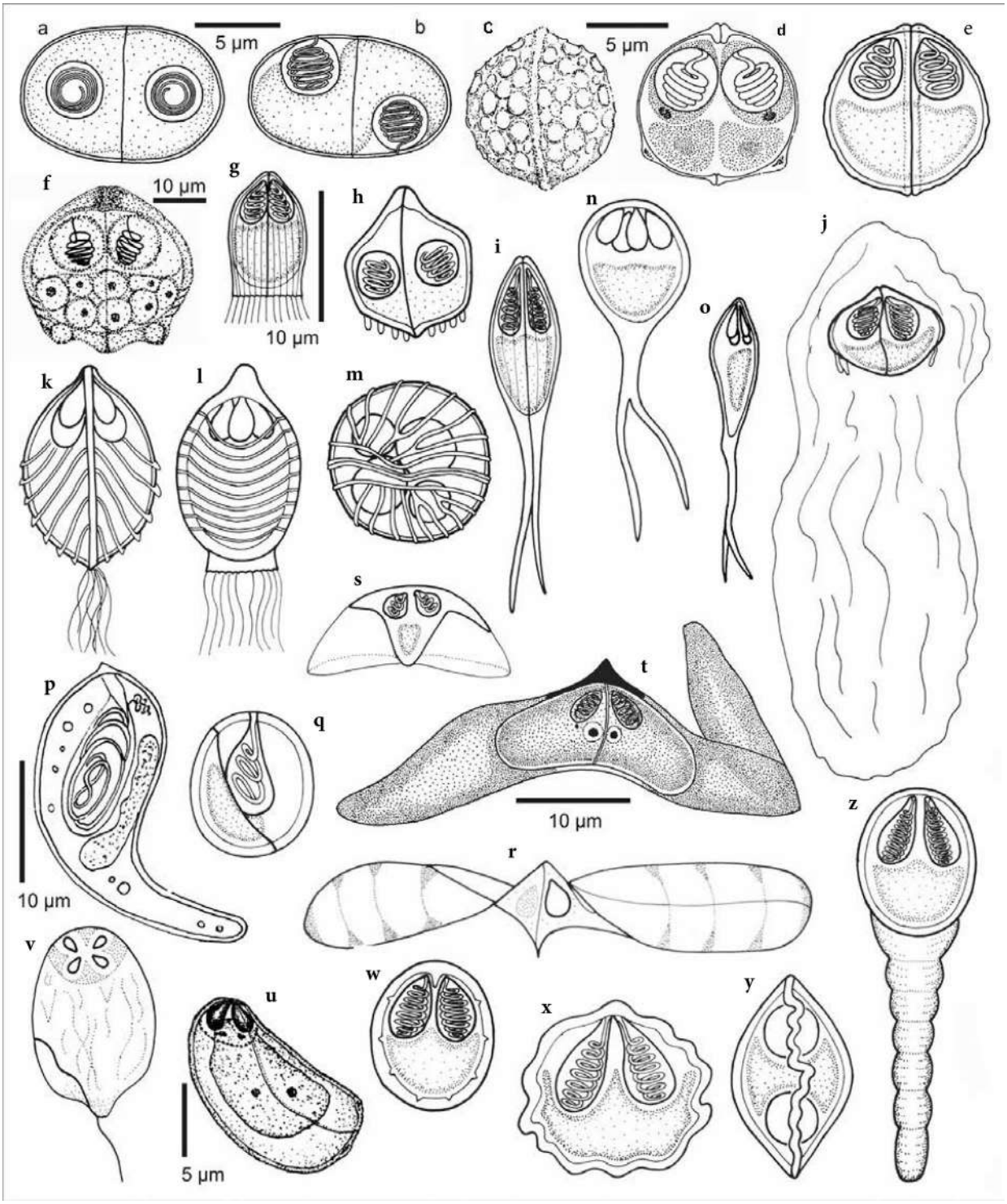

Figure 2. Line drawings of myxosporean spores. *Ellipsomyxa gobii* in (a) apical and (b) sutural view (adapted from Køie, 2003). *Sphaerospora elegans*, (c) pitted spore surface, (d) sutural view (Feist et al., 1991). e) *Sphaerospora renicola* in sutural view. f) *Polysporoplasma sparis* in sutural view (adapted from Sitjà-Bobadilla and Alvarez-Pellitero, 1995). g) *Hoferellus cyprini* in sutural view. h) *Wardia ovinocua*. i) *Myxobilatus gasterostei* in sutural view. j) *Palliatus mirabilis* in sutural view. *Chloromyxum leydigi* in (k) sutural and (l) frontal view. m) *Chloromyxum cristatum* in apical view. n) *Caudomyxum nanum*. o) *Agarella gracilis*. p) *Auerbachia anomala* (adapted from Meglitsch, 1968). q) *Globospora sphaerica*. r) *Alatospora samaroidea*. s) *Pseudoalatospora scombri*. t) *Renispora simae* (adapted from Kalavati, 1996). u) *Parvicapsula asymmetrica* (adapted from Shulman and Shulman-Albova 1953). v) *Neoparvicapsula ovalis*. w) *Myxobolus muelleri*. *Spirosuturia carassii* in (x) frontal and (y) apical view. z) *Unicauda clavicauda*. Sources: Lom and Dyková (2006) and adapted from sources noted in-line. License for all: CC BY.

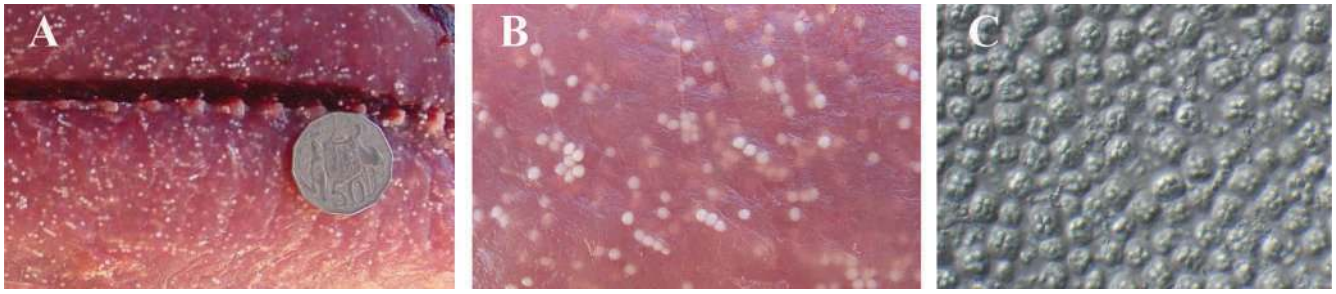

Figure 3. *Kudoa* sp. pseudoplasmodia in the flesh of a tuna collected off Ningaloo Reef, Western Australia. A) Macroscopic view with an Australian 50-cent piece for size reference B) Close up of macroscopic pseudoplasmodia in flesh, and C) Photomicrograph of spores released from pseudoplasmodia showing the 4 valves and 4 polar capsules characteristic of this this group. Source: T. Miller. License: CC BY-NC-SA 4.0.

Whirling Disease

Infections with the myxosporean *Myxobolus cerebralis* can cause the condition called whirling disease in salmoniform fish. As its name suggests, clinical signs of disease are characterized by the distinct erratic whirling patterns swum by affected individuals and a distinct darkening of the caudal region (Jones et al., 2015). *Myxobolus cerebralis* is one of the few known chondrophilic, or cartilage preferring, species. Development of the pseudoplasmodia primarily occurs in the cartilage of the head and vertebrae, and compression of the brain and medulla spinalis results in the abnormal swimming behavior observed in infected fish (Molnár and Eszterbauer, 2015). Discovery of the annelid host, *Tubifex tubifex*, as the obligate invertebrate host required to complete the life cycle, led to a revolution in understanding of myxosporean development and transmission (Jones et al., 2015).

Whirling disease has resulted in substantial decreases in susceptible salmonid populations throughout its known range, which is now considered distributed almost circumglobally wherever *Tubifex tubifex* is found. A notable exception is Australia, which is currently considered free of *Myxobolus cerebralis*. There it remains on the list of nationally notifiable diseases and strict quarantine measures are in force to reduce the possibility of incursion of this parasite, which could have devastating impacts to naïve salmonid populations on the relatively isolated island continent.

Myxosporean Infections and Seafood Marketability

In contrast to the myxozoan infections briefly mentioned in the above sections, some myxosporean species do not cause significant health issues or severe pathology in their host fish, but negatively impact the production and trade of seafood post-harvest. This is primarily due to the presence of unsightly macroscopic cyst-like pseudoplasmodia in flesh or myoliquefaction of infected musculature, external surfaces, or viscera. For example, a number of species of *Kudoa* are known to produce distinctly white, macroscopic pseudoplasmodia in muscle tissue of tuna species that stand out dramatically against the pink/crimson flesh of fresh fillets and render them effectively unmarketable for human consumption (Figure 3) (Moran et al., 1999). Another major marketability issue encountered in fish harvested from the wild or produced in aquaculture is post-mortem myoliquefaction, which results in mushy or butter-like consistency of fish muscle when cooked (Kristmundsson and Freeman, 2014; Langdon, 1991; Langdon et al., 1992; Moran et al., 1999). This is due to the presence of myxosporean pseudoplasmodia (often *Kudoa* or *Unicapsula* spp.) residing in myofibrils of infected fish releasing a suite of protease or proteolytic enzymes once the host has died, presumably an evolutionary adaptation to facilitate rapid release from the host into the environment (Alama-Bermejo et al., 2009; Lester, 1982; Stephens and Savage, 2010). Enzymatic breakdown of muscle tissue via this protease activity is accelerated in the presence of heat, not a particularly desirable combination when the product being marketed is destined for cooking prior to human consumption. High intensity infections can result in fish fillets subjected to heat via cooking displaying the consistency of jelly or peanut butter, which can elicit complaints from patrons visiting a restaurant or guests around the family barbecue.

Myxozoan infections and associated disease or marketability issues have had major negative impacts on wild fisheries and the aquaculture industries worldwide (Jones et al., 2015; Kent et al., 2001; Okamura et al., 2015a). Despite the significant progress made over the last few decades in the understanding of myxozoan biology, much remains unknown. From a biodiversity perspective, while around 2,400 species have been described, it appears that the surface has barely been scratched to discover the total myxozoan species richness of the world's freshwater and marine

ecosystems. In addition, less than 1% of all myxozoan species have had their complete life cycles elucidated and all susceptible hosts for particular species resolved. Further investigations into the diversity, host specificity, ecology, and transmission dynamics are clearly required to help mitigate the impacts of known and emerging diseases associated with myxozoan infections.

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MESOZOA

14

Mesozoa

Mesozoa (Phylum Dicyemida and Phylum Orthonectida)

Sarah R. Catalano

Phylum Dicyemida

Phylum Orthonectida

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Chapter 14

Mesozoa (Phylum Dicyemida and Phylum Orthonectida)

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Introduction

Unseen by the naked eye, the renal appendages (synonymous with kidneys, renal sacs, and renal organs) of benthic cephalopods, including squid, octopus, and cuttlefish (Figure 1), contain thousands of tiny worm-like organisms known as dicyemid mesozoans (Furuya and Tsuneki, 2003; Furuya et al., 2004; Finn et al., 2005). They can colonize 1 or both renal appendages at high numerical densities (Figure 2) and although simple in body structure, their life cycle is complex and not fully characterized. They are not known to infect any other marine organism, although the occurrence of a dispersal, free-swimming embryo form has led to questions as to whether an intermediate host or dormant stage exists.

Over 100 species have been formally described based on morphological characteristics, with documentation from the western and northeastern Pacific Ocean, northern Indian Ocean, Mediterranean Sea, northwestern and eastern Atlantic Ocean, Gulf of Mexico, Antarctic Ocean, and Southern Ocean. Only recently have molecular genetic analyses been applied to this group in an attempt to validate new species descriptions based on classical taxonomic methods and shed light on the unknown position in the Tree of Life for the enigmatic group of organisms.

Taxonomic Classification

Both the dicyemids and orthonectids (a group which parasitize a number of marine invertebrate phyla) have long been considered a class within the phylum Mesozoa (see Stunkard, 1972; Hochberg, 1983; McConnaughey, 1983a; 1983b).

Figure 1. The only known hosts of dicyemid mesozoans including A) squid (*Sepioteuthis australis*, southern calamari), B) octopus (*Octopus kaurna*, southern sand octopus), and C) cuttlefish (*Sepia apama*, giant Australian cuttlefish). Source: S. Catalano. License: CC BY-NC-SA 4.0.

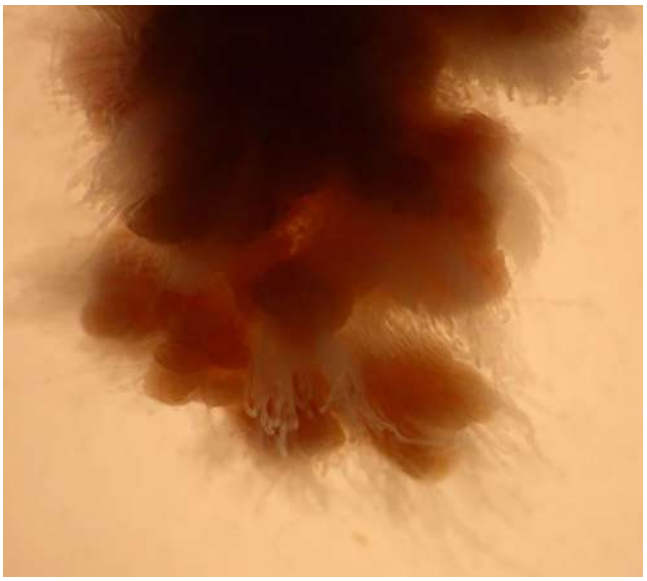

Figure 2. Hundreds of dicyemid mesozoans attached to the renal appendage (in red) of the giant Australian cuttlefish (*Sepia apama*). Each white strand represents 1 individual adult dicyemid. Source: S. Catalano. License: CC BY-NC-SA 4.0.

However, due to distinct differences between these 2 groups in terms of morphology and life cycle stages, it is now accepted to treat each group as separate phyla, phylum **Dicyemida** and phylum **Orthonectida**. The phylum **Dicyemida** contains 3 families, Dicyemidae Van Beneden 1882, Conocyemidae Stunkard 1937, and Kantharellidae Czaker 1994, although the validity of the Kantharellidae is questionable and uncertain due to the single species from this family being inadequately described (Furuya et al., 2007). Nine genera are recognized within the 3 families:

Family Dicyemidae
Dicyema von Kölliker, 1849
Dicyemennea Whitman, 1883
Dicyemodeca (Wheeler, 1897) Bogolopova, 1957
Pseudicyema Nouvel, 1933
Pleodicyema Nouvel, 1961
Dodecadicyema Kalavati & Narasimhamurti, 1980

Family Conocyemidae
Conocyema Van Beneden, 1882
Microcyema Van Beneden, 1882

Family Kantharellidae
Kantharella Czaker, 1994

The largest number of described species are in *Dicyema*, followed by *Dicyemennea*, with the other genera being monotypic or containing a small number of species (Catalano, 2012). Catalano (2012) provides a comprehensive list of the 112 species described up until 2012, however, an additional 12 species were described up until 2019 (Catalano, 2013a; 2013b; Catalano and Furuya, 2013; Castellanos-Martínez et al., 2016).

Typically for generic and species classification, the number and orientation of cells in each tier of the calotte, the presence or absence of abortive axial cells, the presence or absence of syncytial stages, the size of the adult stages, the number of cells comprising the body, the shape of the calotte, the anterior extension of the axial cell, the presence or absence of verruciform cells, and the structure of the infusoriform larvae are distinguishing morphological characters (Hochberg, 1982; 1983). However, recent molecular analyses have shed a level of doubt on some of these morphological characters, particularly calotte cell counts for genera classification, as the placement of a *Dicyema* species, with 4 metapolar cells in its calotte, grouped within the *Dicyemennea* clade, which is known to have 5 metapolar cells in their calottes (Catalano et al., 2015). Further molecular analyses, which include multiple species from all the known genera alongside additional molecular markers, will be needed to resolve and either validate or dismiss the current level of classification based on morphological traits. New species descriptions should now not only include measurements from all life cycle stages (nematogen, rhombogen, vermiform embryo, and infusoriform embryo) along with line drawings and light micrograph images of distinguishing characters (classical morphological measures, for example, as in Furuya, 2009), but also molecular analyses with the *COI* (*c* oxidase subunit I) gene sequenced as a minimum for inclusion in the preliminary phylogenetic tree for dicyemid mesozoans as presented by Catalano et al. (2015). Recently, Drábková et al. (2022) used several phylogenomic methods to generate a phylogeny that shows a common ancestor of the Dicyemids and the Orthonectida with ancestral Platyhelminthes as the basal group from which the Mesozoa arose.

Morphology

The body plan of a dicyemid mesozoan is very simple, comprising 8 to 40 cells with no body cavities, differentiated organs, tissues, or glands (Suzuki et al., 2010), although the dispersal embryo form, known as the **infusoriform embryo**, is morphologically distinct from the remaining 3 forms. The **vermiform adult**, **vermiform embryo**, and **rhombogen adult** (collectively known as the **vermiform stages**, Figure 3), all contain a central, long **axial cell**, which is where developing embryos are derived (Awata et al., 2006). This cylindrical axial cell is then protected by the presence of ciliated **peripheral cells** that surround the axial cell in a single layer, although at the anterior region, the peripheral cells are modified to form a **calotte**. The calotte serves as the dicyemid's anchor—it is inserted into the convoluted surface of the host renal appendage allowing the parasite to maintain a foothold while the remainder of its body hangs free in the surrounding environment obtaining nutrients, as seen in Figure 2 (Furuya et al., 2003a; 2007). Traditionally the number and arrangement of cells in the top 2 tiers of the calotte (known as the **metapolar** and **propolar cells**) has been used to assign new species into 1 of the 9 described genera (Figure 4). In particular, it has been reported that the dicyemids have 4 propolar cells, but different numbers of metapolar cells: *Dicyema* (4 metapolar cells, propolar cells opposite to metapolar cells), *Pseudicyema* (4 metapolar cells, propolar cells alternate with metapolar cells), *Dicyemennea* (5 metapolar cells), *Dicyemodeca* and *Pleodicyema* (6 metapolar cells), *Dodecadicyema* (6 metapolar cells plus 3 micropolar cells: Small cells which form the anterior tip of the calotte, only found in *Dodecadicyema* species) (Figure 4). The species of the family Conocyemidae are characterized by having no metapolar cells, but either parapolar cells or

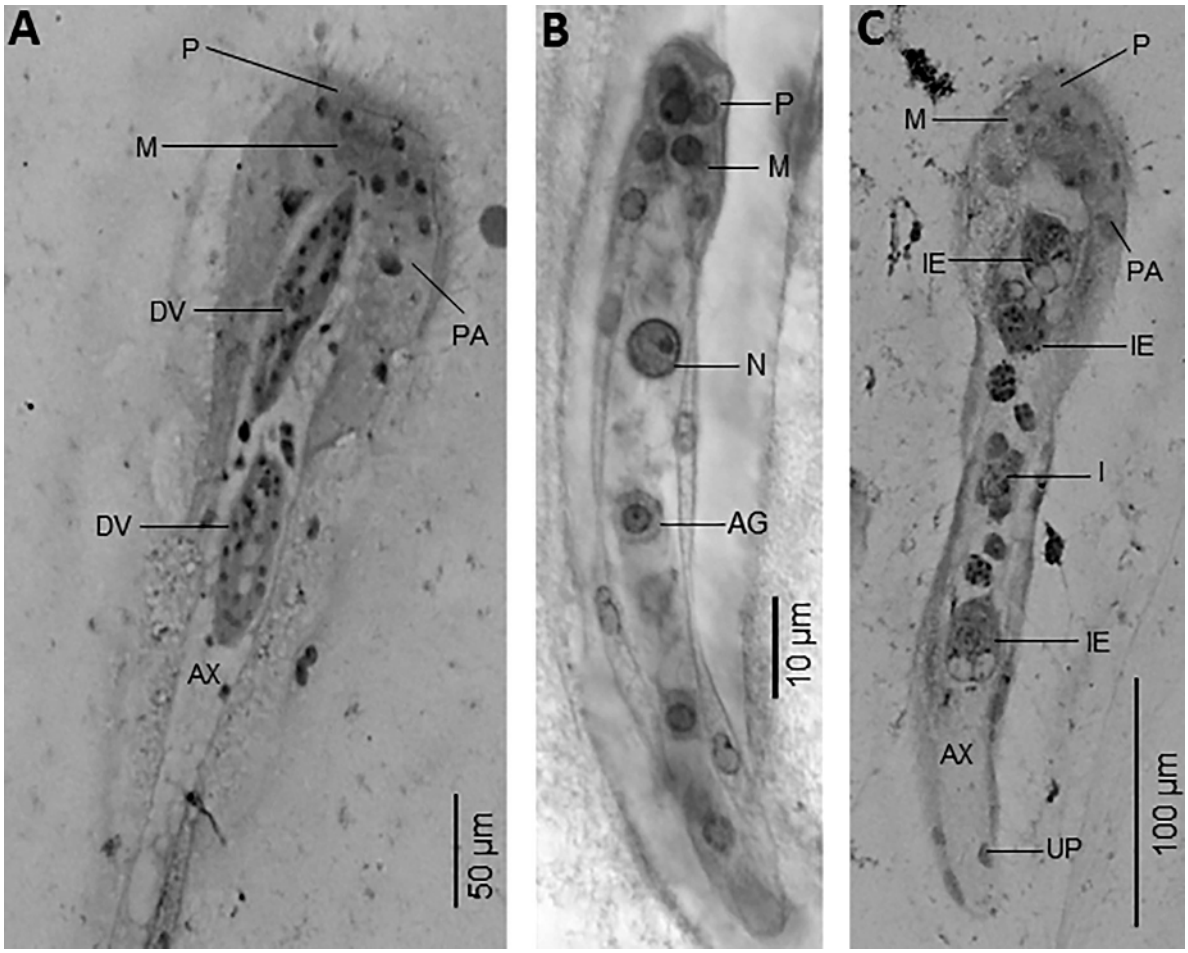

Figure 3. The vermiform stages: A) Adult nematogen (*Dicyema pyjamaceum*) with 2 developing vermiform embryos within the axial cell, B) close up of the vermiform embryo (*Dicyemennea floscephalum*) within the axial cell of a nematogen, C) adult rhombogen (*Dicyema pyjamaceum*) with 3 infusoriform embryos within the axial cell. Abbreviations: AG, agamete; AX, axial cell; DV, developing vermiform embryo; I, infusorigen; IE, infusoriform embryo; M, metapolar cell; N, nucleus; P, propolar cell; PA, parapolar cell; UP, uropolar cell. Adapted from Catalano and Furuya, 2013; Catalano, 2013a. Source: S. Catalano. License: CC BY-NC-SA 4.0.

a **syncytial cell**: *Conocyema* (4 parapolar cells) and *Microcyema* (syncytial cell: A single cell with 6 nuclei which is only found in *Microcyema* species) (Figure 4). The species of the Kantharellidae are different from the other families because there is no cell constancy, with 3 to 9 propolar cells, 2 to 7 metapolar cells, and 2 to 4 parapolar cells (Czaker, 1994) (Figure 4). Nonetheless, whether calotte cell counts represent an accurate way of distinguishing dicyemid genera remains in question, with molecular analyses shedding some doubt on this classical level of classification (see Catalano et al., 2015).

Unlike the vermiform stages, the infusoriform embryo is not long and cylindrical, but rather small and circular (Figure 5). The infusoriform embryo is characterized by 4 large internal **urn cells**, each containing a **germinal cell** which is thought to give rise to the next generation, as well as 2 large **apical cells** at the anterior region and beating **cilia** surrounding the body (Furuya and Tsuneki, 2003). One of the most interesting characters of the infusoriform embryo are the **refringent bodies**, which are contained within the large apical cells. These refringent bodies are composed of a chemical with a high specific gravity that accounts for more than one-third of the body weight of the infusoriform embryo, namely, **magnesium salt of inositol hexaphosphate** (Lapan and Morowitz, 1972; Lapan, 1975). This dense chemical provides the infusoriform embryo with negative buoyancy, which is suggested to allow the embryo to remain close to the sea floor to encounter and infect a new host.

Life Cycle

In contrast to their simple morphology, the life cycle of dicyemid mesozoans involves 2 stages of development

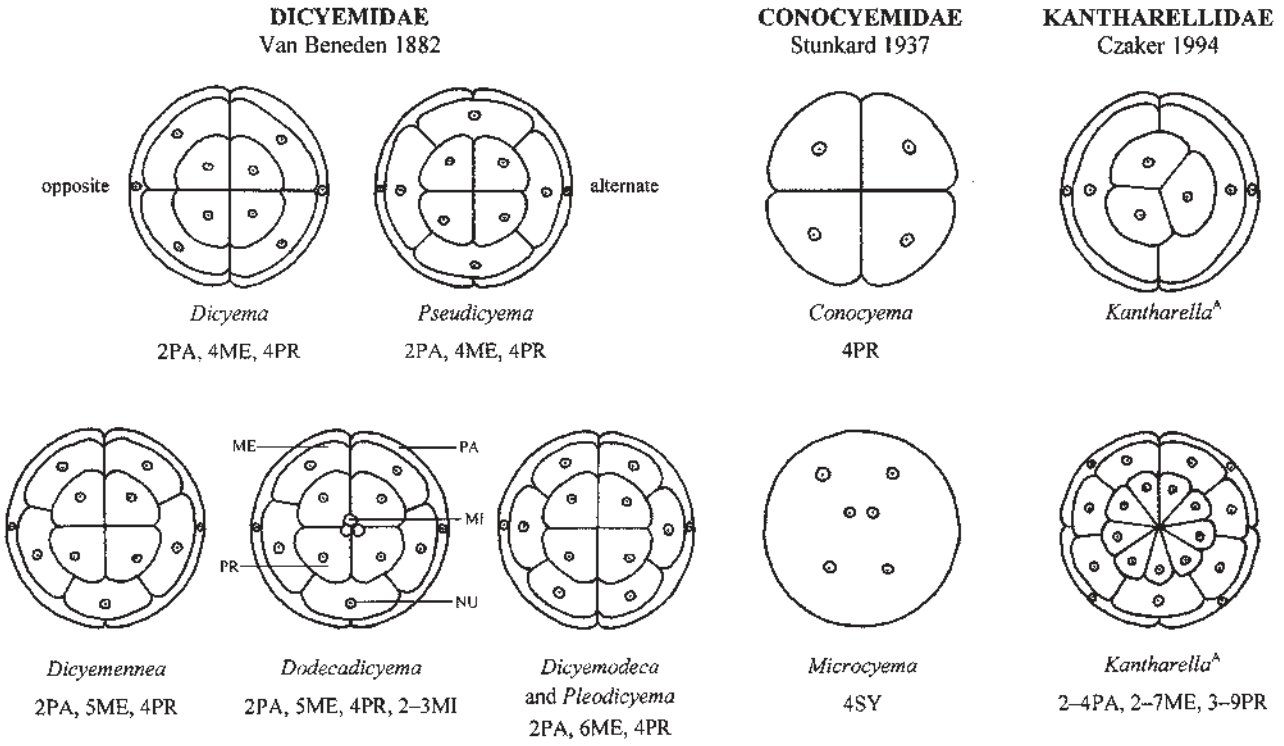

Figure 4. Schematic drawing of anterior end views of calottes showing the arrangement and number of cells characteristic of different genera. Abbreviations: ME, metapolar cell; MI, micropolar cell; PA, parapolar cell; PR, propolar cell; SY, syncytial cell. Adapted from Catalano, 2012. License: CC BY-NC-SA 4.0.

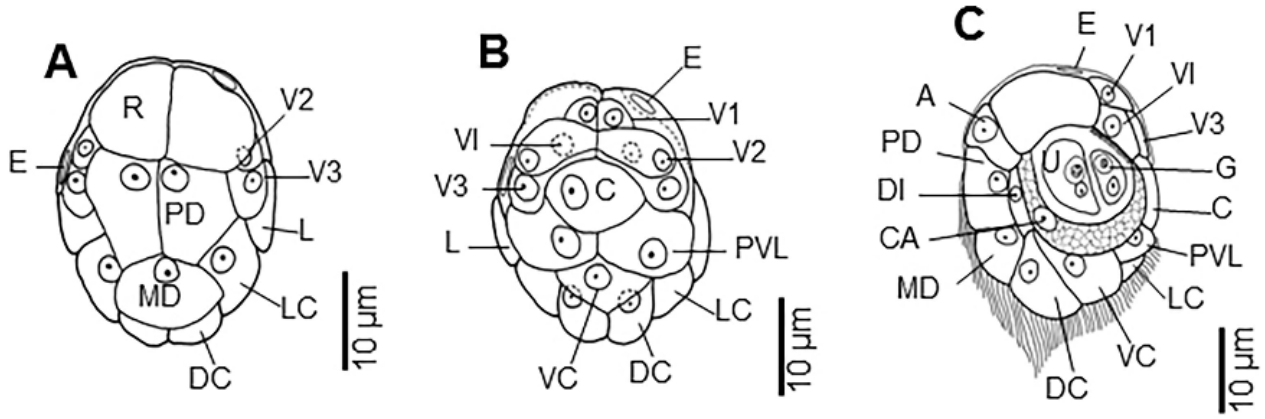

Figure 5. Line drawings of the infusoriform embryo (*Dicyemennea floscephalum*) showing A) dorsal view, cilia omitted, B) ventral view, cilia omitted, and C) sagittal section. Abbreviations: A, apical cell; C, couvercle cell; CA, capsule cell; DC, dorsal caudal cell; DI, dorsal internal cell; E, enveloping cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; MD, median dorsal cell; PD, paired dorsal cell; PVL, posteroventral lateral cells; R, refringent body; U, urn cell; V1, first ventral cell; V2, second ventral cell; V3, third ventral cell; VC, ventral caudal cell; VI, ventral internal cell. Source: Adapted from Catalano, 2013a. License: CC BY-NC-SA 4.0.

(vermiform and infusoriform) and 2 modes of reproduction (asexual and sexual) (Figure 6) (Furuya et al., 2003b; 2007). The vermiform stages, which are restricted to the renal appendages of the host, comprise the adult nematogen, vermiform embryo, and adult rhombogen. The infusoriform stage, which represents the dispersal stage that escapes from the host via the urine to find and infect a new host, comprises the infusoriform embryo.

While the vermiform stages are similar in terms of morphology, comprising 8–40 cells and a worm-like body shape, the infusoriform embryo is distinct, comprising 37–39 cells and being much smaller in size (typically 32–36 µm in length and 26–28 µm in width) with a rounded body shape (Furuya and Tsuneki, 2003). The vermiform stages are formed asexually from an agamete (axoblasts) whereas the infusoriform embryo develops from a fertilized egg produced around a hermaphroditic gonad called the infusorigen (Figure 7).

Within the axial cell of an adult nematogen, the vermiform embryos grow and develop asexually, with more than 1 embryo common within the single axial cell of the adult. Although the exact mechanism is unknown, the vermiform embryo is then released from the nematogen into the fluid around the renal appendages, finds a free surface for attachment and inserts its anterior calotte into the convoluted surface of the host's renal appendage while the rest of its body hangs free in the surrounding urine acquiring nutrients. As it develops further, it transitions into the adult nematogen and will produce its own vermiform embryo and the cycle continues. A high population density in the kidney, as seen in Figure 2—where an accumulation of a chemical factor in the environment is detected (Lapan and Morowitz, 1972; 1975)—is then thought to trigger a shift from asexual reproduction and increasing numbers within the renal appendage to a sexual mode of reproduction and escaping out of the crowded and highly infected host to find and infect a new, potentially naïve, host. This shift is seen in the form of production of the rhombogen adult in place of the nematogen adult, which contains in its single axial cell, 1 or more infusoriform embryos. These dispersal embryos then escape from the host out into the surrounding environment, ensuring that the species will survive beyond the eventual death of the host (Lapan and Morowitz, 1972).

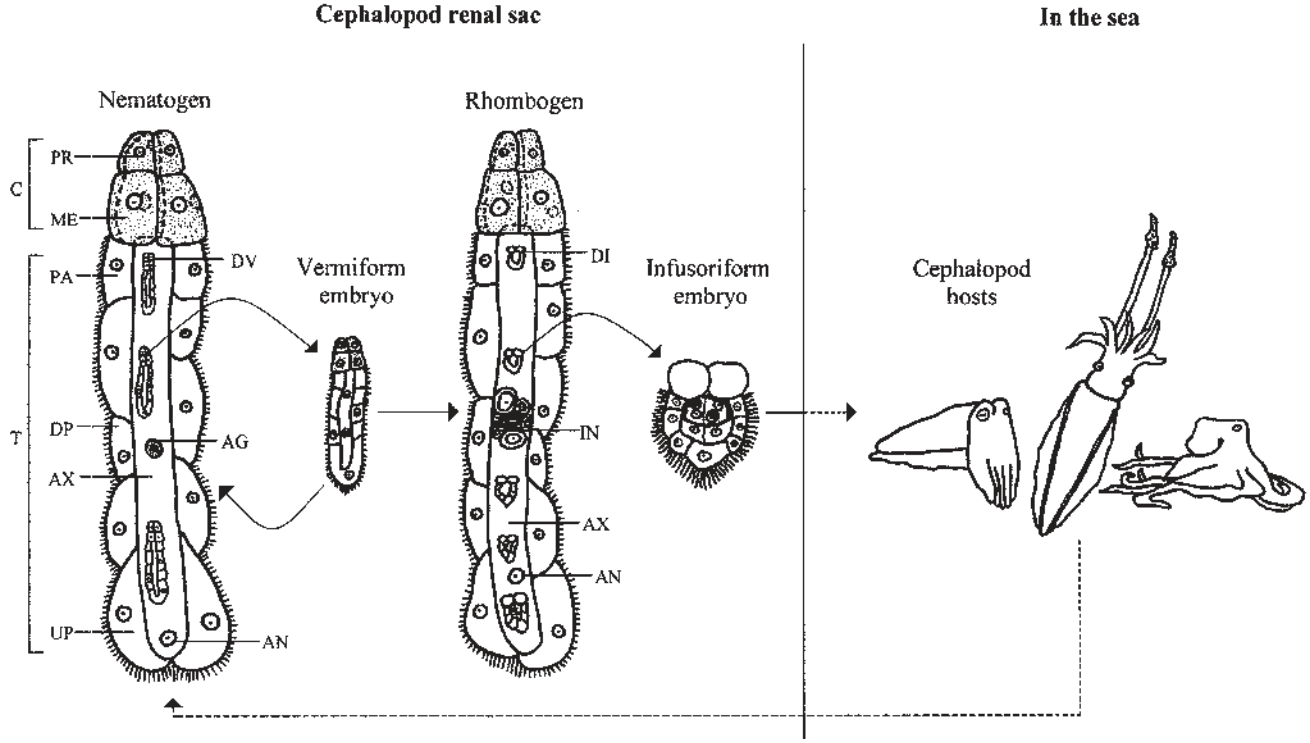

Figure 6. Diagrammatic representation of the morphology and life cycle of dicyemids. The dashed line indicates unknown processes of how the infusoriform embryo finds and infects a new cephalopod in the sea and how it then develops into adult forms. The nematogen, rhombogen, and vermiform embryo represent the asexual vermiform stages; the infusoriform embryo represents the sexual infusoriform stage. Abbreviations: AG, agamete; AN, axial cell nucleus; AX, axial cell; C, calotte; DI, developing infusoriform embryo; DP, diapolar cell; DV, developing vermiform embryo; IN, infusorigen; ME, metapolar cell; PA, parapolar cell; PR, propolar cell; T, trunk cell; UP, uropolar cell. Source: Adapted from Catalano, 2012. License: CC BY-NC-SA 4.0.

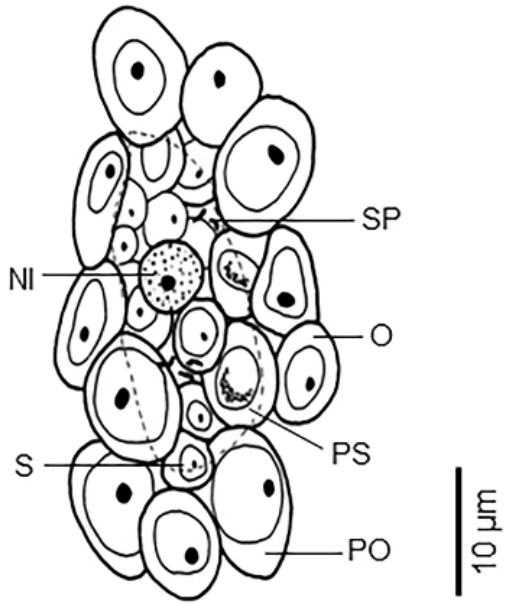

Figure 7. Line drawing of the infusorigen which is located within the axial cell of an adult rhombogen. Abbreviations: NI, nucleus of infusorigen; O, oogonia; PO, primary oocytes; PS, primary spermatocytes; S, spermatogonium; SP, sperm. Source: Adapted from Catalano, 2013a. License: CC BY-NC-SA 4.0.

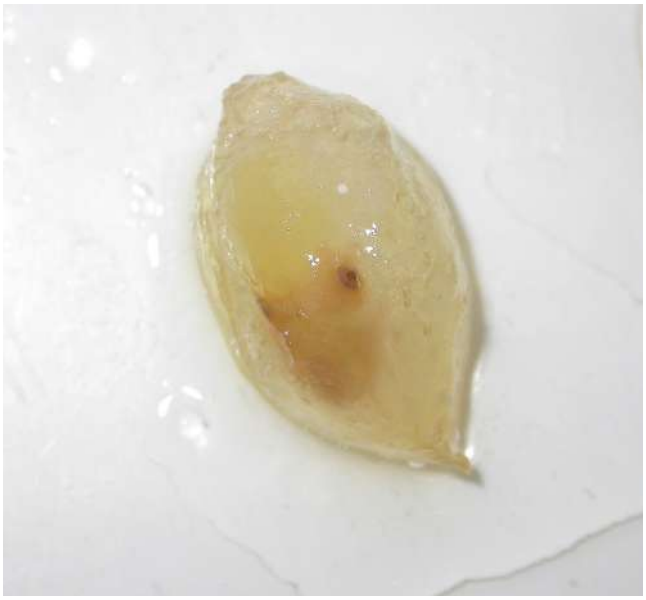

Figure 8. Host eggs have been hypothesized to be the potential stage of new infection for dicyemid mesozoans. Source: S. Catalano. License: CC BY-NC-SA 4.0.

The next stage of the life cycle remains unknown yet quite astonishing given most cephalopods are found to be infected by dicyemid parasites at high intensities. Particularly, it is uncertain how the tiny, infusoriform embryo, with limited swimming capabilities in relation to its host and a short survival time in seawater, then finds a new host, attaches to it or is taken up internally and starts the cycle off again by potentially morphing into the required vermiform stage (adult nematogen) so asexual reproduction can take place and the renal appendages of a new host will then become colonized.

Despite the monstrous challenge that the infusoriform embryo faces of finding and infecting the correct host species with a limited lifespan in a large, fluid, ever-changing environment, dicyemid parasites are still found in almost all benthic cephalopods examined (Catalano et al., 2014). As such, questions have been raised about an intermediate host; however, results from past experimental studies suggest the life cycle is direct (Lapan and Morowitz, 1975). Host eggs were hypothesized to be the potential stage of new infection, as they are abundant and sessile in the environment, allowing a huge number of new individuals to be infected with low energy costs (Figure 8) (Catalano et al., 2013). Additionally, as adult cephalopods have a short lifespan of 1 to 2 years, with mortality common after a single spawning event (Semmens et al., 2007), infection of the host egg stage provides dicyemids with the maximum amount of time for survival. Nonetheless, no dicyemid DNA was recovered from environmental water samples or cuttlefish eggs at the mass breeding aggregation of giant Australian cuttlefish (*Sepia apama* Gray) in South Australian waters (Catalano et al., 2013), leading to the notion that to resolve this unknown in the life cycle, experimental infection is needed. Interestingly, exclusive infection of the asexual stage of the dicyemid (adult nematogen) was found in the left renal appendage of a large giant Australian cuttlefish that had been held in captivity for 2–3 months, recently mated, and naturally died before samples were collected, indicating that dicyemids may persist and continue replicating even after host death (Catalano, 2013b). Furthermore, although the host had died, an immediate priority by the dicyemid was not to disperse, as density increased within the renal appendage with vermiform embryos continuing to produce instead of the dispersal of the infusoriform embryo (Catalano, 2013b). Perhaps a dead host just gets stuck with lingering parasites; or, perhaps, the life cycle of the dicyemid may be more intricate and mysterious than first thought.

Secondary Nematogens

Although not recognized as a regular part of the dicyemid life cycle, an additional form exists, namely the secondary nematogen. This rare form, which in the past has been denied to occur at all (Gersch, 1938), but has been observed by McConnaughey (1951) and Catalano (2013a), is characterized

by containing infusorigen and infusoriform embryos together with young vermiform embryos within the axial cell, in essence having features of both adult nematogens and rhombogens (Figure 9). This form is thought to result by accident in the transitional period of development from a nematogen to rhombogen, with persistence of some axoblasts in good condition through the rhombogen period that have been able to resume their activity and produce, once more, viable vermiform embryos (Catalano, 2013a; McConnaughey, 1951). Whether there is then the possibility for reversal back to a full nematogen form, brought about through competition between these 2 modes of reproduction, and the possibility of infusorigens becoming exhausted while axoblasts are still being produced, is unknown. It is also unknown whether the occurrence of secondary nematogens is species-specific (Catalano, 2013a).

Hosts and Patterns of Infection

The only recorded hosts of dicyemid mesozoans are cephalopods, which include squid, octopus, and cuttlefish (as seen in Figure 1). In general, dicyemid species are highly host-species specific, although typically, 2 or more species are recorded in each host species (Furuya, 1999; 2017). The common octopus, *Octopus vulgaris* Cuvier, has the largest number of dicyemid species recorded from it (11), followed closely by the stubby squid, *Rossia pacifica* Berry, with 9 species (see Catalano, 2012). Hochberg (1990) suggests that *O. vulgaris* and *R. pacifica* may actually represent a host species complex, each with their own distinct dicyemid fauna, and the reported parasites might make up a reciprocal species complex.

When more than 1 dicyemid species do co-occur within a single host individual, generally the calotte shape from each is different, allowing each dicyemid species to colonize a distinct niche or surface of the host renal appendage (Furuya et al., 2003a; Furuya, 2008; Furuya and Tsuneki, 2003). Species of dicyemids that possess similar calotte shapes are rarely found together in a single host individual (Furuya and Tsuneki, 2003). The microhabitat of the renal appendages provides all that the dicyemid requires to complete its life cycle, including a surface for attachment, constant fluid bath, a source of nutrients and a simple exit for release of the dispersal stage (Hochberg, 1982).

In general, the presence of dicyemids is more commonly observed in benthic rather than pelagic cephalopods, which has been related back to the negative buoyancy and sinking ability of the infusoriform embryo. However, other factors, such as host size, age, behavior, and geographic locality likely play a role in the presence of infection, as exceptions

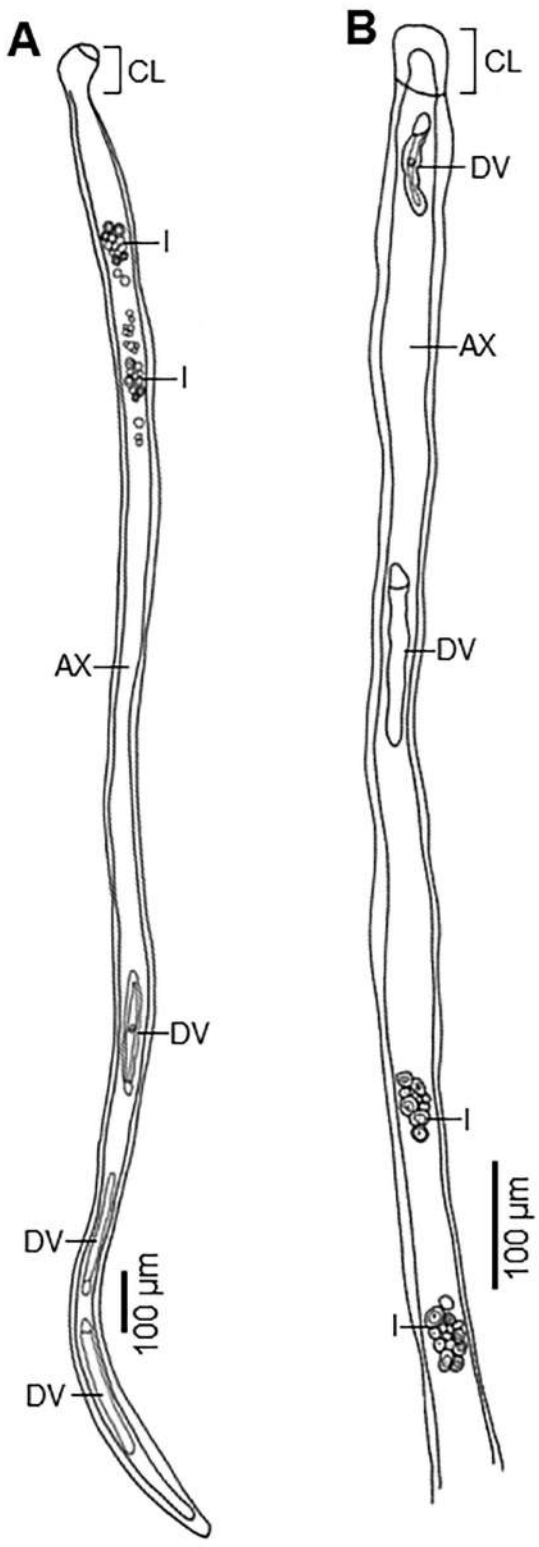

Figure 9. Line drawing of secondary nematogens (*Dicyema furuyi*) from *Sepia papuensis*. Abbreviations: AX, axial cell; CL, calotte; DV, developing vermiform embryo; I, infusorigen. Source: Adapted from Catalano, 2013a. License: CC BY-NC-SA 4.0.

to this notion are observed. For example, the southern dumpling squid, *Euprymna tasmanica* Pfeffer, frequently associates with the sea bottom, burying itself in the sand during the day to hide from predators (Norman and Reid, 2000). Such a strategy would allow for ample opportunity to be infected by the infusoriform embryo; however, in the study by Catalano et al. (2014), adding to the weight of evidence but while not conclusive, no dicyemids were recorded from this host species for 6 individuals collected and analyzed. In contrast, Finn et al. (2005) recorded the presence of dicyemids in 14 out of 18 *E. tasmanica* individuals from the same region, however, the 6 individuals collected in the study by Catalano et al. (2014) were small with a mantle length half of what is typically reached for this species. Other authors have also recorded absence of dicyemids from small host individuals (for example, Furuya et al., 1992b; Furuya and Tsuneki, 2005; Castellanos-Martínez et al., 2011). Furuya and colleagues (2004) correlated this to the complexity of the renal appendage, stating large host individuals have a more developed and complicated external surface compared to smaller host individuals, therefore they are able to provide more attachment sites and surface area for vermiform stages.

Interestingly within a single host individual, different stages (either exclusively the asexual stage or exclusively the sexual stage) have been recorded in each renal appendage, such as the adult nematogen (asexual stage) in the left renal appendage and the adult rhombogen (sexual stage) in the right renal appendage (Figure 10) (Finn et al., 2005; Catalano et al., 2014). This suggests that dicyemids infect the renal appendages independently of one another, at different times, and do not or cannot move from 1 renal appendage to the other. This also elucidates that the cue which mediates the transition from the asexual to the sexual stage is parasite driven rather than host-mediated, or else both renal appendages would be equally affected and contain the same stage at any one time (Lapan and Morowitz, 1975; Finn et al., 2005; Catalano et al., 2014).

True Parasite or Commensal Organism?

The true nature of the dicyemid mesozoan as a parasitic, commensal, or mutualistic organism remains unresolved with arguments for and against each option presented in the literature. Some authors support the parasitic way of life, stating that the delicate brush borders of the host renal appendage surface is damaged by the dicyemid attaching and maintaining its foothold, while others support the dicyemid as a commensal organism, stating they have little effect, either positive or negative, on the host (Finn et al., 2005; Furuya and Tsuneki, 2005). The third notion is that these organisms are mutualistic, with the beating cilia on their bodies facilitating host excretion of ammonia and urine, while also allowing the dicyemid to receive nutrients, taken up through the peripheral cells by endocytosis (Lapan, 1975; Hochberg, 1990; Furuya et al., 2004).

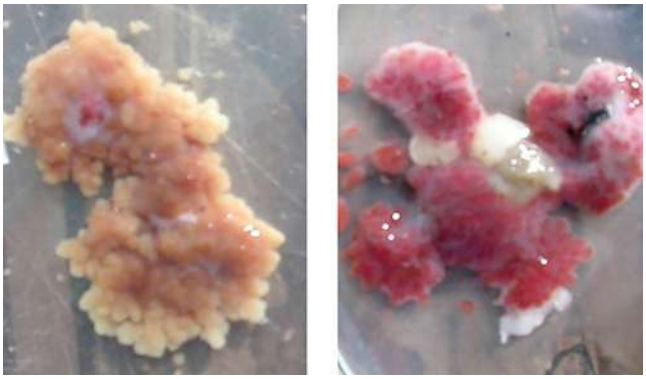

Figure 10. The left (brown) and right (red) paired renal appendages of cuttlefish, which can harbor different stages of dicyemid mesozoans in each renal appendage. Source: S. Catalano. License: CC BY-NC-SA 4.0.

Molecular Analyses: Mitochondrial Mini-circle Molecules

Few studies have focused on the molecular genetics of the dicyemid mesozoans. For those that have reported molecular analyses, the mitochondrial (mt) cytochrome *c* oxidase complex unit genes (*COI*, *COII*, and *COIII*) have typically been sequenced, although some studies have reported sequences for nuclear genes from dicyemid species (Ohama et al., 1984; Katayama et al., 1995; Pawlowski et al., 1996). Quite unusually, the mt genome architecture of the dicyemids departs from the typical ~ 16 kb circular metazoan genome. In addition to a putative circular genome (Boore, 1999), the mt *COI*, *COII*, and *COIII* genes have been found to be located on separate mini-circle molecules, each with their own non-coding region (NCR) (Watanabe et al., 1999; Awata et al., 2005, Catalano et al., 2015). While the gene coding region defines genome metabolic functionality by specifying proteins, the NCR can define the architecture and regulation of the genome, often harboring the replication origin of the mini-circle and the promoters for transcription (Le et al., 2002; Burger et al., 2012). Although no specific origin of replication was found in the *COI* mini-circle molecules of 10 dicyemid species sequenced in the study by Catalano et al. (2015), palindrome sequences with the potential to form stem loop structures were identified in 5 species, suggesting that these palindrome regions may be involved in initiating mini-circle replication. Nonetheless it is quite

bizarre to have single mt genes on single mini-circle molecules, as the typical mt genome, where all the genes are linked together on a chromosome will ensure the complete genetic information is transmitted when the mitochondrion replicates. This is otherwise challenging with a fragmented mt genome structure as observed in the dicyemid mesozoans. Further molecular studies are needed for the dicyemid mesozoans, particularly to confirm the validity of classifications based on traditional morphological traits, with sequences of DNA containing thousands of characters with orders of magnitude more than morphological analyses (Poore and O'Hara, 2007).

Position in the Tree of Life

The position of the dicyemids in the Tree of Life has long fascinated researchers, with the Belgian biologist Édouard Van Beneden providing the first attempt to classify the dicyemids (Van Beneden, 1876). His belief was that this group occupied an evolutionary intermediate position between the Protozoa (unicellular animals) and the Metazoa (multicellular animals), and hence he created the intermediate name Mesozoa Van Beneden, 1876. Since then, numerous attempts have been made to classify the dicyemids, however, often leading to additional confusion rather than resolution. In particular, the dicyemid mesozoans have been suggested to be members of the Spiralia (based on developmental studies and encoding of a 'spiralian peptide' in the dicyemid *DoxC* gene (Furuya et al., 1992a; Kobayashi et al., 1999), highly simplified bilaterians (based on tool-kit *Pax6* and *Zic* genes; Aruga et al., 2007), ancient multicellular animals (based on 5S rRNA gene; Ohama et al., 1984), relatives of nematodes (based on 18S rRNA sequences; Pawlowski et al., 1996), closely affiliated to annelids (based on 18S and 28S rRNA sequences; Petrov et al., 2010), and a sister group to the clade consisting of annelids and molluscs (amino acid sequences of innexin; Suzuki et al., 2010). Recently, the transcriptome of *Dicyema japonicum* was sequenced with the authors presenting support for the placement of Dicyemida with the Orthonectida in phylum Mesozoa, which then forms a sister group to the clade of Mollusca and Annelida (Lu et al., 2017). However, differences in internal features and stages of their respective life cycles still shed a level of doubt on the dicyemids and orthonectids grouping together within a single phylum. Further transcriptome sequence data from additional dicyemid and orthonectid species, including representatives from all of the described genera, will be required to validate these findings and confirm the definite position in the Tree of Life of the dicyemid mesozoans.

Collection and Staining Methods

As dicyemid mesozoans are minute and comprise only a few cells, they rapidly degrade following host death so collection should be targeted from fresh material. After euthanasia, place the body of the cephalopod ventral side up in a tray and open the mantle cavity with a sterile scalpel blade to expose the paired renal sacs. Using forceps and scissors sterilized in absolute ethanol to avoid cross contamination, remove the left and right renal appendages, and smear small pieces onto glass microscope slides. A smear is made by holding the renal appendage between the forceps in one hand and the glass slide in the other, and then with slight pressure, moving the renal appendage across the glass slide from left to right covering the slide surface from the top to the bottom in straight parallel lines. If the host is infected by dicyemids, typically small white strands will be seen on the glass slide when it is held up to the light. At a minimum, 4 smears should be made per renal appendage (8 smears per host), although the number of smears can be increased for larger host individuals. Smears will then need to be fixed immediately in 70% ethanol to avoid parasite desiccation, with Lock-Mailer™ jars (Ted Pella, Inc.) proving to be ideal for field sampling and storage before slides are stained and mounted upon returning to the laboratory.

Although a range of staining methods have been used by past authors, a trial performed by Catalano et al. (2014) suggests staining with Ehrlich's acid haematoxylin diluted 20 parts of MilliQ water to 1 part stain for 20 minutes, dehydration in an ethanol series and counterstaining in eosin (70% ethanol for 10 minutes, 90% ethanol for 10 minutes, eosin 1% alcoholic solution diluted 20 parts of MilliQ water to 1 part stain for 2 minutes, and 100% ethanol for 15–20 minutes) as ideal for visualization of distinguishing morphological characters. Stained smears can then be mounted in Canada balsam, dried on a hot plate at 50 °C and examined with a compound microscope at magnification up to × 1,500, with drawings and measurements made with the aid of an ocular micrometer and drawing tube.

To confirm host species identification, it is desirable to collect a section of the host tissue (for example, mantle tissue) in 100% DNA-grade ethanol for molecular analysis. A section of each renal appendage can also be collected and stored in 100% DNA-grade ethanol for molecular analysis of the dicyemid parasite to complement traditional morphological classification. Depositing stained slides in registered museum collections should be mandatory for all new dicyemid species descriptions.

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Part III

ENDOPARASITIC PLATYHELMINTHS

PLATYHELMINTHES

15

Platyhelminthes

Introduction to Endoparasitic Platyhelminths (Phylum Platyhelminthes)

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Phylum Platyhelminthes

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Chapter 15

Introduction to Endoparasitic Platyhelminths (Phylum Platyhelminthes)

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Introduction to Platyhelminths

The phylum Platyhelminthes includes the cestodes, trematodes, and monogeneans (which are classified in one treatment as monopisthocotylids and polyopisthocotylids; see Brabec et al., 2023). Most worms classified into Platyhelminthes have bodies that are dorsoventrally flattened, so they are sometimes referred to as flatworms. They may be leaf shaped or oval and some are more or less rounded, and some, such as tapeworms grow to large sizes. For example, parasitic flatworms range in size from those that are nearly microscopic such as species of *Gyrodactylus* that live on the gills of fishes and have a maximum body length of less than 500 µm to the giant cestodes of whales like *Tetragonoporus calyptocephalus* that live in the intestine of sperm whales and can attain a length of more than 30 m. Adult flatworms lack a **coelom** and are called **acoelomate** but they do possess a well-developed **mesoderm**, which becomes parenchyma, reproductive organs, and musculature.

Platyhelminths also are bilaterally symmetrical and thus have a definite anterior end with associated sensory and motor nerve elements. The nervous system is elaborate in many species enabling them to live in a wide variety of habitats, including inside other animals, lakes and streams, moist terrestrial environments, and ocean sediments worldwide. The bodies of other animals are quite hospitable to some platyhelminths, so they are commonly parasitic. Platyhelminths can even serve as hosts for other platyhelminths; for example, some **cercariae** (free-swimming transmission stages of trematodes that have left their snail host) can and do penetrate planarians and **encyst**, developing to **metacercariae** that are the stages that are infective to the next host in a complex life cycle (Fried and Rosa-Brunet, 1991).

A **plesiomorphic** condition of platyhelminth physiology is their apparent inability to synthesize fatty acids and sterols de novo, which may help explain why platyhelminths are most often symbiotic with other organisms, either as commensals or parasites (Meyer and Meyer, 1972). Free-living acoel turbellarians, sometimes considered illustrative of ancestral platyhelminths, also seem to lack this ability, indicating that this loss occurred before the parasitic forms evolved from the basal species of the group. Being soft bodied, platyhelminths have left a relatively poor fossil record, but some evidence suggests they have been on Earth for eons, for instance, fossil tracks from a slab of Permian siltstone have been interpreted as those of a land planarian.

General Platyhelminth Morphology

The outer covering of these animals is called the **tegument** and the structure and function varies among species in the major taxonomic groups. Generally speaking, turbellarians and some free-living stages of trematodes and cestodes have a tegument composed of **ciliated epithelium** (Figure 1), which in some cases is their primary mode of locomotion. This epithelium consists of a single layer of cells and contains many **glandular cells** and **ducts** from **subtegumental glands**. **Sensory nerve endings** are abundant in the tegument. In some platyhelminths, cells that produce adhesive secretions are paired with those that produce releasing secretions; the combination is known as a **duo-gland adhesive system**.

Adult trematodes and cestodes have no external cilia except in larval stages such as **miracidia** (**miracidum**, singular) in trematodes and **coracidia** (**coracidium**, singular) in some

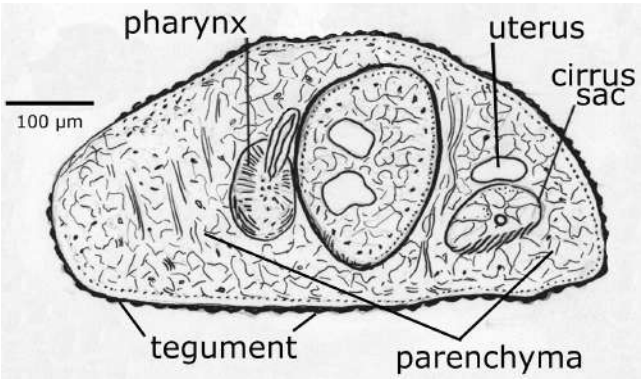

Figure 1. Cross section of a parasitic trematode showing the lack of ciliated epithelium. Source: H. W. Manter. License: CC BY.

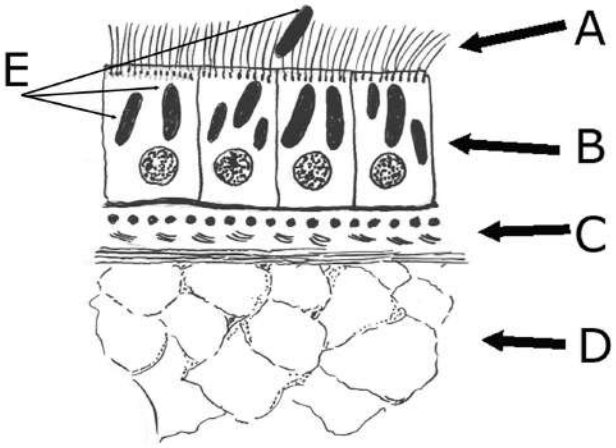

Figure 2. Example section through the body of a turbellarian flatworm. Ciliated tegument (A), epithelial or tegumentary cells (B), circular, longitudinal, and diagonal muscles (C), parenchyma (D), and rhabdites (E). Source: H. W. Manter. License: CC BY.

cestodes. During metamorphosis of these parasitic forms, the larval epidermis is replaced by a syncytial adult tegument (see Figure 2). The **syncytium** is a continuous cellular matrix without the normal intercellular membranes and with nuclei of which are in cell bodies (**cytons**) located beneath a superficial muscle layer. Thus, the name Neodermata (**neo** = new, **derma** = skin; Greek) has been used in classifications at the subphylum level to distinguish such worms from free-living species that retain the ciliated epithelium as adults. Most of a platyhelminth's body is made up of **parenchyma**, a loosely arranged mass of fibers and cells of several types. Some of these cells are secretory, others store food or waste products, and still others have huge mitochondria and function in regeneration. The internal organs are so intimately embedded in the parenchyma that dissecting them free of the surrounding tissue is nearly impossible. The bulk of the parenchyma probably is composed of **myocytons** (non-contractile part of muscle cells).

Muscle fibers course through the parenchyma. Contractile portions of muscle fibers are rarely striated and are usually arranged in 1 or 2 longitudinal layers near the body surface, just beneath the syncytial epidermal layer. Circular and dorsoventral fibers also occur.

The **nervous system** of platyhelminths is a ladder type, with paired ganglia near the anterior end, nerves running anteriad to sensory or holdfast organs, and longitudinal nerve trunks extending posteriad subterminal to the body (meaning, to nearly the end of the body). The number of trunks varies, but most trunks are lateral and are connected by transverse commissures. The nervous system and morphological variation could be used for classification and determination of species, but the techniques of staining and study are quite difficult to master, so not many parasitologists use these characteristics for diagnostic purposes.

Sensory elements are abundant and may be distributed in a variety of patterns, depending on the species. Tactile cells, chemoreceptors, eye spots, and statocysts have been reported from platyhelminths and these have received various levels of study.

The **digestive system** of parasitic platyhelminths is typically a blind sac, also called a **cecum**, although a few trematodes, such as the species that live in the intestines of bats, the morphologically minuscule *Anenterotrema* spp., have only a mouth, and perhaps a pharynx, but no gut at all. Most platyhelminths with a digestive system have a mouth near their anterior end, and most trematodes have a muscular **pharynx** with which they suck in food through the mouth. The gut varies from a simple bi-lobed/bifurcating sac to a highly branched tube, but only rarely do trematodes have an anus. Digestion is primarily extracellular, with phagocytosis by intestinal epithelium (**gastrodermis**), which may contain both secretory and phagocytic cells (Bogitsh, 1993). Undigested wastes are regurgitated back out through the mouth. Relative to this fact, Libbie Hyman (1951, p. 6) said, "The value of an anus cannot be overstated. It permits the animal to feed continuously without waiting for the intestine to be emptied of the previous meal. It also permits a more thorough digestion of food by allowing the food to remain longer in the intestine and by permitting a one-way flow of digestive juices."

Note that cestodes completely lack a digestive system of any sort during all stages of development (see Figure 3).

The functional unit of the **excretory system** of almost all platyhelminths are arrays of **flame cells**, or **protonephridia**. These single cells are arrayed through the parenchyma and

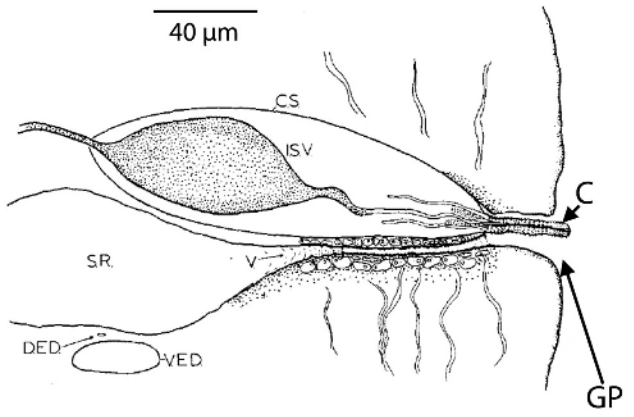

Figure 3. Transverse section through a mature segment of a cestode (tapeworm) showing the dorsal excretory duct (DED) just above the ventral excretory duct (VED). This view is from the posterior end of the worm looking anteriad (meaning, toward the anterior). The seminal receptacle (SR) in this species expands after a very short vagina (V). Dorsal to the vagina is the cirrus sac (CS) with internal seminal vesicle (ISV) shown. The cirrus (C) is shown with minute spines and is shown protruding slightly through the genital pore (GP). In this species the vagina enters the genital pore ventral to the cirrus and cirrus sac. Source: S. L. Gardner, HWML. License: CC BY.

each flame cell comprises a tuft of flagella that extends into a delicate tubule, which may consist of another cell interdigitating with the first (Hertel, 1993; Rohde, 2001). As is the case with the nervous system, ultrastructural studies aimed partly at uncovering characters of evolutionary significance have shown that platyhelminth excretory systems are far more complex than originally thought. Rohde (2001) showed that detailed structure of the flame cell system in various species is correlated more with evolutionary relationship than to habitat in which the animals are living. Protonephridial systems have at least 3 types of flame cells and as many kinds of tubule cells (Rohde, 2001). Excess water, which may contain soluble nitrogenous wastes, is forced into the tubule, which joins with other tubules, eventually to be eliminated through 1 or more excretory pores. Filtration occurs through minute slits formed by **rods**, or extensions of the cell, collectively called a **weir** (Old English *wer*: A fence placed in a stream to catch fish). In parasitic platyhelminths the weir is formed by rods from both the terminal flagellated cell (the **cyrtocyte**) and a tubule cell and is thus referred to as a **2-cell weir**. Because excreta are mainly excess water, this system is often referred to as an **osmoregulatory system**, with excretion of other wastes considered a secondary function. Some species have an excretory bladder just inside the pore (see Figure 4 to see a line drawing of some platyhelminth structures).

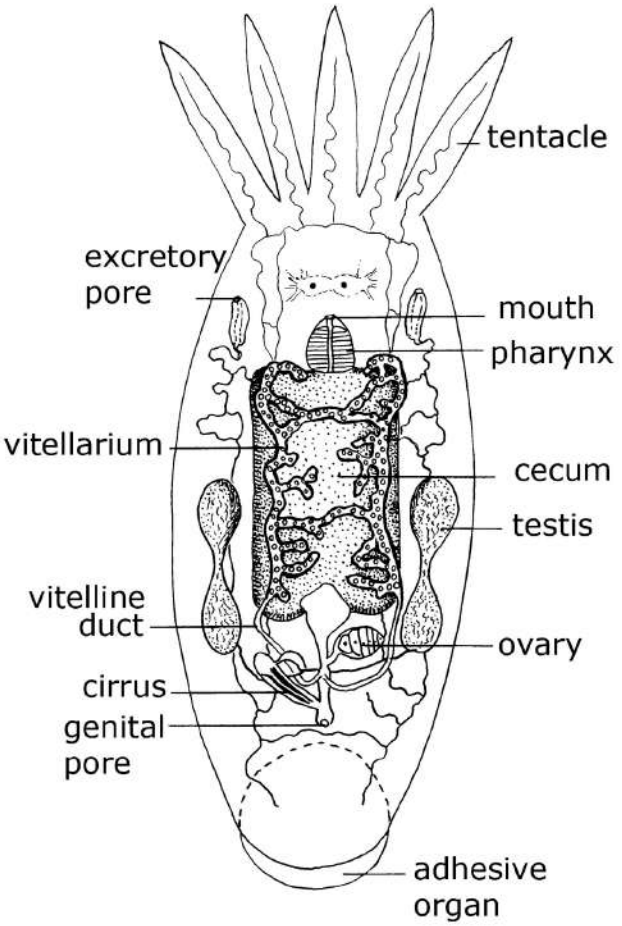

Figure 4. Dorsal view of a marine dwelling flatworm *Temnosewellia semperi* (Weber, 1890) (phylum Platyhelminthes: order Rhabdocoela: family Temnocephalidae) that normally occurs on the external carapace of freshwater crabs. Source: Adapted from Bresslau and Reisinger, 1933. License: CC BY.

Reproductive systems follow a common basic pattern in all Platyhelminthes. However, extreme variations of this basic pattern are found among different groups. Most species are **monoecious** (meaning that structures for both sexes are present in a single organism), but a few are **dioecious** (with separate sexes among individual animals). Because the arrangements of structures in the reproductive systems of platyhelminths are important **autapomorphic** and **synapomorphic characters** and include numbers, sizes, shapes, cellular make up, and more, and are used to identify parasites, they are considered in more detail in each specific group. Most hermaphrodites have the ability to fertilize their own eggs, but many do not do so except under exceptional circumstances and cross-fertilization is the norm. Some turbellarians and cestodes have the potential to practice **hypodermic impregnation**, which is sperm transfer through piercing the

body wall with a male organ, the **cirrus**, and injecting sperm into the parenchyma of the recipient. How sperm find their way into the female system is not known, but the sperm end up in the genital tract of the female part of the worm, whether we know how it happens or not. Most worms, however, deposit sperm directly into the female tract. Larvae usually develop within egg membranes, but a few species are viviparous or ovoviviparous. In species that live as parasites and some turbellarians, egg yolk is supplied by cells other than the ovum that are from the **vitelline glands**, and eggs are thus **ectolecithal**. Asexual reproduction is also common in trematodes and a few cestodes.

Introduction to Platyhelminth Systematics

Historically, the phylum has included 4 classes: Turbellaria, Monogenea (also classified as Monopisthocotyla and Polyopisthocotyla; see Brabec et al., 2023), Trematoda (Digenea), and Cestoda, generally corresponding to: free-living forms, ectoparasitic single-host worms, endoparasitic flukes with 2 or more hosts (1 almost always a mollusc), and tapeworms, respectively. In this book, the focus is on the subphylum Neodermata, particularly the Monogenea, Trematoda (Digenea), and Cestoda. This list of classes implies that the higher classification is settled, but this is not true. Investigations into the phylogenetic relationships of Platyhelminthes is an active area of research in invertebrate biology with many workers attacking evolutionary problems from a variety of directions. The older literature in this area is organized following traditional classifications, for example, see Grassé (1961), Hyman (1951), and for cestodes, the Zoology of Tapeworms (Wardle and McLeod, 1952).

Although *parasitic* is not necessarily a valid criterion for separating taxa, *parasitic* platyhelminths have been shown to form a monophyletic group, having been derived from a common ancestral species called the Neodermata. As noted above, this is based on the fact that all known species in this group shed their epidermis at the end of their larval life and when they transition to adults (a general synapomorphy for these worms).

Some phylogenetic studies (Littlewood and Olson, 2001; Brabec et al., 2023) indicate a common ancestor of both Cestoda and species of the proposed class Monopisthocotyla (see Brabec et al., 2023). Morphological or developmental characters such as the nature or origin of the egg yolk, spermiogenesis, body wall musculature, or structure of the excretory organs (especially the flame cells), are used in platyhelminth classification. Molecular characters that have been used include 18S and 28S ribosomal DNA sequences, genes for cytochrome oxidase, NADH dehydrogenase, elongation factor 1-α, and immunochemistry of neurotransmitters (Litvaitis and Rohde, 1999; Mariaux and Olson, 2001; Raikova et al., 2001). Phylogenies based on molecular characters do not always agree with those based on morphology (Littlewood and Bray, 2001) and phylogenies based on molecular characters do not always agree among themselves.

Extant species of flatworms (Metazoa: Acoelomata: Platyhelminthes) represent a lineage of diploblastic metazoa that are considered to show evolutionarily static pictures of the hypothetical ontogenetic stages ultimately showing the development of triploblastic-coelomate (Metazoa: Coelomata) organisms. Most authors agree that the basal extant taxon of the parasitic flatworms is related to species of *Stenostomum* (subphylum Catenulida), and *Stenostomum* spp. have been used to root many of the phylogenetic trees that have been developed to examine the evolutionary relationships of the various parasitic flatworms (Ehlers, 1986; Litvaitis and Rohde, 1999; Brooks and McLennan, 1993). The major structural feature dividing catenulid platyhelminths from the rest is the lack of a frontal organ, which is a terminal or subterminal pit with mucoid gland cells and sometimes cilia. Catenulids lack this organ, although some species have lateral pits. Some authors doubt that frontal organs are homologous among the taxa that possess them. Nevertheless, Catenulida appear as basal and as a sister taxon to all remaining Platyhelminthes (except Acoela and Nemertodermatida) in the consensus tree of Littlewood et al. (1999), although it should be recognized that a consensus tree is simply a way to summarize disparate trees and a consensus tree does not represent a phylogeny (Swofford et al., 1996).

While not all scientists agree upon taxon names or hierarchical levels, the classification produced by Brooks and McLennan (1993) was based on their phylogenetic tree and utilizes the full range of rules of classification in the use of superclasses, subsuperclasses, infraclasses, cohorts, and subcohorts in addition to some of the more commonly used terms such as classes and orders. Rohde's (1996) phylogeny is based both on a small amount of data from 18S ribosomal DNA and on reassessment of structural features, including information on spermiogenesis. This phylogeny differs from that of the morphological phylogeny of Brooks and McLennan (1993) mainly in placement of Temnocephalidea and Udonellidea. The consensus tree provided by Littlewood and Olson (2001) was developed using both morphological and a limited set of molecular data.

The multigene phylogeny of the parasitic flatworms published by Brabec et al. (2023) includes phylogenetic trees (shown modified in Figures 5A and 5B) that are based on a more recent assessment than Littlewood and Olson's (2001). Their work was done to examine hypotheses of the evolution and origin of endoparasitism in the Platyhelminthes and

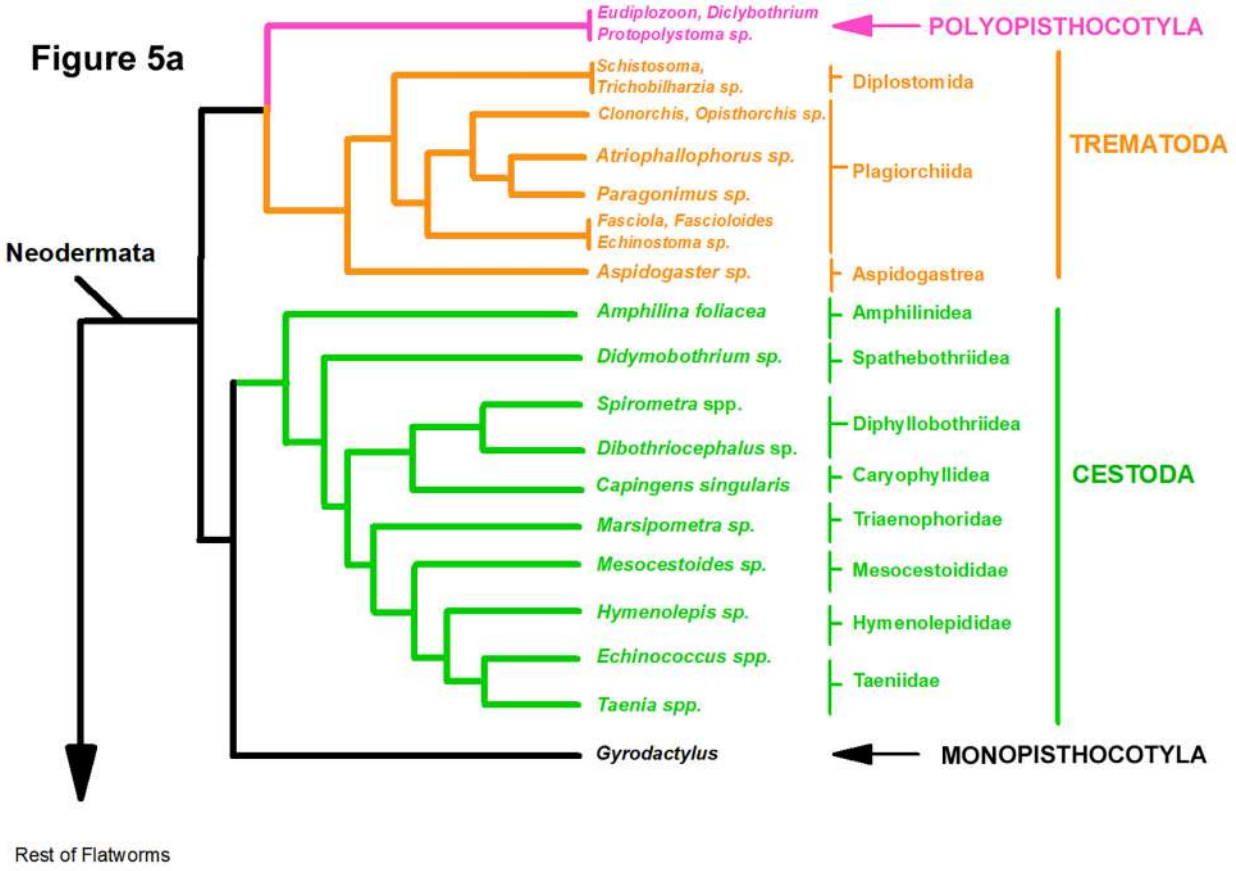

Figure 5A. Phylogeny based on 83 species of helminths of which 51 were parasites. Tree based on Bayesian inference algorithms showing a common ancestor of the Monopisthocotyla and the Cestoda while the Polyopisthocotyla shares a common ancestor with the Trematoda (see Brabec et al., 2023). Source: Adapted from Brabec et al., 2023. License: CC BY.

included the phylogenetic analyses of 225 genes from 83 taxa (51 of which were parasitic forms). Two equally plausible trees were shown by these authors who used 2 different tree construction algorithms, 1 showing members of the class Monopisthocotyla (ectoparasites) as sister to the class Cestoda (Figure 5A) and the other tree showing species of the class Monopisthocotyla sharing a common ancestor with the trematodes, species of the class Polyopisthocotyla (including *Protopolystoma* and other genera) and cestodes (Figure 5B). Their main conclusions are:

1) The Neodermata includes those flatworms that lose their epidermis upon transitioning from free-living larval forms into sexually reproducing adult forms.
2) The mode of living as parasitic flatworms evolved independently in the Neodermata.
3) Complex life histories of the cestodes and the trematodes originated independently.

Brabec and colleagues' (2023) analysis of the origins and diversification of the flatworms represents the tip of the molecular iceberg, hinting that phylogenetic analyses of genomic and proteomic data will eventually become common operations for biologists in the future.

The classification of Platyhelminthes will likely undergo more changes based on new data producing new phylogenies, but the book Interrelationships of the Platyhelminthes edited by Littlewood and Bray (2001) will be the standard reference on platyhelminth systematics for some years to come (Gardner, 2002) even though the conclusions within Littlewood and Bray (2001) have more recently been subjected to rigorous testing by Brabec and colleagues (2023). This is the way science works, hypotheses are produced, tested with new information, and new hypotheses supersede the old ones (Hull, 1988).

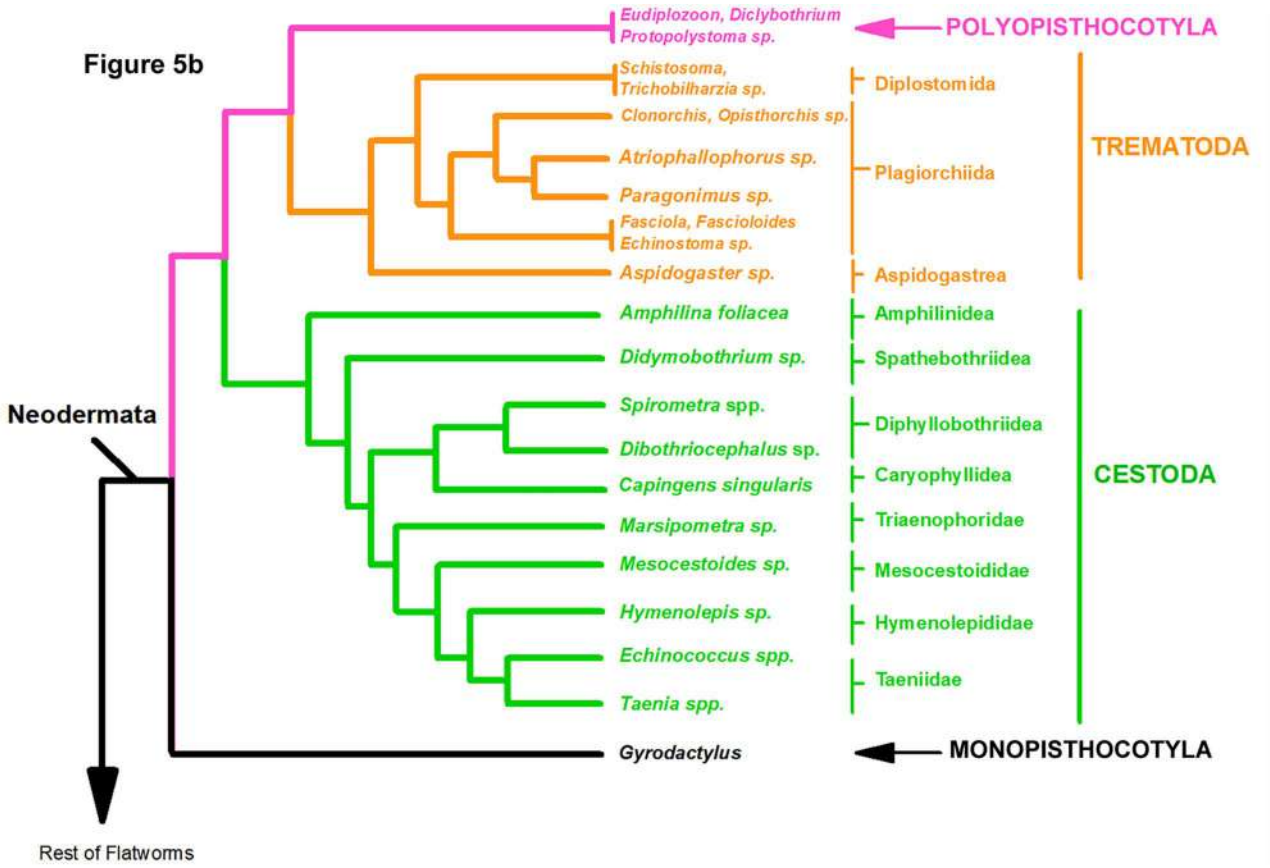

Figure 5B. Phylogeny based on 83 species of helminths of which 51 were parasites. Tree based on the maximum likelihood algorithm showing a common ancestor of the Polyopisthocotyla with the Trematoda and the Monopisthocotyla sharing a common ancestor with the rest of the Neodermata (see Brabec et al., 2023). Source: Adapted from Brabec et al., 2023. License: CC BY.

Note about Placement of Monogenea and *Transversotrema* in the Book

Note that the Monogenea (lately proposed to be classed as Monopisthocotyla and Polyopisthocotyla by Brabec et al., 2023) are covered in Part V of the book (Ectoparasites). Some species of this group live inside their host (endoparasitic) while most species are ectoparasitic. *Transversotrema* trematodes are also included in the ectoparasite part.

Acknowledgement

This section was partially adapted with permission from Roberts et al. (2014, p. 191–195, 309). The license for this adaptation is CC BY-NC-SA 4.0.

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CESTODES

16

Cestoda

Introduction to Cestodes (Class Cestoda)

Scott L. Gardner

Phylum Platyhelminthes

Class Cestoda

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 16

Introduction to Cestodes (Class Cestoda)

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Introduction

Cestodes, also called tapeworms, are **acoelomate**, meaning that they do not have a body cavity lined with tissue derived from the embryonic **mesoderm**, and their bilateral symmetry, well-organized reproductive, osmoregulatory, nervous, and reproductive organs, place these animals in the monophyletic phylum Platyhelminthes. The name Cestoidea was established for these animals by Rudolphi (1809), although many current treatments refer to the class Cestoda, which is used here. Relatives of the class Cestoda include the digenetic trematodes, the Turbellaria, and the sister taxon to the cestodes, the Monogenea.

Cestodes have long excited in humans a sense of bewilderment, fascination, and sometimes even fear, because they seem to appear spontaneously within a host and, when present, they are occasionally pathogenic in various ways. People's interest in them may also be due to the fact that they are ubiquitous. Nearly every species of vertebrate examined by biologists has been shown to host 1 or more species of cestodes. Since there are about 68,000 known species of vertebrates, and only around 4,800 species of cestodes yet been described, it follows that an immense number of cestode species is yet to be discovered.

In addition to Rudolphi, the pioneering works of Karl von Siebold, Friedrich Küchenmeister, Rudolf Leuckart, Maximilian Braun, Constantin Janicki, Friedrich Zschokke, Gerald D. Schmidt, Robert Dollfus, Marietta Voge, Alekseĭ Andreevich Spasskii, Lidija Petrovna Spasskya, Masashi Ohbayashi, and others laid the foundation for the study of tapeworms, or **cestodology**. A vast literature on this group has accumulated through the years; even so, much remains unknown, and work to discover the diversity of cestodes is urgent. Due to varied pressures, such as anthropogenic deforestation, desertification, and general overharvesting and obliteration of nature—just as is true for species considered to be charismatic megafauna—more species of cestodes may be lost due to extinction than science is able to discover each year.

Morphology of Tapeworms

Although considerable variation of morphological characteristics occurs among different orders of cestodes, there are underlying **synapomorphies** that unite the various orders into the class Cestoda. The following generalized description is supplemented within the text of this book, especially where specialization has modified the basic pattern. Tapeworms usually consist of a chain of segments called **proglottids**, each of which contains 1 or more sets of reproductive organs although some species are **monozoic**. The proglottids are continuously produced near the anterior end of the animal by a process of **asexual budding** also called **strobilization**. Each bud moves toward the posterior end as a new one takes its place, and during the process, the budding segment or proglottid becomes sexually mature. This means that the segment has the full complement of male and female sex organs but does not yet have eggs (see Figures 1 and 2A). The **gravid** (meaning, full of eggs; see Figures 2B and 2C) or senile **terminal segments** either shed their eggs directly into the intestine (**anapolytic**) and then they eventually detach, or they may detach while still full of eggs (**apolytic**) and either disintegrate in the intestine leaving the eggs or the segments to exit the host digestive system in the feces. Sometimes the segments exit the body and begin to crawl away from the pile of feces. The entire body of a cestode consisting of repeating segments is called the **strobila** (see Figure 3A), and a segmented strobila is said to be

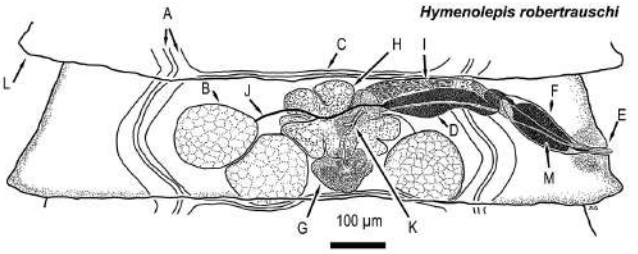

Figure 1. Mature proglottid (segment) of *Hymenolepis robertrauschi* from a grasshopper mouse (*Onychomys* sp.) collected in New Mexico, United States. A) Osmoregulatory canals, small canal is dorsal; wider canal is ventral; B) testis, here 3 are visible and are a characteristic of species of the genus *Hymenolepis*; C) lateral osmoregulatory canal passing ventrally across segment; D) external seminal vesicle; E) cirrus; F) cirrus sac, also called cirrus pouch; G) vitelline gland; H) ovary: I) seminal receptacle, J) vas efferens; K) ootype; L) vellum of segment. Source: S. L. Gardner, HWML. License: CC BY.

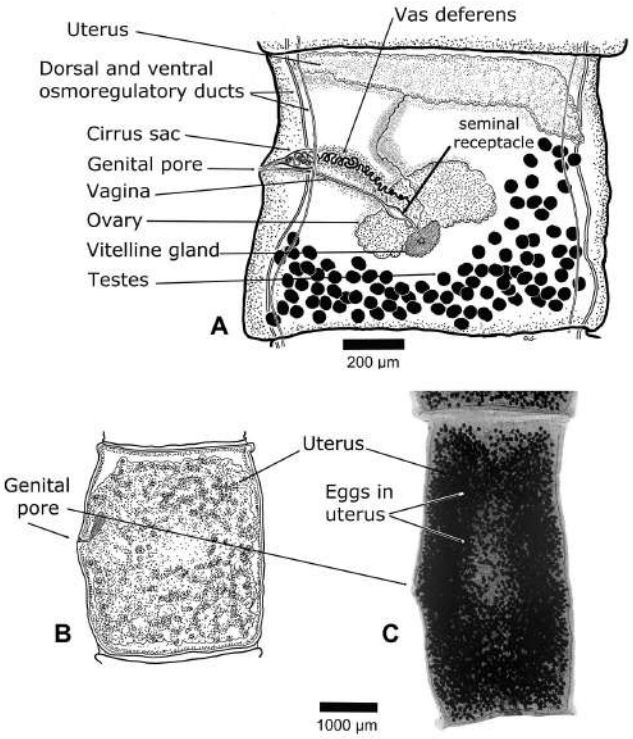

Figure 2. General structure of a craspedote tapeworm of the genus *Mathevotaenia* showing mature and gravid segments, also called proglottids. A) A fully mature proglottid showing male and female sex organs showing the longitudinal excretory ducts, testes, genital pore, cirrus sac, vitelline gland, lobed ovary, and seminal receptacle. The vasa efferentia (tubules that run from each testis to the vas deferens) are not shown; B) the gravid proglottid with eggs in the early stages of development; C) the terminal and fully gravid proglottid showing eggs filling the uterus. This species was collected in 1984 and described in 2023. Source: Adapted from Gardner and Grappone, 2023. License: CC BY.

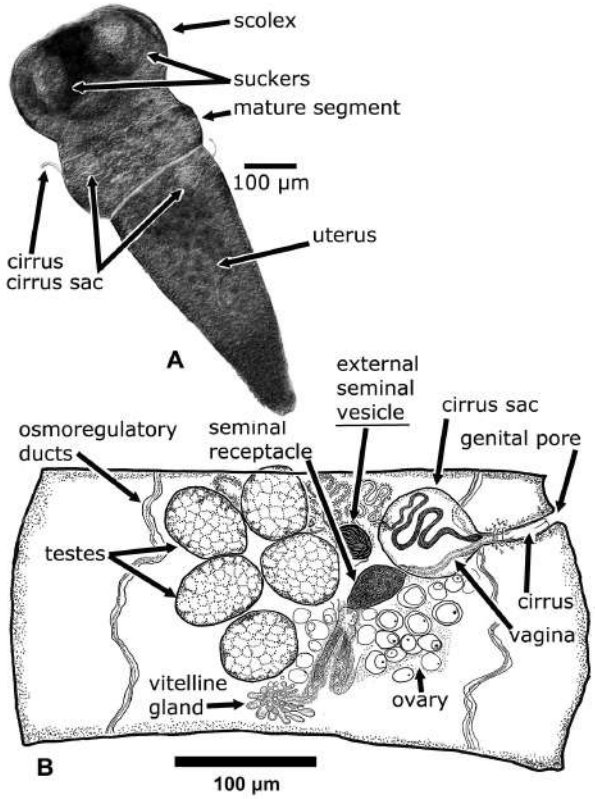

Figure 3. *Pritchardia boliviensis*. Known individuals of this species represent examples of a very small tapeworm, which as an adult has only a scolex and 3 discernible segments: One pre-mature segment, 1 mature segment, and 1 gravid segment. A) Photomicrograph of a whole animal; B) drawing of a mature segment of this same species with structures labeled. These cestodes are common in the small intestines of the small marsupials in the Andean foothills of South America but are very difficult to discover as they must be obtained from recently-collected mammals. Source: Adapted from Gardner et al., 2013. License: CC BY.

polyzoic. In some groups of cestodes, the body consists of a single segment, and is then said to be **monozoic**. If each proglottid or segment overlaps and is wider at the posterior part than the anterior part of the following segment, the whole strobila is said to be **craspedote**, if not, it is called **acraspedote**. Often, between the holdfast organ, called the **scolex** (Figures 3A, 4A, 6A, and 7B), and the first segments of the strobila there is a smooth, relatively undifferentiated zone called the **neck**. This may be long or short, or absent altogether. The neck, or in its absence the posterior part of the scolex, contains **germinal cells** that have the potential for budding of the segments, a process called **strobilization**. The compact germinal cells visible in the nascent proglottids are called the **anlagen**.

There is usually a scolex at the anterior end that is the principal means of attachment or locomotion of these animals. Depending on the group, the scolex may have suckers, grooves, hooks, spines, glandular areas, or combinations of these. In some instances, the scolex is quite simple, lacking any of these specializations, or it may be absent altogether. In a few species it is normal for the scolex to be lost and replaced in function by a distortion of the anterior end of the strobila; this called a **pseudoscolex**. A few species are capable of penetrating into the gut wall of the host where the scolex, and sometimes a considerable length of strobila, are encapsulated by host immune reactions, while the remainder of the strobila dangles into the lumen of the gut.

Following are descriptions of the organ systems of cestodes. Since the taxonomy of cestodes is based primarily upon the anatomy of the reproductive organ systems, an understanding of these systems, particularly, is essential to have a clear understanding of these interesting animals.

Organ Systems

Nervous system

The nervous system appears to be a modified ladder-type, with a **longitudinal cord** near each lateral margin and **transverse commissures** in each segment. The 2 lateral cords are united in the scolex in a complex arrangement of ganglia and commissures. The nervous system is rarely used as a **taxonomic character**, although the lateral cords are convenient points of reference for the location of other structures. There are abundant characters of the nervous system of these animals that can be used for morphological descriptive and comparative purposes, but few authors use these characters for this purpose.

Osmoregulatory system

As in other groups of worms in the phylum Platyhelminthes, the organ of osmoregulation is the **protonephridium**, or **flame cell**. These unicellular glands remove excess fluid from the **parenchyma tissues** and discharge this fluid from the body by a series of **collecting tubules**. The largest of these tubules are called the **osmoregulatory** or **excretory canals** (Figures 2A and 3B) and are typically of two pairs, one ventrolateral and the other (usually smaller) dorsolateral on each side. These canals may be independent throughout the strobila or may **ramify** and **anastomose** in each proglottid. Commonly, a transverse canal near the posterior margin of each segment unites the ventral canals while the dorsal canals remain simple. The dorsal and ventral canals join in the scolex, usually in association with complex branching, sometimes associated closely with the posterior part of the **apical organ** or the **rostellar pouch** (Figures 4A and 4C) depending on the species. Posteriad, the 2 pairs of canals unite into an **excretory bladder** with a single pore. In polyzoic species this bladder is lost with the detachment of the **terminal proglottid**, and thereafter the canals empty independently at the end of the strobila. In a few instances the major canals also empty through short, lateral ducts. The major function of the osmoregulatory system seems to be water balance, but some excretion of metabolic wastes also probably occurs. The dorsal canals carry fluid anteriad toward the scolex and the ventral canals carry fluid posteriad. Occasionally, the dorsal canals are absent. The arrangement of major canals is of taxonomic importance.

Muscular system

Most cestodes possess well-defined, longitudinal bundles of muscle fibers along with scattered dorsoventral groups of muscles. The scolex is well supplied with muscles and nerve fibers, making it extraordinarily motile. In the strobila, the

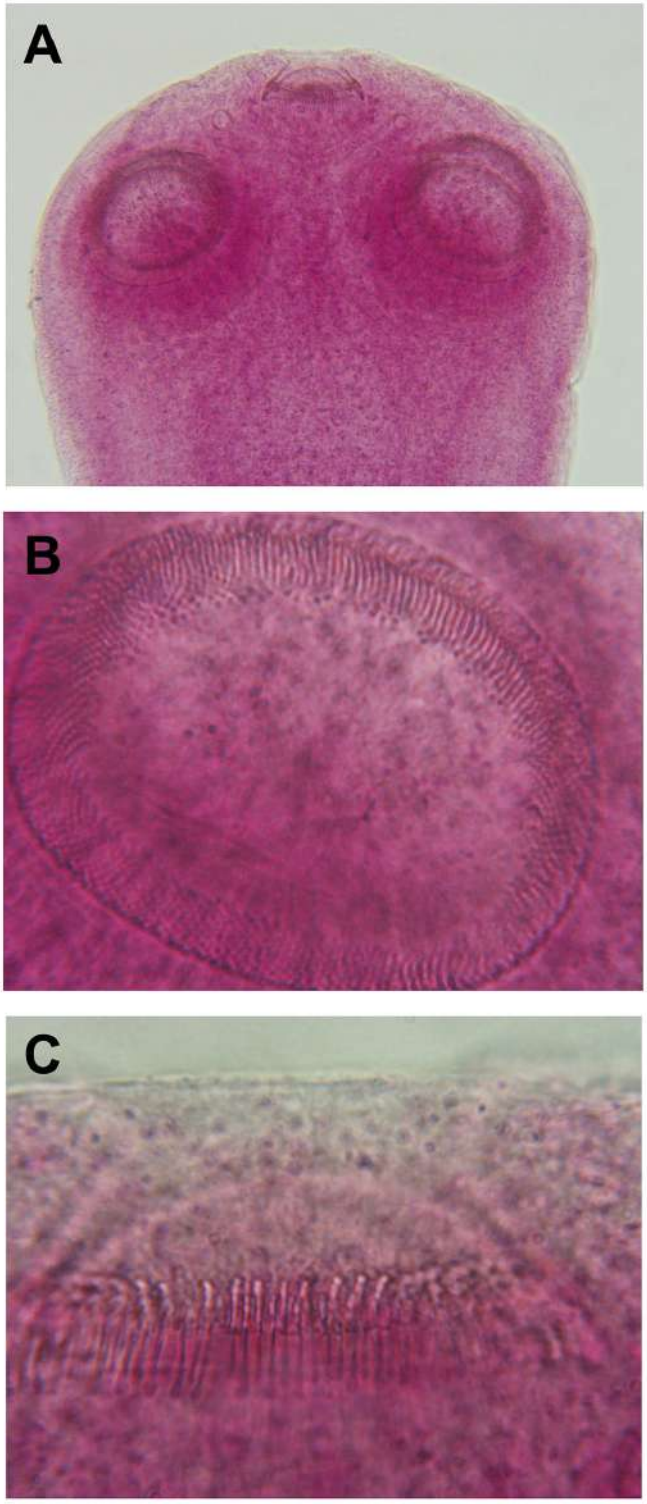

Figure 4. A) A species of *Raillietina* with hooks visible on the retracted rostellum and small hooklets visible on the suckers; B) a highly magnified view of one of the suckers showing the small hooks arranged around the margins of the sucker; C) a closer look at the hooks arranged around the rostellum of the scolex; they alternate long and short and are about 20 µm-long and 2 µm in maximum width. Source: S. L. Gardner, HWML, 2023. License: CC BY.

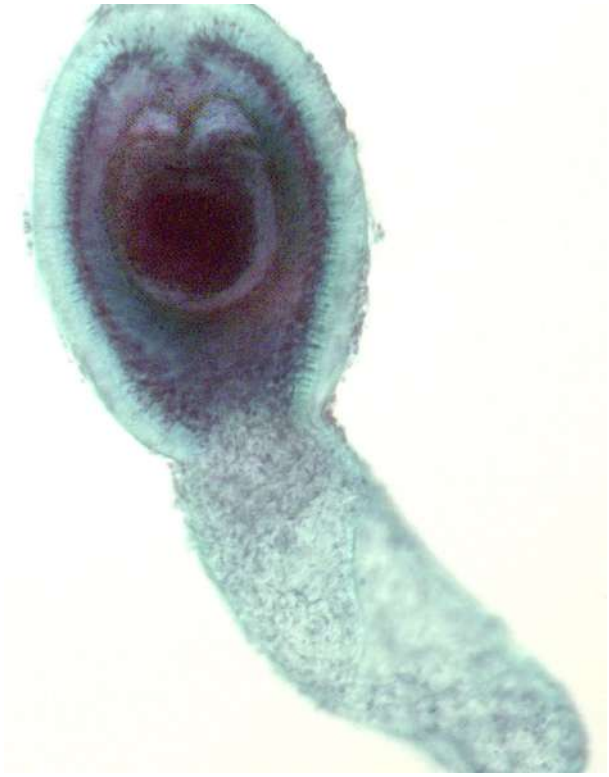

Figure 5. Larva of *Hymenolepis diminuta* (Rudolphi, 1819) grown from an experimentally infected *Tenebrio molitor* Linnaeus 1758. The scolex can be seen inverted in the enter of the larva. Stained in Semichon's acetic carmine and counterstained with fast green, mounted on a microscope slide in Canada balsam. Source: S. L. Gardner, HWML. License: CC BY.

Figure 6. Eggs of *Hymenolepis weldensis* Gardner and Schmidt, 1988 from a Sandhills pocket gopher (*Geomys lutescens* Merriam 1890) from near Cedar Point Biological Station, near Ogallala, Nebraska, United States. The eggs were imaged after they were removed from the gravid uterus of a living tapeworm. The eggshells cracked under pressure of the coverslip while on the microscope slide. The larvae, or **embryophores**, can be seen pushing out of the eggs. In this stage, the embryos are motile and the hooks can be seen thrusting and trying to penetrate the intermediate host, which is probably a beetle of the family Tenebrionidae, although the life cycle is still unknown for this species. Source: Adapted from Gardner and Schmidt, 1988. License: CC BY-NC-SA 4.0.

longitudinal muscle bundles often are arranged in a definite layer within the parenchyma, dividing it into a well-defined cortex and medulla. The arrangement of these muscles is of taxonomic importance but is not much used for this purpose.

Reproductive systems

Almost all known cestodes are **monoecious**, or **hermaphroditic**, with the exception of a few species from birds and stingrays, which are **dioecious** or **gonochoristic**. Most commonly, each proglottid, or segment, contains 1 complete set each of male and female reproductive organs, although a few species have 2 complete sets in each segment, and some have many. A few rare species in birds have 1 female and 2 male sets in each proglottid. After its origin in the neck, and as the segment moves toward the rear of the strobila, as described above (Figures 1, 2, and 3), the reproductive organs mature and **embryonated** eggs are formed. Most commonly, the male organs mature first and produce sperm, which are stored until maturation of the ovary. Early maturation of the testes is called **protandry** or **androgyny** and is used as a taxonomic character. In fewer species the ovaries mature first which gives rise to a condition known as **protogyny** or **gynandry**. This is also used as a taxonomic character.

Male reproductive system.

Depending on the species, the male reproductive system (Figures 1, 2, and 3) may have as few as 1 up to many hundreds of **testes**, each of which has a fine **vas efferens** that transmits sperm toward the **genital pore**. If there are numerous testes, these vasa efferentia unite into a common **vas deferens** which enables transfer of sperm toward the genital pore. The vas deferens may be a simple dilation, or it may expand into a spheroid, often pear-shaped, or piriform external seminal vesicle or it may be highly convoluted, with the convolutions functioning in sperm storage. Eventually, the vas deferens leads into a **cirrus pouch** or **cirrus sac**, which is a muscular sheath containing the terminal portion of the male system. Depending on the species of cestode, inside the cirrus pouch, the vas deferens may form a convoluted ejaculatory duct or form an expanded internal seminal vesicle. Distally, the duct is modified into a muscular cirrus,

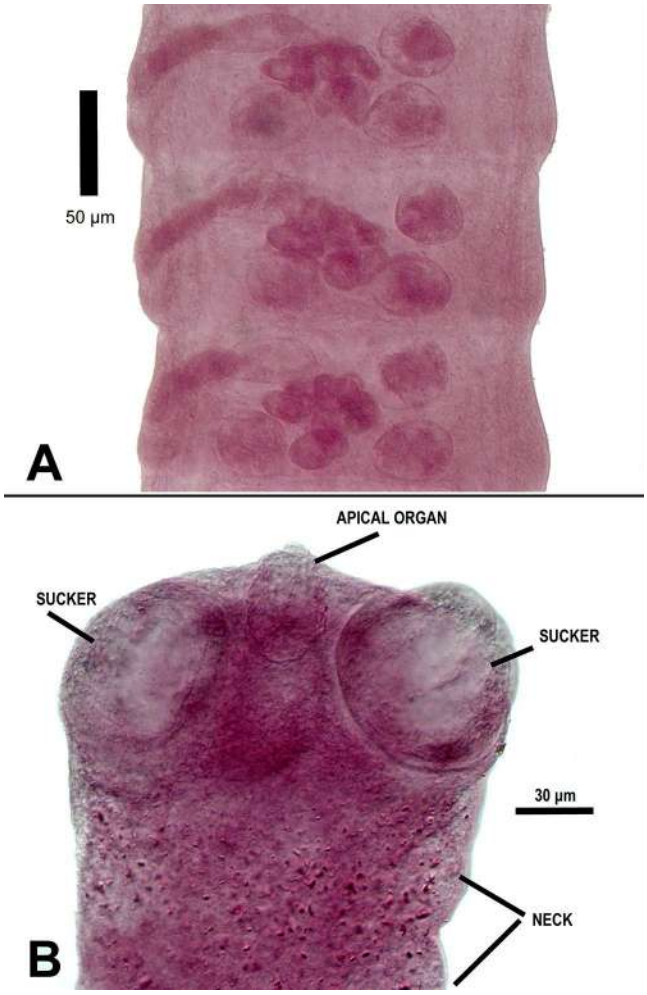

Figure 7. A) Example of a cyclophyllidean cestode in the family Hymenolepididae (*Hymenolepis tualatinensis* Gardner, 1985); B) scolex of the same specimen. Source: S. L. Gardner, HWML. License: CC BY.

the male copulatory organ. The cirrus may be spinous or not and may vary considerably in size, including length and diameter, among species. The cirrus can **invaginate** into the cirrus pouch and **evaginate** through the **cirrus pore**. Often, the male and female genital pores open into a common depressed chamber called the **genital atrium**. This atrium may be simple, or armed with a variety of spines, stylets, or hooks and may be glandular or possess accessory pockets. Also, depending on the species, the cirrus pore or the **atrial pore** may open on the margin or somewhere on a flat surface of the proglottid.

Female reproductive system.

The female reproductive system consists of a single **ovary** which may be large or small, compact or diffuse, and may be located almost anywhere within the proglottid, depending on the species. Associated with the ovary are **vitelline cells**, or **vitellaria**, which contribute to eggshell formation and nutrition for the developing embryo. These may be in a single compact vitellarium called the **vitelline gland** or scattered as follicles in various patterns. After an ovum matures in the ovary it leaves the ovary through a single **oviduct** that may have a controlling sphincter, the **ovicapt**. Fertilization of the ovum usually occurs in the proximal oviduct. Cells from the vitelline glands pass through a common vitelline duct, sometimes equipped with a small vitelline reservoir, and join with the fertilized ovum that is now called a **zygote**. Together they pass into a zone of the oviduct surrounded by unicellular glands called **Mehlis' glands**. The lumen of this zone is known as the **ootype**. The Mehlis' glands secrete a very thin membrane around the zygote and associated vitelline cells. Eggshell formation is then completed from within by the vitelline cells. Leaving the ootype, the developing egg passes into the **uterus** where embryonation is completed and a larval cestode comes into being.

The form of the uterus varies considerably among groups and may consist of a simple or convoluted tube, a reticular, lobated or simple sac, or may be replaced by other structures. In some groups the uterus disappears and the eggs, either singly or in groups, are enclosed within hyaline egg capsules imbedded within the parenchyma. In other groups one or more fibro-muscular structures, the **paruterine organs**, form within and attached to the uterus. In this case the eggs pass from the uterus into the paruterine organs, which assume the function of a uterus. The uterus then usually disintegrates.

Eggs (Figure 6) are released from the worm through a preformed uterine pore in many groups. In others, the proglottid splits or fragments, thus releasing the eggs. In many apolytic species, the gravid proglottids detach from the strobila and are passed from the host, where they crawl about on feces or soil scattering eggs as they go. In most anapolytic species the eggs are first discharged, then the **senile segments** break off and are released from the strobila, either singly or in chains.

The female genital pore, also called the vaginal pore, usually opens near the cirrus pore and often, but not always, this is in the **genital atrium** that is the termination of both the male and female reproductive tracts. The vagina may be armed distally with minute spines and may have 1 or more sphincters along its length. Near the proximal end, usually close to the ovary, there is usually a dilation called the seminal receptacle that stores sperm received in copulation. From the seminal receptacle a duct continues into the ootype.

There is a dichotomy in number of eggs produced among species, some of which have a reproductive potential that truly staggers the imagination. Within the family Taeniidae, individuals of most species of *Echinococcus* produce only a few hundred eggs per day versus individuals of most species of *Taenia* that can produce hundreds of thousands, up to millions, of eggs per day (Moore, 1981).

Acknowledgement

This section was modified from Schmidt (1986).

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17

Eucestoda

Introduction to Cyclophyllidea Beneden in Braun, 1900 (Order)

Scott L. Gardner

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Cyclophyllidea

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 17

Introduction to Cyclophyllidea Beneden in Braun, 1900 (Order)

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Introduction

Cestodes in the order Cyclophyllidea are the most-commonly encountered cestodes in amphibians, reptiles, birds, and mammals. Interestingly, they are mostly absent from fishes, with just a single species known from bony, or teleost, fishes, such as the elephant fish in Africa. In terms of diversity of species, the Cyclophyllidea is the largest order of all the cestodes with more species in this group than all other orders combined. As with most cestodes, almost all cyclophyllidean cestodes use an intermediate host as a necessary first step in their life cycle. In some species, the intermediate stages serve as an amplification stage in which a single egg of a cestode that is eaten by an intermediate host may proliferate into millions of potential larvae that will each grow into an adult cestode if the correct species of definitive host eats the infected intermediate host. This is common in the family Taeniidae. The characters of this group of animals are what most people relate to when they think of cestodes.

A character that serves to place a cestode firmly in the cyclophyllidean group is the presence of a **scolex**, or anterior holdfast, that usually has 4 simple, rounded suckers, arranged symmetrically, usually with 2 arranged dorsally and 2 arranged ventrally. There is usually a **rostellum** on the apical part of the scolex and, if the rostellum is present, it may or may not be supplied with **hooks**. The state of having hooks, in cestode parlance, is termed armed. There may be a neck, or not.

The strobila, or the repeating segments that make up the cestode, may be variable, but it usually has distinct **metamerism**, meaning repeating duplicated segments or **proglottids**. Most have segments or proglottids that are hermaphroditic, meaning that they have both male and female gonads in one segment. Some species may have a **strobila** that is all male and another separate strobila that is all female, this phenotype is called gonochoristic, but these species are relatively rare and occur in just a few species of shorebirds. The **genital pores** are usually found on the lateral surface of the segment, but in species of Mesocestoididae, the genital pore is ventral and centrally located in the segment.

The second main character that places a given species of cestode in the order Cyclophyllidea is the single, compact **vitelline gland** that is usually situated posterior to the ovary in the segment. Depending on the species, the uterus can be variable and can be a simple tube, a reticulated mass, or a paruterine organ. There is no uterine pore in individuals within the Cyclophyllidea.

List of Families

Mostly following Schmidt (1986), families of Cyclophyllidea include: **Mesocestoididae** Perrier 1897, **Dioecocestidae** Southwell 1930, **Progynotaeniidae** Fuhrmann 1936, **Taeniidae** Ludwig 1886, **Amabiliidae** Ransom 1909, **Acoleidae** Fuhrmann 1906, **Davaineidae** Fuhrmann 1907, **Hymenolepididae** Perrier 1897, **Catenotaeniidae** Spasskii 1950, **Dilepididae** Railliet et Henry 1909, **Anoplocephalidae** Cholodkovsky 1902, **Nematotaeniidae** Lühe 1910, **Dipylidiidae** Stiles 1896, **Paruterinidae** Fuhrmann 1907, and **Metadilepididae** Spasskii 1959. The most recent summary of the families of cestodes in the Cyclophyllidea by Mariaux and colleagues (2017) also includes the **Gryporhynchidae** Spasskii & Spasskaya, 1973.

Due to its potential for zoonotic infections, species in the family Taeniidae Ludwig 1886 are the most commonly studied and species from 2 genera from this family are discussed in some detail in the following sections, including both *Taenia* and *Echinococcus*.

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18

Eucestoda

Taenia (Genus)

Sumiya Ganzorig and Scott. L. Gardner

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Cyclophyllidea

Family Taeniidae

Genus *Taenia*

doi:10.32873/unl.dc.ciap018
2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 18

Taenia (Genus)

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Introduction

The genus *Taenia* Linnaeus, 1758 belongs to the family **Taeniidae** Ludwig 1886, in the order **Cyclophyllidea** van Beneden in Braun, 1900. The name **taenia** means **band** or **ribbon**, derived from Greek (Maggenti et al., 2017). Carolus Linnaeus established the genus *Taenia* in 1758, in the 10th edition of Systema Naturae to include the species that were known at that time as parasites of humans and dogs, namely *Taenia solium, T. vulgaris, T. lata*, and *T. canina*. Pork tapeworm *T. solium* is a nominal type species, *T. vulgaris* is now recognized as a synonym of the pork tapeworm (*T. solium*) and the remaining 2 species do not belong to the genus. It was shown later that *T. lata* is a synonym for the broad fish tapeworm *Diphyllobothrium latum* Linnaeus, 1757 (now called *Dibothriocephalus latus*) and *T. canina* is a synonym of the common dog tapeworm also called the flea tapeworm *Dipylidium caninum* (Linnaeus, 1758). Up to that time, the genus *Taenia* was one of the first helminth genera along with the species recognized along with species of *Fasciola* and *Ascaris*.

All species of *Taenia* require 2 mammalian hosts (definitive and intermediate) to complete the life cycle via a predator-prey relationship. Interestingly, except for the 3 human taeniid parasites (*T. solium*, *T. saginata*, and *T. asiatica*), all other *Taenia* species, in the adult stage, inhabit the alimentary tract of terrestrial carnivores and in the larval stage (also called the **metacestode** stage) they occur in various herbivorous mammals. Many species are of medical and veterinary importance, and besides the 3 *Taenia* that are found only in humans as definitive hosts, several other species may infect humans.

Highlights for *Taenia*

- **First cestode genus.** It is the first genus established for cestodes
- **Most studied.** It is one of the most studied genera, but its taxonomy, systematics, and species diversity still remain controversial and conflicting
- **Many species infect humans.** Almost one-fourth of *Taenia* species may infect humans, 3 of them are specific to humans and referred as human-*Taenia* that infect millions of people around the globe annually
- **Economically important.** Besides the zoonotic species, a number of species infect millions of livestock and other important animals worldwide resulting in enormous economic damage
- **Carnivore-herbivore life history.** Species of *Taenia* have a unique life cycle that requires 2 obligate mammalian hosts, an intermediate herbivore and a definitive predator host
- **Many reproduce asexually.** One-fourth of all the species may multiply asexually at the metacestode stage
- **Some species can hybridize.** Hybridization between closely related species may occur in areas where they are geographically sympatric, such as *T. saginata* and *T. asiatica*
- **Large tapeworms of humans.** The *Taenia* species are some of the largest of the tapeworms of humans and may reach a length of several meters
- **Long life span.** *Taenia* may live as long as their hosts
- **Cosmopolitan distribution.** *Taenia* species with anthropogenic associations are mostly cosmopolitan, although endemic species are known from each zoogeographic region.

Morphology of *Taenia* Species

The strobila or body is ribbon-like with many proglottids. The immature and mature proglottids are wider than they are long, with relative length increasing posteriad in the strobila. The rostellum usually has 2 rows of hooks of typical shape; the hooks of the anterior row are larger, alternating with those of the second row. The rostellum rarely has just 1 row of hooks, or hooks may be absent as in adults of *Taenia saginata*. There is a single set of reproductive organs in each proglottid. The genital pores alternate irregularly. The female genital organs are situated posteriorly in the segment. The ovary is bi-lobed and is situated at the median. The vitelline gland is simple, situated posterior to the ovary. The testes are abundant, mostly anterior and lateral to the female organs. The uterus arises as a median, longitudinal tube. The gravid uterus has lateral branches and is often secondarily branched.

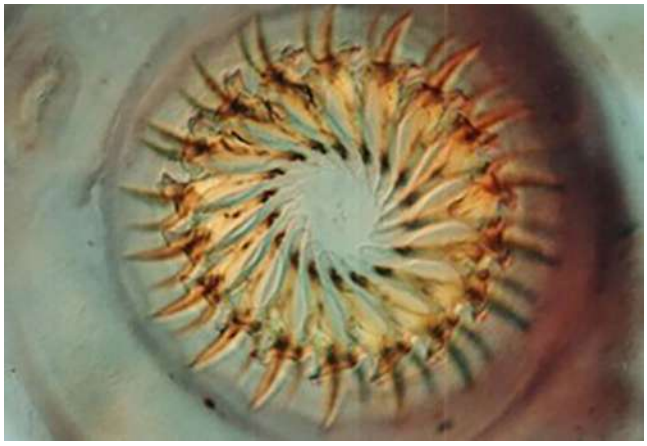

Figure 1. Rostellar hooks of *Taenia taeniaeformis*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

Figure 2. Rostellar hooks and suckers of *Taenia kotlani* from a snow leopard. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

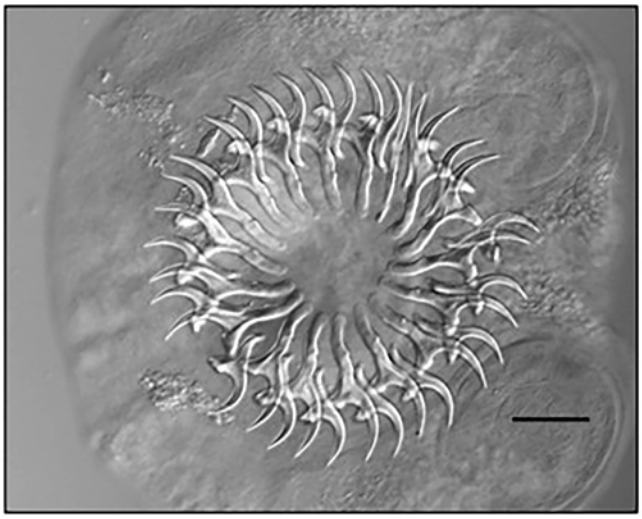

Figure 3. Rostellar hooks of *Taenia polyacantha*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

The eggs are spherical, each with a thick-walled embryophore, and composed of thick walls (this description comes from that provided by Rausch, 1994).

Asexual Reproduction of Metacestodes

The phenomenon of asexual multiplication in the larval stage is common in trematodes, but not in cyclophyllid cestodes as only fewer than 1% of all cestodes have proliferative or asexually reproducing larvae (Mackiewicz, 1988). However, a large number of *Taenia* species (about one-fourth) have been reported to be able to multiply asexually at the metacestode stage (*Taenia multiceps, T. serialis, T. endothoracicus, T. krepkogorski, T. parva, T. selousi, T. twitchelli, T. crassiceps*, *T. polyacantha*, and *Versteria mustelae*) (See Moore and Brooks, 1987). *Taenia retracta* also was found to multiply at the metacestode stage (Karpenko and Konyaev, 2012). Species of *Echinococcus* (another taeniid genus) also multiplies asexually at the larval stage, while only a few other cestodes are capable of producing asexually proliferative larvae, including 1 mesocestoidid, *Mesocestoides vogeae*; a dilepidid (family Dilepididae) *Paricterotaenia paradoxa*; and 3 species of hymenolepidids, *Staphylocystis pistillum*, *S. scalaris*, and *Pseudodiorchis prolifer* (Mackiewicz, 1988; Galan-Puchades et al., 2002).

Identifying *Taenia*

Species belonging to this genus have the largest body sizes of all the cestodes in the order Cyclophyllidea, their length is usually measured in tens of centimeters or even several meters. Cestodes belonging to this genus exhibit a set of unique morphological characters, including: Gross anatomy (strobila length and number of proglottids or segments); rostellum of the scolex with or without hooks (commonly called armed or unarmed) and those that do have hooks having 2 rows of characteristically shaped hooks (Figures 1–4); a single set of reproductive organs with a bi-lobed ovary, many testes, and a laterally branched gravid uterus filled with spherical eggs possessing thick and radially striated shells (Figures 5–9). Larval stages are mostly cysticercus-type with scolex invaginated within, or associated with, a bladder; or modification such as strobilocercus, armatetrathryridium, coenurus, pseudocoenurus, or polycephalic metacestodes (Figures 10 and 11). The **cysticercus** is the basic type of larval form for *Taenia* cestodes, characterized by a single

Figure 4. Rostellar hooks of *Taenia crassiceps*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

Figure 5. Young proglottids of *Taenia crassiceps*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

bladder with 1 scolex; a **strobilocercus** possesses an elongated segmented body, while an **armatetrathyridium** (**fimbriocercus**) has an unsegmented body. A coenurus type-larva has a bladder filled with fluid and an internal germinal layer that produces multiple scolices that bud off of this germinal layer. **Polycephalic type** larval forms are more rare and have several scolices arising from a central bladder, such as found in *T. endothoracica* (Kirschenblatt, 1948) (Figure 12).

Identification of *Taenia* spp. based only on morphological criteria is not easy due to the overlap of characters. So, other criteria such as biological (such as host or site of infection) and geographical (such as location or distribution) are used in combination. Hook morphology, size, and number are the most significant features for the identification of *Taenia*

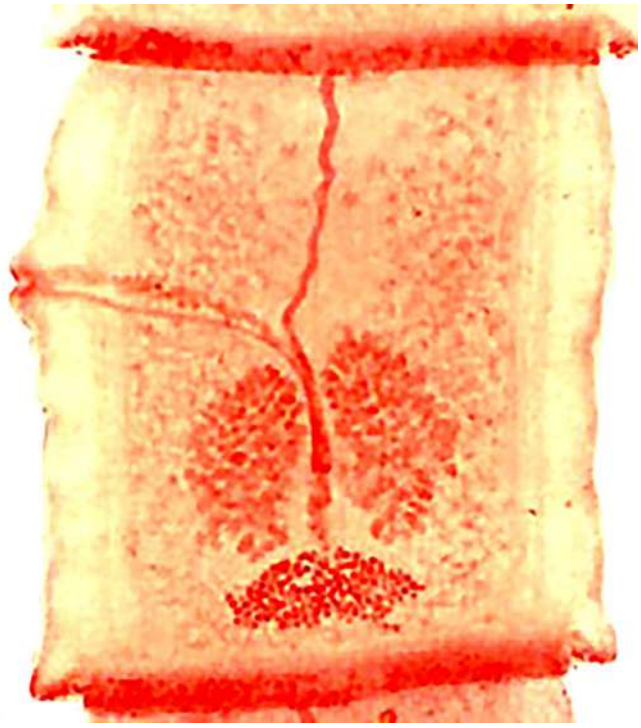

Figure 6. Mature proglottid of *Taenia crassiceps*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

spp. in both the adult and larval stages. This is especially important for the identification of larval stages, because the metacestode, in addition to the soft body tissues, such as the strobilocercus or hemistrobilocercus, possesses only a scolex armed with hooks. A study on hook morphometrics (Tufts et al., 2016) showed that hook shape and length were important characteristics for the identification of larvae of Taeniidae. Knowledge of the morphology of adult worms, including the characteristics of mature and gravid segments are needed for proper identification.

Loos-Frank (2000) provided characteristics for the 44 species and subspecies of the genus *Taenia*. Besides hook morphometrics, the most important characteristics were number and distribution of testes, cirrus sac or pouch position, and the presence of a vaginal sphincter. The dimensions of the cirrus pouch, number of uterine branches, and size of ovarian lobes were of lesser importance.

For study of these animals and to identify them using morphology, a freshly collected specimen must be relaxed in water, and then killed and fixed using appropriate methods followed by staining and mounting of the specimens on microscope slides in gum Damar. All these steps are crucial for correct identification. In some species, even the combination of various identification criteria does not enable an accurate identification. However, progress in

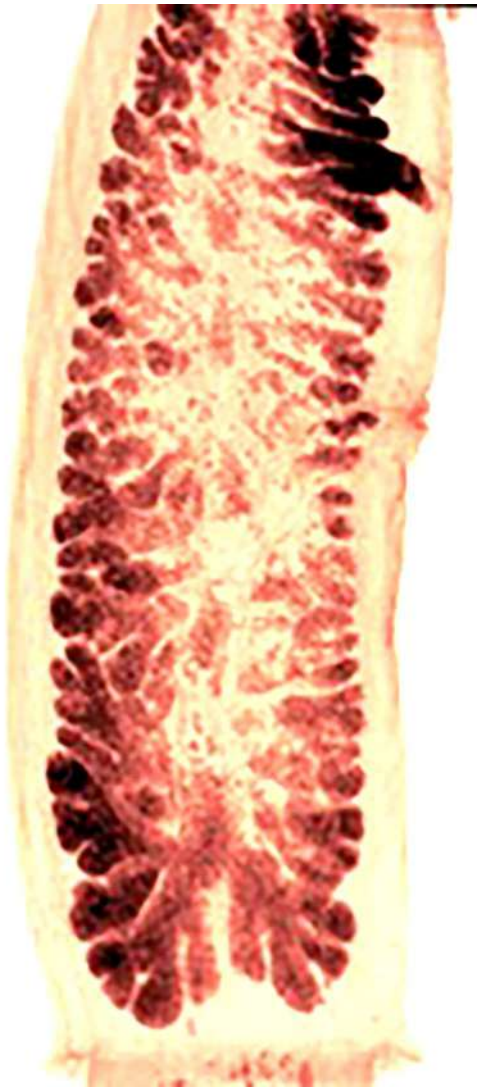

Figure 7. Gravid proglottid of *Taenia crassiceps*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

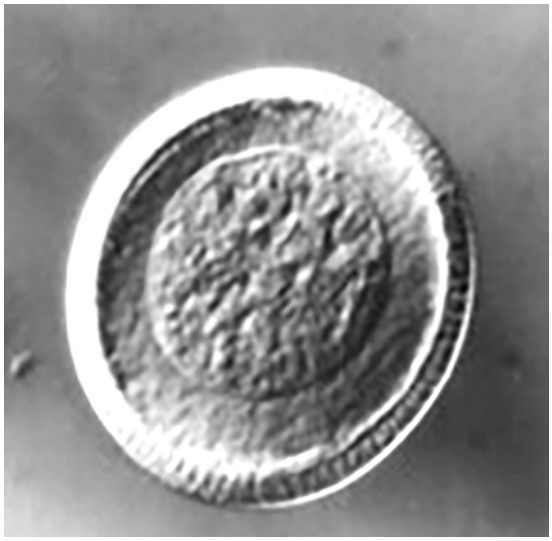

Figure 8. Egg of *Taenia kotlani*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

Figure 9. Egg of *Echinococcus multilocularis*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

molecular techniques, such as DNA sequencing of various genes has provided improved tools for the precise identification of taeniid cestodes.

Sequencing of the mitochondrial and nuclear genes has helped not only to accurately identify *Taenia* spp., but also to provide valuable genetic characterization which has supported and validated species and genera. Molecular markers for the precise identification of taeniid cestodes include partial fragments of mitochondrial *cox1*, *cytb*, *nad1*, and/or nuclear DNA sequences of 12S rDNA,18S rDNA, phosphoenolpyruvate carboxikinase (*pepck*), DNA polymerase delta (*pold*) and others. Relatively recently, complete mitochondrial genome sequences have been made available for all three human-*Taenia* species, and *T. crassiceps*, *T. hydatigena*, *T. multiceps*, and *T. pisiformis* (Jeon et al., 2007; Jia et al., 2010). Analysis of the complete mitochondrial genome revealed highly variable genes such as *nad6*, *nad5*, *atp6*, *nad3,* and *nad2* (Jia et al., 2010). Cryptic species within some closely related species were found, for example *T. polyacantha* and *Taenia=Hydatigera taeniaeformis* isolates. Lavikainen and colleagues (2008) reported essential nucleotide differences in 2 mitochondrial gene sequences in isolates belong to *T. polyacantha* which has a distribution across a huge geographic area extending from Europe to North America and suggested that these represented cryptic morphological species. In this case, the molecular data and the morphological data appear

Figure 10. Cysticercus of *Taenia hydatigena* with evaginated scolex. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

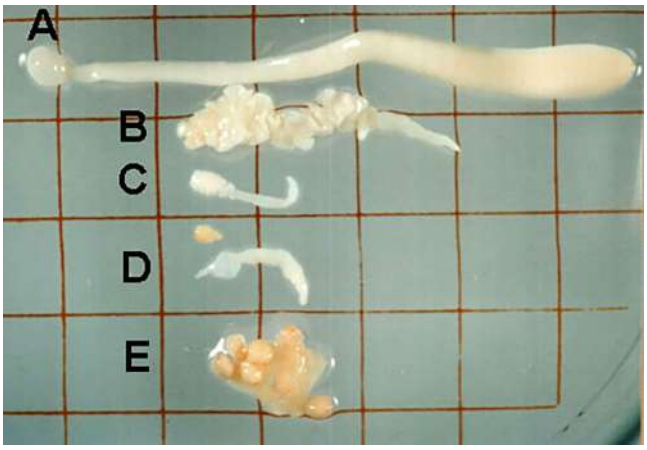

Figure 11. Different types of metacestode in *Taenia*. From top: A) Strobilocercus of *T. taeniaeformis*; B) armatetrathyridium of *T. martis*; C) *T. polyacantha*, tetrathyridium of *Mesocestoides* sp.; D) strobilocercus of *T. retracta*; E) polycephalic metacestode of *T. endothoracicus*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

to converge, as Rausch and Fay (1988) previously described 2 subspecies of *T. polyacantha* based on differences in the numbers and sizes of rostellar hooks; this could be evidence of post-glacial (Pleistocene Epoch) incipient speciation. Recently, Lavikainen and colleagues (2016) described *Hydatigera kamiyai* based on a Japanese isolate of *T. taeniaeformis* known to be restricted to both arvicoline rodents (voles) and mice belonging to the genus *Apodemus* as intermediate hosts.

Systematics and Phylogeny

As the oldest cestode genus to be described, and the first that had a Latin name ascribed to species in the genus, *Taenia* was used by taxonomists for many species not necessarily belonging to this genus. Because of the propensity of some

Figure 12. Multistrobilate larval form of *Taenia endothoracicus* from a wild gerbil collected and examined in western Mongolia. The adults occur in canids, most likely foxes. Source: S. L. Gardner, HWML. License: CC BY.

taxonomists to split species and assign other species to this genus, there were at one time more than 100 species recognized, but over time, with more accurate methods, the species number has steadily declined. Approximately half of them, or about 40 to 50 species, remained valid for a time, but this number is still decreasing. There are two primary reasons for this: The first being that some species were initially misidentified and are now excluded from the list; and the second reason is due to disagreement among researchers about the number of nominal genera of the subfamily Taeniinae. The species widely regarded as *Taenia* spp. have been placed in from 1 to 6 different genera: The Russian scientist Abuladze (1964) listed 64 species and placed them into 6 separate genera including: *Taenia* Linnaeus, 1758, *Hydatigera* Lamarck, 1816, *Tetratirotaenia* Abuladze, 1964, *Taeniarhynchus* Weinland, 1858, *Multiceps* Goeze, 1782, and *Fossor* Honess, 1937. Verster (1969) recognized only 1 genus (*Taenia*) and validated 32 of 70 species that were described as belonging to genus *Taenia* sensu stricto while Schmidt (1986) lists 88 species in the genus *Taenia* and partly followed Abuladze (1964) in recognizing 3 additional genera: *Insinuarotaenia* Spasskii, 1948, *Taeniarhynchus* Weinland, 1858, and *Monordotaenia* Little, 1967. At about the same time, a new genus named *Fimbriotaenia* had been created by Kornyshin and Sharpilo (1986). However, Rausch (1994), and Loos-Frank (2000) retained only the type genus in their works. Loos-Frank (2000) updated the previous revision made by Verster (1969) and included a list containing 44 species and subspecies belonging to *Taenia* sensu stricto. Here it is important to mention

that classifications produced by the researchers above, are based on morphology of adult cestodes with data included on metacestode stages.

More recent studies based on DNA barcoding, gene sequencing of nuclear and mitochondrial DNA (*COI*, NADH, and other genes), revealed that some old genera could be validated on the base of modern data. It was recently found (Nakao et al., 2013a; 2013b; Lavikainen et al., 2016) that analysis of both nuclear and mitochondrial DNA sequences strongly supports the validity of the genus *Hydatigera* Lamarck, 1816 which is not recognized by most researchers (Verster, 1969; Rausch, 1994; Hoberg et al., 2000; Loos-Frank, 2000). Also, Nakao and colleagues (2013a; 2013b) based on genetic data, proposed a new genus *Versteria* Nakao et al. (2013) for *Taenia mustelae* Gmelin, 1790, an eponym in honor of the late Anna Verster from South Africa.

Based on the above results, the up-to-date family Taeniidae now consists of 4 valid genera: *Taenia*, *Echinococcus*, *Versteria*, and *Hydatigera*. With the resurrection of the genus *Hydatigera* and establishing the new genus *Versteria*, 40 valid species remain in *Taenia* sensu stricto (Lavikainen, 2014). The species *T. mustelae* (Gmelin, 1790) and *T. brachyacantha* (Baer and Fain, 1951) are removed from *Taenia* and placed into the genus *Versteria*. Finally, the genus *Hydatigera* now includes *T. taeniaeformis*, *H. kamiyai*, *T. krepkogorski* Shulz and Landa, 1934, and *T. parva* (Baer, 1924).

The phylogeny of the genus *Taenia* and other taeniid cestodes has been studied by many researchers using both morphological and molecular data. In recent times, with increasing genetic material accumulated in GenBank and other sequence databases, in silico phylogenetic studies are increasing. Hoberg and colleagues (2000; 2005) provided thorough phylogenetic analyses of *Taenia* based on 27 morphological characters of valid species. This analysis did not support the idea of tribes (Taeniini, Fimbriotaeniini) and genera (*Hydatigera*, *Fimbriotaenia*, *Fossor*, *Monotdotaenia, Multiceps*, *Taeniarhynchus*, and *Tetratirotaenia*) created by previous researchers, and diagnosed monophyly for *Taenia* (Hoberg et al., 2000).

The phylogeny of *Taenia* based on partial sequences of mitochondrial *cox1* and *nad1* genes was studied by several researchers in the mid-1990s and beyond (Okamoto et al., 1995; De-Queiroz and Alkire, 1998). Those studies included a limited number of examined species (Lavikainen et al., 2008; Lavikainen, 2014). However, even these preliminary studies suggested important findings on origins of human *Taenia* species (De Queiroz and Alkire, 1998) and showed distinct placement of *T. mustelae* and *T. taeniaeformis* in the new phylogenetic trees (Okamoto et al., 1995; De Queiroz and Alkire, 1998). De Queiroz and Alkire (1998) suggested that *T. saginata* and *T. asiatica* are sister taxa and likely represent a single colonization of humans, and *T. solium* represents an independent colonization event. Recent studies based on longer mitochondrial DNA regions or complete genes, and nuclear DNA sequences, such as two protein-coding genes, phosphoenolpyruvate carboxykinase (*pepck*) and DNA polymerase delta (*pold*) were used to estimate the phylogeny of the Taeniidae (Lavikainen et al., 2008; 2010; 2016; Nakao et al., 2013a; 2013b). These studies show that *Taenia* is a highly diverse assemblage, and contrary to Hoberg and colleagues (2000), is paraphyletic, meaning that the classification puts some of the species that are actually in other genera together (Lavikainen et al., 2008). Several species, including *T. mustelae*, *T. taeniaeformis*, *T. krepkogorski*, and *T. parva* were found to be distantly related to other *Taenia* spp. and these results supported creation of the new genus *Versteria* and resurrection of the old genus name: *Hydatigera* (see Lavikainen, 2014).

Phylogenetic analysis using the mitochondrial *cox*1 gene partial nucleotide sequences from cestodes with different types and degrees of asexual multiplication during metacestode stages indicate that asexual development and multiplication among taeniid cestodes was independently derived and these characteristics have no value in higher taxonomy. However, taeniid cestodes with larvae that have a armatetrathyridia (*Taenia polyacantha*), strobilocercae (*T. taeniaeformis*), pseudocoenurae- or polycephalic-type (*T. endothoracicus*) metacestodes are branched distinctly from all other taeniids (Figure 13). So far, according to the newest taxonomy of Taeniidae the phenomenon of asexual multiplication is found in representatives from all 4 genera: *Taenia*, *Hydatigera*, *Versteria*, and *Echinococcus*.

Distribution and Hosts

Geographic and host distribution of species of *Taenia* sensu lato are highly variable. All the human *Taenia* and the species that are closely associated with livestock and domestic carnivores are well-known and are represented mostly by geographically cosmopolitan species (*T. solium*, *T. saginata*, *T. hydatigena*, *T. multiceps, T. ovis*, *T. pisiformis*, *T. serialis*, *T. solium*, and *T. taeniaeformis*). Distribution of the rest of the species in the genus is limited at various geographic scales. The large variety of both ungulates and carnivores in Africa supports the existence of at least 13 endemic species of *Taenia*, which makes Africa the area with the highest area of endemicity of species in the genus. In the Holarctic zoogeographic region more than 20 species have been reported, however, only 7 species (*T. arctos*, *T. crassiceps*, *T. intermedia*, *T. krabbei*, *T. laticollis*, *T. macrocystis*, and *T. polyacantha*) are fully distributed throughout the Holarctic

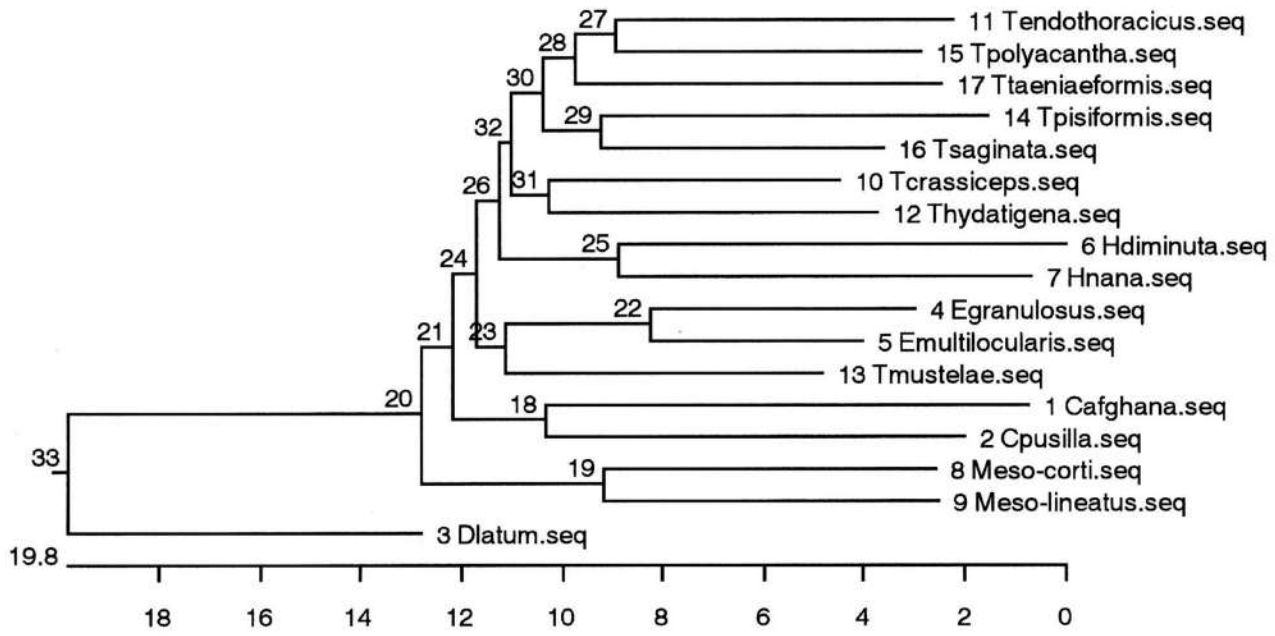

Figure 13. Phylogenetic tree of cyclophyllid cestodes constructed from neighbor joining (NJ) analysis of the mitochondrial *cox*1 gene partial nucleotide sequences. Source: S. Ganzorig. License: CC BY.

region. The distribution of about 5 to 8 species is limited to the Palearctic region (*T. endothoracicus*, *T. kotlani*, *T. martis*, *T. parenchumatosa*, and *T. retracta*) and the Nearctic region (*T. omissa*, *T. pencei*, *T. pseudolaticollis*, *T. rileyi*, and *T. taxidiensis*). The Australian region has no endemic species and those in the Oriental and Neotropical regions are poorly known but *T. talicei* is known from larval forms in rodents of the genus *Ctenomys* in Bolivia and the life cycle has been recently worked out (Rossin et al., 2010). The life cycle of *T. saigoni* found in *Macaca* spp. in Vietnam remains unknown (Loos-Frank, 2000). Specific identification of the bicephalic metacestode found in rats in Malaysia is also lacking (Kamiya et al., 1987). Many of the definitive hosts are endangered or rare and have been protected by local or international conventions. So far, collecting adult cestode specimens from hosts in the mammalian order Carnivora is now impossible or difficult in many areas.

Progress has been made to enable the study of alternative definitive host models for taeniid species. Included in these successes were alternate hosts for *Echinococcus multilocularis*, *Taenia crassiceps*, *T. hydatigena, T. pisiformis*, and a few other species (Kamiya and Sato, 1990; Sato et al., 1993; Toral-Bastida et al., 2011). As models, immunosuppressed laboratory rodents were used to obtain sexually mature cestodes from infection with metacestodes. The alternative host model might be helpful for the study of unknown metacestodes from various intermediate hosts, as well as specific determination of taeniid eggs.

The definitive hosts for *Taenia* cestodes represent 8 families of Carnivora (Abuladze, 1964; Loos-Frank, 2000). Of these, the canids and felids host the majority, or about 18 to 17 species, respectively. Other carnivores, such as mustellids and hyaenids are found to be hosts for up to 10 species. So far, by the greatest number of *Taenia* species parasitized, the carnivores could be placed in the following order: canids, felids, mustelids, hyaenids, ursids, viverrids, herpestids, and procyonids. Rodents, lagomorphs, and ruminants serve as the main intermediate hosts for *Taenia* spp. The small mammals (rodents, lagomorphs, and insectivores) and large mammals (various ruminants) are principal intermediate hosts for half equally of all *Taenia* species, respectively.

Human *Taenia* and Other Species of Medical Importance

As mentioned briefly above, human forms of *Taenia* include 3 species, *T. solium*, *T. saginata*, and *T. asiatica*, with humans serving as the sole known definitive host. Human *Taenia* is characterized by wide distribution (*T. solium* and *T. saginata* have a worldwide distribution), great size (up to 25 m), and great longevity with individual cestodes being known to live for the lifespan of the host, which can amount to decades in an individual. Humans become infected with *T. solium* and *T. asiatica* when they consume raw infected pork

or pig liver and with *T. saginata* when they eat raw infected beef. Due to the pathogenicity in humans, *T. solium* is called pork tapeworm and *T. saginata* is called beef tapeworm. Infection of humans with adult cestodes of these 3 species is called taeniasis. Pork tapeworm (*T. solium)* can cause cysticercosis in humans, also.

As a parasite of humans, *Taenia solium* has a cosmopolitan distribution and has been known about since antiquity. According to the World Health Organization (WHO, 2022), *T. solium* is a leading cause of foodborne-related deaths. The burden is heaviest in countries of Africa, Asia, Central America, and South America.

Taenia asiatica (Eom and Rim, 1993), also referred to colloquially as Asian *Taenia*, is the most recent human species of *Taenia* to have been described. For a long time, it was misdiagnosed as *T. saginata* due to the similarity in their morphological characteristics. It was first identified in Taiwanese Aboriginal people (Eom and Rim, 1993). Humans serve as the definitive host, and infection by this species causes taeniasis. Intermediate hosts include domestic pigs and wild boar, and *T. asiatica* has also been successfully transmitted experimentally to goats, cattle, monkeys, and mice. Humans infected by eating raw or undercooked meat containing larvae of *T. asiatica* suffer from invasive cysticercosis. Distribution of this species is restricted to warm temperate, subtropical, and tropical Asian countries, such as South Korea, Taiwan, Philippines, Thailand, Vietnam, Japan, southeast China, and Nepal (Ale et al., 2014). A survey in Laos (Sato et al., 2018) found *T. asiatica* hybridizing with *T. saginata*.

Morphologically the adult *Taenia asiatica* is very close to *T. saginata* but may be distinguished by the unarmed rostellum and a large number of uterine branches. Differences are also observed in the metacestode stage as it possesses a wartlike formation on the external surface of the bladder wall. The metacestodes' preferred location is liver and visceral organs, but not in the muscle. Furthermore, it differs by the nature of its intermediate host (pigs versus cattle) and cysticercus development which develops more rapidly in *T. asiatica*) (Eom and Rim, 1993). Nucleotide sequences of nuclear and mitochondrial genes are a reliable method to distinguish *T. asiatica* from *T. saginata*, *T. solium*, and hybrids. The hybridization of *T. asiatica* and *T. saginata* for the first time was reported by Okamoto et al. (2010) in specimens from Thailand, where all 3 human *Taenia* species are sympatric. Later, hybridization was also found in Laos (Sato et al., 2018).

Within the Asia-Pacific region, where all 3 human *Taenia* species occur, it is important to discriminate among these species. A loop-mediated isothermal amplification method (LAMP) for a differential identification of *Taenia* tapeworms from humans was applied by Nkouwa and colleagues (2012). The results suggested a reliable and easy method for identification of all 3 species in the sympatric area, even in field conditions. A LAMP is a single tube technique for the amplification of DNA and does not require a thermal cycler or other expensive equipment.

Other *Taenia* Species that Can Harm Humans

The metacestode stages of 8 *Taenia* species are known to infect humans, namely, *T. crassiceps, T. ovis, T. taeniaeformis, T. hydatigena*, and *T. martis* cause cysticercosis in people*;* while infection by eggs of *T. multiceps, T. serialis*, and *T. brauni* may cause coenurosis. Infection with strobilocercae of f *T. taeniaeformis*, a parasite of wild and domestic felids, has afflicted humans in several countries including Argentina, Denmark, Taiwan, and others. Parasite of canids, *T. crassiceps*, *T. ovis*, *T. hydatigena*, *T. multiceps*, *T. serialis*, and *T. brauni* can infect humans when eggs are accidentally ingested, and these develop into metacestode stages, individually called a cysticercus or coenurus (Miyazaki, 1998). In these cases, the human is acting as an intermediate host (albeit a dead end one), so the location of metacestodes is exactly the same as those found in natural intermediate hosts.

Taenia martis has been found to infect humans, causing cysticercosis in the eye and brain (Brunet et al., 2015). This species is a specific parasite of carnivores belonging to the family of Mustelidae and rodents are the usual intermediate hosts. Transmission to humans probably occurs by the same route as that method that infects the intermediate hosts which is via the oral route with food or water contaminated with *T. martis* eggs.

The majority of the zoonotic *Taenia* species (6 from 8 reported) parasitize various canids as adults, including pet dogs. Domestic pets and wild animals (specifically, carnivores) may cause risk of infection by this cestode to humans. It is important to mention that the larval stages of *T. multiceps*, *T. serialis*, *T. brauni*, and *T. martis* may affect the central nervous system and eye in humans, resulting in significant damage to health, similar to the deleterious effects of *T. solium*.

***Taenia* Species of Veterinary Importance**

About half of the known *Taenia* species are of veterinary importance. All the human *Taenia* species at the metacestode stage also cause cysticercosis in livestock and some wild ungulates. *Taenia saginata* encysts in striated muscles of cattle, *T. solium* infects muscles and other organs of pigs, and *T. asiatica* infects the visceral organs of pigs and wild boar. Carcasses or internal organs of livestock infected with the cysticercae of these cestodes need to be destroyed, which causes great economic loss. Other widely distributed species

that cause cysticercosis in livestock and wild ungulates are *T. hydatigena* (which encysts in visceral organs) and *T. ovis* (which infects the skeletal muscles and heart of sheep). Coenurosis caused by *T. multiceps* is a serious disease of the central nervous system of livestock and wild ungulates.

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19

Eucestoda

Echinoccocus (Genus)

Akira Ito and Scott. L. Gardner

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Cyclophyllidea

Family Taeniidae

Genus *Echinococcus*

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Chapter 19

Echinococcus (Genus)

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Introduction

Species of *Echinococcus* have captured human interest from antiquity. The hydatid, which is the metacestode stage of *E. granulosus*, has been known since the time of Hippocrates (~ 460–377 BCE) (Eckert and Thompson, 2017) and Pallas (1776) first recognized the metacestode cyst as the living larval stage of taeniids (and this was confirmed by Goeze in 1782).

In this section, the taxonomy as well as the life cycles and pathogenicity of *Echinococcus* spp. are the focus since this dynamic parasite infects livestock and wild animals, as well as humans at times.

Beyond this basic introduction, 2 issues of Advances in Parasitology (Thompson et al., 2017a; 2017b) focusing on *Echinococcus* and echinococcosis are among the best resources to consult for understanding the species and their pathogenicty.

Morphology

Adult species of tapeworms in the genus *Echinococcus* are characterized by some clear synapomorphies, including: 1) Small bodies consisting of only a **scolex** and 3 (or at the most 4) **proglottids**; 2) characteristically shaped **hooks** on the **rostellum** (Figures 1 and 2); 3) mature proglottids with many **testes** arranged medially, generally not crossing the lateral **excretory ducts**; and 4) testes extending anteriad of the **cirrus sac** almost to the edge of the segment and posteriad, distal to the **vitelline gland** (Rausch, 1993; Rausch and Bernstein, 1972; Gardner et al., 1988; 2013).

The whole animal reaches a maximum length of less than about 7 mm, and usually shorter, depending on the species.

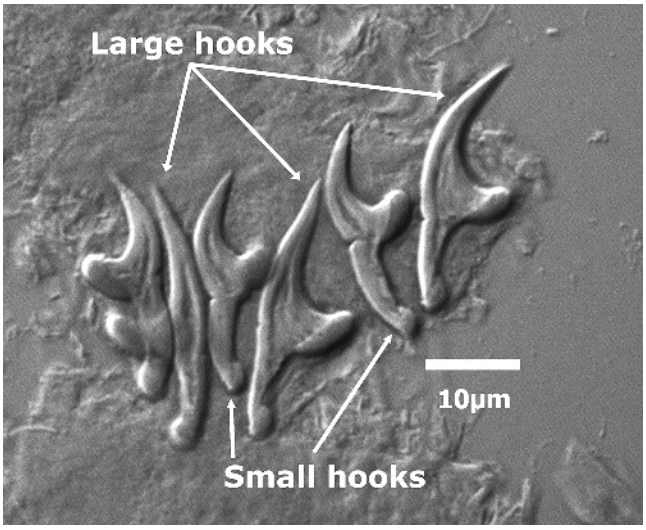

Figure 1. Hooks of *Echinococcus multilocularis* collected from the area near Har Us Lake, Hovd, Mongolia. Specimen number NK223782. Taeniids typically have 2 rows of hooks on the rostellum with 1 row consisting of smaller hooks and another row with larger hooks. Source: S. L. Gardner, HWML. License: CC BY.

Figure 2. Two hooks (large and small) from a protoscolex of *Echinococcus multilocularis* from near Taos, New Mexico, United States from a deer mouse *Peromyscus maniculatus*. The specimen was cleared in lactophenol on a microscope slide under a number 1 coverslip. Using a small amount of pressure with a pencil eraser on the coverslip, the protoscolex was squashed gently enabling the hooks to be separated from the protoscolex for viewing and imaging. Images were made with Normarsky optics using a Zeiss Axiophot TM microscope. Source: A. T. Dursahinhan and S. L. Gardner, HWML. License: CC BY.

As with most cyclophyllidean cestodes, the anterior end (as noted) includes a scolex with 4 muscular **suckers**, lacking hooks or spines on the suckers, and a rostellum on the apical

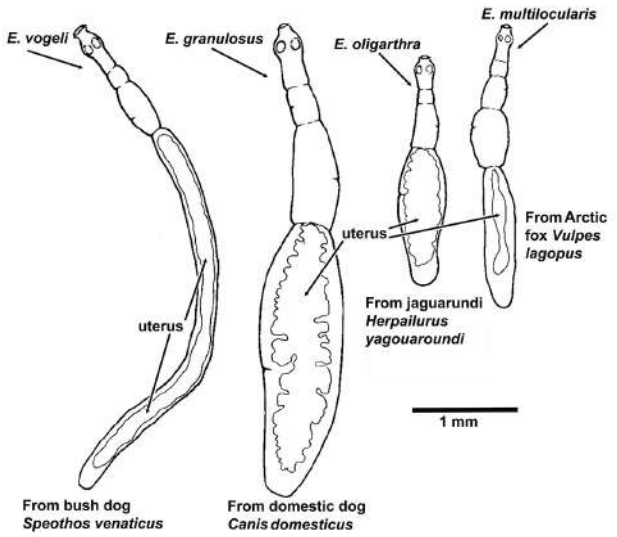

Figure 3. Comparisons among *Echinococcus vogeli*, *E. granulosus*, *E. oligarthra*, and *E. multilocularis*. Note the longer, thinner gravid proglottid in *E. vogeli* compared to the other species in the figure. The eggs are not shown in the uteri in this figure. Source: S. L. Gardner, HWML. License: CC BY.

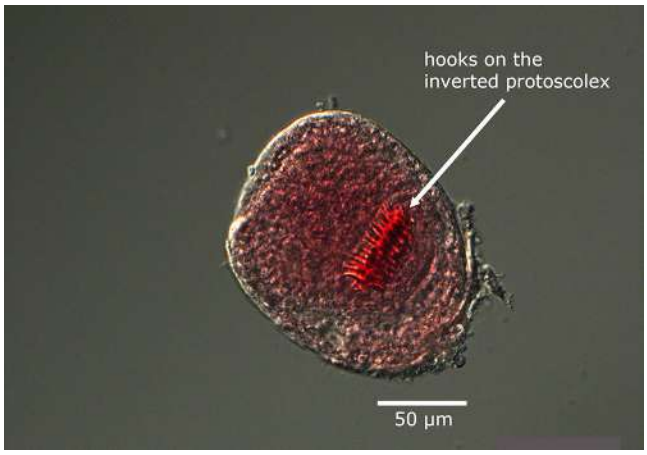

Figure 4. Protoscolex from the cyst of *Echinococcus multilocularis* obtained from a deer mouse *Peromyscus maniculatus* collected from near Taos, New Mexico, United States. The specimen was stained using Semichon's acetic carmine (which is the usual method). Source: A. T. Dursahinhan and S. L. Gardner, HWML. License: CC BY.

end of the **scolex** that is supplied with 2 rows of hooks that alternate surrounding the rostellum and are of characteristic shapes and sizes, depending on the species (see Figure 3) (Rausch, 1993; Rausch and Bernstein, 1972; Gardner et al., 1988; 2013).

Each adult cestode in its carnivore host is derived from a single **protoscolex** (Figure 4) that is produced by asexual budding in a hydatid cyst of its intermediate host. Each fully developed protoscolex can transform into an adult tapeworm in the small intestine of the carnivore that consumes it while feeding on the infected intermediate host. The adult

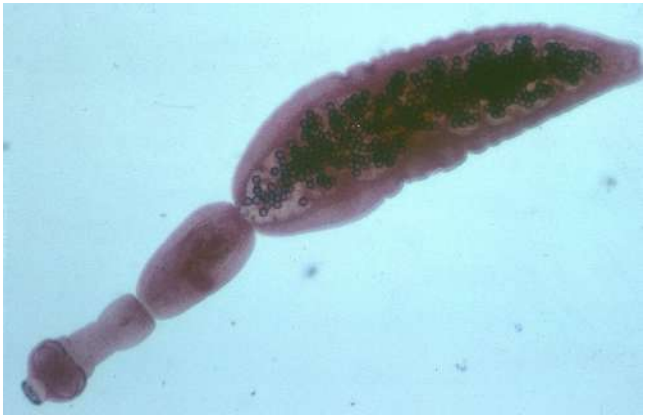

Figure 5. Adult *Echinococcus granulosus* from the intestine of a dog. Eggs can be seen in the last gravid segment. Source: S. L. Gardner, HWML. License: CC BY.

Figure 6. Posterior gravid segment of *Echinococcus multilocularis* from an experimental infection in a dog in Alaska, United States. Source: R. L. Rausch. License: CC BY.

Table 1. *Echinococcus* taxonomy.

Species	Genotypes and strains, genotype denoted as G1–G10
Echinococcus granulosus Batsch 1796	G1, G2, G3, sheep/buffalo strains
E. equinus Williams and Sweatman 1963	G4, horse strain
E. ortleppi Lopez-Neyra and Soler Planas, 1943	G5, cattle strain
E. canadensis Webster and Cameron, 1961	G6, G7, camel and pig strains; G8, American cervid strain; G10, Nordic cervid strain
E. felidis Ortlepp, 1937	Lion species, warthog intermediate hosts
E. multilocularis Leuckart, 1863	Canid final hosts, rodent intermediate hosts
E. shiquicus Xiao et al., 2005	Canid final hosts, lagomorph intermediate hosts
E. oligarthra Diesing, 1863	Felid final hosts, hystricognath/echimyid intermediate hosts
E. vogeli Rausch and Bernstein, 1972	Canid final hosts, hystricognath intermediate hosts

tapeworms then live in the intestine of carnivores and embed the anterior end (scolex) deep in the base of the **villi** (also called the **crypt of Lieberkühn**) in the mucosal layer of the host's duodenum. When observed after cutting the host's intestine open longitudinally, severe infections (numbering in the hundreds of thousands of cestodes) make the intestine appear to be covered with felt. Each worm can produce a few hundred **eggs** per day and, along with the thousands of other adults in an infected dog, together can produce hundreds of thousands of eggs each day (see Figures 5 and 6) (Rausch, 1993; Rausch and Bernstein, 1972; Gardner et al., 1988; 2013).

Taxonomy: From Morphological to Molecular

Echinococcus is one of the major groups in the family Taeniidae (Knapp et al., 2011; Nakao et al., 2010a; 2013a; 2013b; Romig et al., 2015; Thompson and McManus, 2002) (see Figure 7). In the past, 4 morphospecies had generally been accepted as valid taxa, namely, *E. granulosus*, *E. multilocularis*, *E. vogeli*, and *E. oligarthrus* (see Rausch and Bernstein, 1972). *Echinococcus granulosus* is the most common species and it is distributed worldwide. Early systematists, such as Robert L. Rausch (1921–2012) and others, did not have reliable tools for differentiation of *E. granulosus* so they proposed several intraspecies variations or strains rather than name distinct species (Rausch, 1967; 1995; 2003; Moro and Schantz, 2009); these are referred to as G1–G10 (although the G9 genotype is unresolved; see Table 1) (McManus, 2013; Thompson and McManus, 2002; Rostami et al., 2015). Studies using molecular approaches have revealed that the broad umbrella of *E. granulosus* is properly differentiated into at least 5 independent species, namely: *E. granulosus* sensu stricto (s. s.; dog and sheep species, G1, G2, G3), *E. equinus*

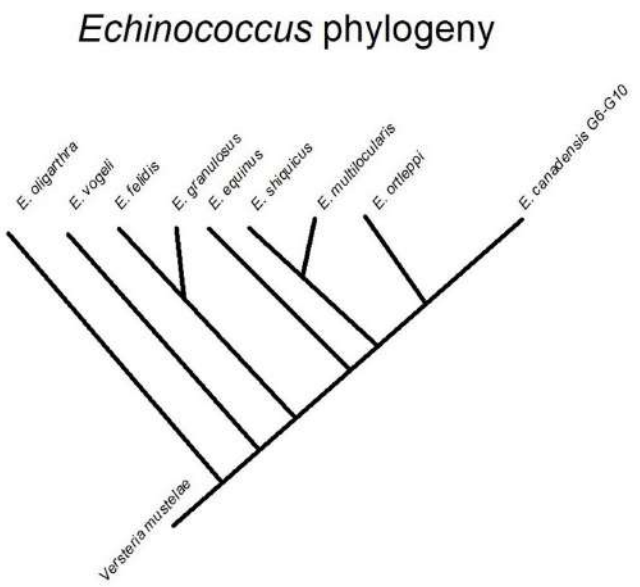

Figure 7. *Echinococcus* phylogeny. Estimated evolutionary relationships among all known species of *Echinococcus*. *Echinococcus canadensis* genotypes G6–G10 are shown as a single branch/species in this tree. The tree was based on a maximum likelihood analysis of mitochondrial genomes and nuclear protein-coding genes. Source: Adapted from Nakao et al., 2013b. License: CC BY-NC-SA 4.0.

(horse species, G4), *E. ortleppi* (cattle species, G5), *E. canadensis* (G6, G7, G8, G10), and *E. felidis* (see Hüttner et al., 2008; Nakao et al., 2007; 2010b; 2013b).

Subsequent to and including the pioneering work on *Echinoccocus granulosus* using mitochondrial DNA analyses by Bowles and colleagues (1992; 1995), there have been many molecular studies published on *Echinococcus* (see Bretagne et al., 1996). Among them, Nakao and colleagues (2007)

reconstructed the phylogenetic relationships of *E. oligarthra* (= *E. oligarthrus*, see the change recommended by Nakao et al., 2013b), *E. vogeli*, *E. multilocularis*, *E. shiquicus*, *E. equinus*, *E. ortleppi*, *E. granulosus* sensu stricto (G1), and 3 genotypes of *E. granulosus* sensu lato (s. l.; G6, G7, G8) inferred from complete mitochondrial genomes. Nakao and colleagues (2007) suggested that:

1) The 3 *E. granulosus* genotypes corresponding to the camel, pig, and cervid strains are monophyletic and their high level of genetic similarity supports taxonomic species unification of these genotypes into *E. canadensis*;

2) Sister species relationships are confirmed between *E. ortleppi* and *E. canadensis*, and between *E. multilocularis* and *E. shiquicus*;

3) The basal positions on the phylogenetic tree are occupied by the Neotropical endemic species *E. oligarthra* and *E. vogeli* whose definitive hosts are derived from carnivores that migrated from North America around the time of the formation of the Panamanian land bridge;

4) Host-parasite biogeographic comparisons suggest that the ancestors of *E. oligarthra* and *E. vogeli* originated in South America and at the same time there was a speciation event that gave rise to all other species of *Echinococcus*. An alternate explanation is that the ancestors of *Echinococcus* originated in North America or Asia depending on whether the ancestral definitive hosts were canids or felids (Nakao, 2013b).

Echinococcus shiquicus is a species from the Tibetan plateau, China (Xiao et al., 2005; 2006) that was discovered in part thanks to conversations that took place during a small international meeting on echinococcosis and cysticercosis organized by Akira Ito held in Chengdu, China in July 2000 (Ito et al., 2003a; 2003b). One of the coauthors of a study presented there, J. M. Qiu, the head of Echinococcosis Research at the Sichuan Center for Disease Control and Prevention at that time, mentioned a unique species of *Echinococcus* during his conference session. Qiu thought *E. shiquicus* might just be an aberrant form of *E. multilocularis* since the adult stage looks like a stunted *E. multilocularis*, but the larval stage of *E. shiquicus* appears to be unicystic, whereas the larval stage of *E. multilocularis* is multilocular or alveolar.

The year 2000 was important for the study of echinococcosis since several groups working independently in China from around 1990 finally met at the conference in Chengdu (Ito et al., 2003a; 2003b) and in another, bigger meeting in Poznań, Poland in September that same year (Craig and Pawłowski, 2002). The United States National Institutes of Health (US NIH) R01 Project on Parasitic Zoonosis (echinococcosis) transmission in China (principal investigator: P. S. Craig) also commenced in October 2000 and continued for 8 years. Ning Xiao also conducted a molecular analysis of *Echinococcus shiquicus* under the direction of Minoru Nakao and published the work as his PhD thesis (Xiao et al., 2005; 2006).

Other studies on the taxonomy of *Echinococcus* include a reevaluation by molecular approaches using fresh eggs from lion feces in Uganda to support revision of *E. felidis*, which was initially described in 1934 from African lions (Ortlepp, 1934) but later included as a subspecies or strain of *E. granulosus*. Adult worms in a lion intestine fixed in formalin were also reevaluated later by Anna Verster in South Africa (Hüttner et al., 2008; 2009; Hüttner and Romig, 2009). From this work, *E. felidis* and *E. granulosus* sensu stricto (G1) are now considered to be sister species (Nakao et al., 2013b).

Further molecular studies on *Echinococcus canadensis* revealed that *E. canadensis* (G6/G7) and *E. canadensis* (G8/G10) are sister species but are still different species (Nakao et al., 2013c; Laurimäe et al., 2018). It was also confirmed in later studies that the G1 and G3 strains of *E. granulosus* s. s. differ from each other (Kinkar et al., 2018b).

Based on molecular analyses of *Echinococcus granulosus* sensu stricto, the genetic bottleneck effect was discovered when samples from the Middle East, China, and Peru were studied (Casulli et al., 2012; Moro et al., 2009; Nakao et al., 2013b; Yanagida et al., 2012). It was initially suggested that *E. granulosus* s. s. (G1) emerged in western Asia and expanded anthropogenically worldwide. However, further studies in Africa strongly suggest that the origin might be in Africa (Wasserman et al., 2016; Ito and Budke, 2017; Ito et al., 2017).

Molecular analyses of *Echinococcus* specimens offer great numbers of new findings. For example, see Álvarez Rojas et al. (2014), Hüttner and Romig (2009), Ito et al. (2017), and Romig et al. (2015) for good, updated reviews on *E. granulosus* sensu lato.

Life Cycle: Complicated through Global Transport of Livestock, Wild Animals, and Humans

The *Echinococcus* life cycle is completed through predator (carnivore) and prey (omnivore and herbivore) interactions; meaning that an infected intermediate host is eaten by a definitive host where the larvae mature into adults in the small intestine. The definitive hosts for *Echinococcus* are carnivores, either canids or felids (Figure 8). As there are many updated reviews on this topic (Nakao et al., 2013b; Romig et al., 2015), new findings may be discovered about the host animals of *E. shiquicus* and *E. felidis*, as well as other aspects of the life cycle involving all other affected species.

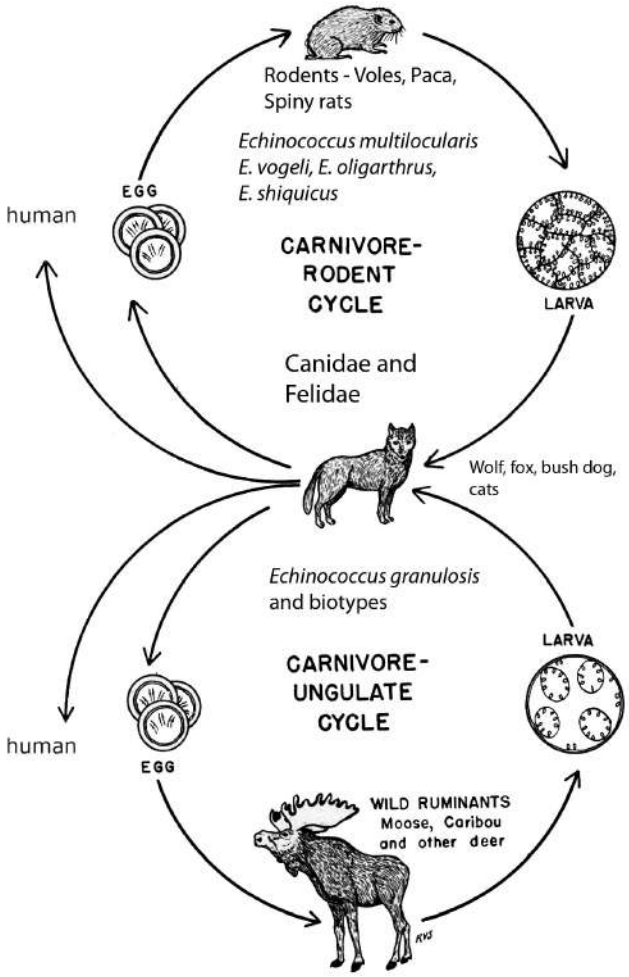

Figure 8. The general life cycle or life history of species of the genus *Echinococcus* showing the carnivore-rodent cycle and showing the carnivore-ungulate cycle. All *Echinococcus* species are not listed here. Source: S. L. Gardner, HWML. License: CC BY.

Both *Echinoccocus shiquicus* and *E. multilocularis* are co-endemic in their areas of overlap in the Qinghai Tibet plateau, China.

Echinococcus shiquicus

The main intermediate hosts for *Echinococcus shiquicus* are rodents (and not the plateau pika *Ochotona cruzoniae*) (Ma et al., 2012; Wang et al., 2018) and the definitive hosts are the red fox (Jiang et al., 2012) and domesticated dogs (Boufana et al., 2013). There are no known human cases of *Echinococcus shiquicus*, although this may be because the human population in the endemic area is relatively small and because the local foxes generally keep away from people. But it is possible that *Echinococcus shiquicus* may be able to infect humans since it is the sister species of *E. multilocularis*.

Echinococcus multilocularis (Figure 9)

Of all known species of cestodes, and among the Taeniidae and *Echinococcus* in particular, *E. multilocularis* is the most serious for human health since it causes alveolar echinococcosis (AE), also called alveolar hydatid disease. AE occurs in humans when the egg of *E. multiolocularis* is ingested and the larvae lodge in various organs, but usually the liver, and grow over time. The growth of the cysts in both humans and rodent intermediate hosts is via **exogenous budding** (growth of the cyst from the surface of the original cyst). This growth is slow in humans and very rapid in rodents. For the adults, the main definitive host is the red fox *Vulpes vulpes*, but all other carnivores may be suitable definitive hosts and almost all species of wild carnivores, both canids and felids, and domesticated dogs and cats, are presumed to be suitable definitive hosts. This hypothesis has been tested and demonstrated in many laboratory studies where the parasite life cycle has been maintained.

A similar broad intermediate host-range has been shown through experimental infections for these cestodes in the case of intermediate hosts, which are mainly rodents, particularly *Microtus* voles, but many other herbivores may also serve as intermediate hosts. *Ochotona* (see Li et al., 2018; Wang et al., 2018) and *Lepus* (see Xiao et al., 2004) species live in the definitive hosts' territories and, so, are expected to be suitable intermediate hosts. It is possible that *Echinoccocus multilocularis* has been shown to be widely distributed in all countries in Eurasia other than the tropical areas. Even if there are no data, with no records showing positive infections of animals in a geographic area, it does not mean that the areas are free of this parasite, but rather that there is simply a lack of surveillance (Botero-Cañola et al., 2019; Gardner et

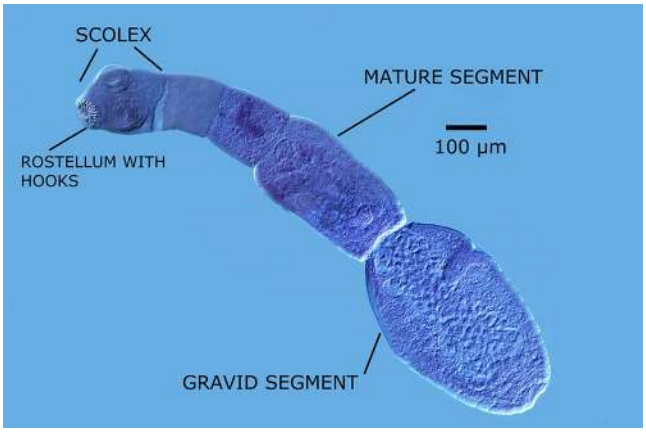

Figure 9. Mature specimen of *Echinococcus multilocularis* from Alaska, United States. Note that the posterior segment that would be full of eggs is missing in this specimen. Source: R. L. Rausch. License: CC BY.

al., 2013; Bagrade et al., 2016; Beck et al., 2018; Lass et al., 2016; Massolo et al., 2014; Umhang et al., 2015).

Human living-environments are often invaded by wildlife (Gottstein et al., 2015; Liccioli et al., 2015; Mackenstedt et al., 2015; Robartdet et al., 2011). There are countless examples of the borderless world with wildlife and domesticated animals and humans in urbanized cities in Europe (Switzerland, Germany, France, Italy, and others), Japan (Ito et al., 2003a; 2003b), and Canada. The best method for avoiding accidental alveolar echinococcosis (AE) in city life is to keep wildlife far from cities. Vaccination of foxes or domesticated dogs, or deworming with praziquantel, will not be successful since *Echinococcus multilocularis* is a wildlife parasite! As this parasite is very pathogenic and without treatment kills humans at a rate of about 97%, surveillance for the presence of the cestode in geographic regions should be completed with the direct evidence of the parasite itself, not simply with molecular evidence (Morishima et al., 2006; 2016). Without any direct evidence of adult worms from dogs, all laboratory work might be in error due to the contamination of the tools used to collect specimens or analyze data in a laboratory. What this means is that sequencing of environmental DNA is not an appropriate method to identify and diagnose this species (because of potential DNA contamination from other sources in the laboratory, in the process of collection, or in the process of transportation and sample preparation). Therefore, direct sequencing of a single egg, or recovered strobilae, using several genes should be employed to avoid introducing accidental artifacts. Another global concern is the migration of *E. multilocularis* through anthropogenically mediated transfer of foxes, and perhaps also rodents, from Europe to North America (Nakao et al., 2013b).

The extent of the genetic diversity inherent in *Echinococcus multilocularis* was first reported by Bretagne and colleagues (1996). They described 3 different geographic genotypes, named: North American, Asian, and European. Tang et al. (2004; 2006) reported *E. multilocularis*-like species with some different biological characteristics and expected it to be an independent species with a previous name, *E. sibiricensis*. This was later proved to be an intraspecies variant and called the Inner Mongolian genotype (Nakao et al., 2009; 2010b). Later, Ito and colleagues (2010; 2013) and Gardner and colleagues (2013) confirmed that this genotype is widely distributed in Mongolia and even in Russia (Konyaev et al., 2013) and have called it the Mongolian genotype (instead of the Inner Mongolian genotype).

The North American genotype is well known to be distributed widely in wildlife (Rausch, 1995; Rausch and Schiller, 1951; Schantz et al., 1995; Storandt and Kazacos, 1993; Storandt et al., 2002), but has been known to occur only very rarely in humans (Yamasaki et al., 2008). However, there have been quite a few cases of AE confirmed in Canada (Catalano et al., 2012; Gesy and Jenkins, 2015; Gesy et al., 2013; 2014; Jenkins et al., 2012; Santa et al., 2018a; 2018b; Shurer et al., 2018). Molecular analysis has revealed that all these AE cases and parasites from wildlife, including wild voles, do not have the North American genotype, but instead have the European genotype. So, it may be concluded that European *Echinococcus multilocularis* appeared only recently in North America (Nakao et al., 2013b). Recent ecological niche-modeling work by Botero-Cañola and colleagues (2019) has shown a possible expansion of the range of *E. multilocularis* in North America. However, the previous purported absence of this parasite in New Mexico (United States) may have been due to nobody having looked for it before, rather than representing an actual geographic range expansion (Botero-Cañola et al., 2019).

Echinococcus felidis

Although the intermediate hosts for *Echinococcus felidis* have been presumed to be several herbivore species living in African lion territory, based on molecular data, only hippopotamuses, warthogs, and pet dogs have been included definitively (Halajian et al., 2017; Mulinge et al., 2018). Several other carnivores, including leopards, lions, and hyenas, are presumed to be additional definitive hosts. These new findings indicate that the environment for wildlife has been complicated by increased pet dog ownership. Although humans are aberrant hosts for the *Echinococcus* life cycle, Macpherson (1983), in work done in Africa, revealed humans as a suitable intermediate host. Macpherson's work leads to the question about whether humans may be involved in the life cycle of *E. granulosus* s. s. or *E. felidis*, in addition to wildlife.

Echinococcus granulosus sensu lato (s. l.)

As described above, human cystic echinococcosis (CE) cases are mainly caused by *Echinococcus granulosus* s. s. (G1), with its cosmopolitan distribution (88.44%), followed by *E. canadensis* (11.07%) and *E. ortleppi* (Álvarez Rojas et al., 2014; Romig et al., 2015; Ito and Budke, 2017; Ito et al., 2017). Recent studies on mitochondrial genes of *E. granulosus* s. s. (G1) or *E. granulosus* s. l. worldwide more strongly reveal a dynamic genetic polymorphism (Álvarez Rojas et al., 2013; 2017; Carmena and Cardona, 2013; 2014; Hassan et al., 2017; Kinker et al., 2018b; Laurimäe et al., 2016).

Echinococcus vogeli and *E. oligarthra*

Recent molecular studies contributed data from these two species in the Americas. Since *Echinococcus granulosus* s.

s. was introduced into the Americas long ago, *E. vogeli* and *E. oligarthra* may be co-distributed with *E. granulosus* in the Americas (Ávila et al., 2017; das Neves et al., 2017).

Therefore, through the acceleration of globalization in the 21st century, the distribution of *Echinococcus* spp. has a much more complicated and chaotic trajectory in the 20th century. More studies from molecular approaches are essential to clarify the origin and spread of the parasite on a global scale (Kinkar et al., 2018a).

Implications of Dual Infections in Intermediate and Definitive Hosts

Recent studies on *Taenia solium* (Yanagida et al., 2014) and *T. asiatica* (Okamoto et al., 2010; Yamane et al., 2013) and those reviewed by Ito and colleagues (2016), have revealed that outcrossing may occur when infections with multiple tapeworms takes place in the definitive host, which is humans. The most recent data in China, where 3 human types of taeniasis occur, show that taeniasis may be caused by *T. solium*, *T. asiatica*, and *T. saginata*. These species are highly co-endemic, indicating that all *T. asiatica* and *T. saginata* are hybrids and dual infection with these 2 species, or even a triple infection with 3 including *T. solium*, is not rare (Li et al., 2018). These molecular studies on inter-species or intra-species hybridization in other species of Taeniidae strongly suggest that intra- and inter-species genetic diversity of *Echinococcus* spp. is a possibility. Indeed, there are several reports revealing that 2 species are confirmed from the same definitive and intermediate host animals. Although there is a report that individuals of *E. multilocularis* and *E. granulosus* may occupy a different part of the small intestine (Thompson and Eckert, 1983), that may not always be true.

How can coinfection with *Echinococcus granulosus* s. s. and *E. canadensis* be tested? These 2 species may occupy the same part of the small intestine, and dual infection may cause outcrossing and hybridizations. As the definitive host slowly acquires immunity to reinfection, meaning that the new infections from separate incidents of carnivory of intermediate hosts can occur for at least several weeks after the first establishment of the cestodes in a canid (Kouguchi et al., 2016), it is easy to imagine dual infections with adult *Echinococcus*, especially with different species of predators when they have different chances for catching infected intermediate host prey. If the immunity to the intestinal tapeworm is species-specific, dual infection with different species is easily established. However, there have been very few reports showing 2 or 3 different-aged tapeworm infections among any cestode infections except those caused by *Vampyrolepis nana* (synonymous with *Hymenolepis nana*). Even in *V. nana*, dual infection happens only when the definitive host gets a primary infection with cysticercoids which follows autoinfection by a large number of second-generation tapeworms (Ito, 2015; 2016). As far as is known, there is no answer explaining why there are no reports of multiple different-aged tapeworm infections even though premunition versus the crowding effect has often been implicated. More experimental infection studies are necessary to understand this issue in tapeworms and especially in the Taeniidae.

An easier explanation is that the definitive hosts get a dual infection from the intermediate host which is coinfected with different species. Then, how do the intermediate hosts get coinfected with different species? As reinfection immunity in the intermediate mammalian host has been shown to be very rapid, usually only 1 population with 1 chance of infection can be established. It is the basic background for production of vaccines against echinococcosis and cysticercosis in livestock (Ito and Smyth, 1987; Lightowlers, 1996; 2006; Lightowlers et al., 1996; 2003). If oncospheres of different species cause reinfection immunity that are species-specific, eggs of different species may cause infection in the same individuals (Álvarez Rojas et al., 2013; Gauci et al., 2018; Oudini-M'rad et al., 2016). Ecological competition for strobilization in the definitive host's intestine between the established tapeworms and newcomers may be one reason the newcomers cannot be established other than affecting intestinal immunity. *Echinococcus* species may be much easier to establish if they occupy different parts of the same host's intestine. If dual infection in the same part of the intestine happens, it may be much easier to speculate that dual infection happens through only a single incidence of eating intermediate hosts which are coinfected with different species. Cross-fertilization in 1 population (Lymbery et al., 1989) or mixed populations may happen. Hybrids in *Echinococcus* spp. in wildlife may be more common than two genotypes of *Taenia solium* (Yanagida et al., 2014) crossed with *T. asiatica*, and crossed with *T. saginata* in humans (Okamoto et al., 2010; Yamane et al., 2013).

Host Range

The predator-prey interaction is the essential factor maintaining the *Echinococcus* life cycle. So, herbivores and omnivores are the intermediate hosts, and carnivores are the definitive hosts. However, cannibalism not only in carnivores but also in omnivores or even in herbivores is not rare but rather common, especially in a stressful environment. There are no data on what happens with echinococcosis due to cannibalism.

There are reports indicating that carnivores including foxes and dogs may be coinfected with 2 different stages. One manifestation is metacestodes in the liver and simultaneously adult *Echinococcus multilocularis* in the intestine in red fox

(Ishino, 1941), metacestodes in dogs (Antolová et al., 2018; Losson and Coignoul, 1997; Meyer et al., 2013; Skelding et al., 2014), and metacestodes of *E. granulosus* s. s. in cats (Armúa-Fernández et al., 2014; Burgu et al., 2004; Konyaev et al., 2012). When eggs of *Echinococcus* or other helminths are ingested by the suitable intermediate hosts, oncospheres hatch and invade the intestinal tissue and migrate to the suitable organs and tissues to differentiate into the metacestode stage, a hydatid. However, there are not sufficient data on the fate of eggs ingested by non-suitable mammalian hosts, including definitive hosts. There is evidence that metacestodes cannot develop into adults in the intermediate host, but carnivores as the definitive hosts for *Echinococcus* spp. may become the intermediate hosts, as well. The mechanism remains unresolved. See the section on *Taenia* for additional information on alternative rodent definitive hosts.

Pathology in Echinococcosis

The larval stage of *Echinococcus* spp. is implicated in human pathologies that may be differentiated into 2 main types: Cystic and cerebral. Cycstic echinococcosis (CE) involves endogenous budding, versus exogenous budding, which occurs in cases of alveolar echinococcosis (AE). In CE, approximately 70% of the cysts are established in the liver, whereas, in AE, over 97% are established in the liver. Differences in tropism, or preference for establishment and growth in various organs in humans by different species of these cestodes, is not clearly established for the various species, perhaps due to confusion relative to identification of the species that cause echinococcosis (Nguyen and Duyet, 2017; Ito et al., 2017). CE is relatively rare, but most recent molecular studies on human cerebral CE cases in children show that they are caused mainly by *E. canadensis* (Shirmen et al., 2018). In contrast, since almost all AE cases are established in the liver, AE cases in the brain, lung, or any other organ are thought to derive from a metastasis of hepatic AE. However, there is no evidence that only 1 oncosphere invades the host tissue. Rather, after multiple eggs are ingested, it may be common that multiple oncospheres simultaneously hatch and invade the intestinal tissue, penetrating and traveling to the liver via the hepatic-portal system where they may lodge and begin to grow via exogenous proliferation (Rausch, 1954; Rausch and Schiller, 1954; Rausch and Jentoft, 1957; Aoki et al., 2015). When advanced hepatic AE cases are confirmed, the big hepatic lesion may be not from a single lesion, but instead from multiple primary lesions fused together. It is possible that non-hepatic AE cases may be caused by both metastasis of the original infection as well as primary infection with the oncosphere larvae disseminating to any area of the body after passing through the liver to the heart.

Although mice show a difference in fertile and sterile AE cysts (Nakaya et al., 1997) and species of *Peromyscus* appear to manifest a larval form in the liver substantially different in structure from those that develop in arvicoline voles (Rausch and Richards, 1971), such host differences need additional investigation, such as those conducted using newer techniques by Islam and colleagues (2018).

Echinococcus granulosus s. l., *E. felidis*, and *E. oligarthra* develop into typical cystic lesions, whereas *E. multilocularis* develops into an alveolar lesion, and often mutlifocal lesions which are likely to have been established by metastasis (see Figure 1.6 in Thompson, 1986). However, multi-organ AE cases may be due to a primary multi-organ infection with multiple oncospheral invasions. *Echinococcus shiquicus* and *E. vogeli* are polycystic and are intermediate in pathogenicity between the CE and AE forms.

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Eucestoda

Proteocephalidae La Rue, 1911 (Family)

Tomáš Scholz and Roman Kuchta

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Onchoproteocephalidea

Family Proteocephalidae

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Chapter 20

Proteocephalidae La Rue, 1911 (Family)

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Introduction

The cestode order Onchoproteocephalidea (1 of the 19 currently recognized orders; see Caira and Jensen, 2017) does not contain human parasites and only very few species are able to be pathogenic in cultured hosts (Williams and Jones, 1994). This order is composed of 2 previously separate orders, Proteocephalidea Mola, 1928, from freshwater and terrestrial hosts, and part of the order Tetraphyllidea Carus, 1863, parasites of marine elasmobranchs (see below). The number of species is not extraordinarily high; de Chambrier and colleagues (2017b) recognize as valid 316 species of Proteocephalidae, whereas Caira and colleagues (2017) list 246 species from elasmobranchs, including 188 species of *Acanthobothrium* Blanchard, 1848 (family Onchobothriidae Braun, 1900).

Members of the order Onchoproteocephalidea have an unusually wide host spectrum (also known as, great host range), which includes elasmobranchs, teleost fishes, amphibians, reptiles, and a mammal. The taxonomic history of these cestodes serves as an excellent example of how opinions of researchers about taxonomic relevance/importance and homology of morphological traits have had to be changed based on the methodological tools used and the available knowledge of evolutionary history of the group in question.

Taxonomic History

The current order Onchoproteocephalidea was established by Caira and colleagues (2014) and includes the former order Proteocephalidea and some taxa of the family Onchobothriidae, which previously formed part of the Tetraphyllidea (see Caira and Jensen, 2017). The focus here is only on the former order Proteocephalidea represented by members of a single family, Proteocephalidae La Rue, 1911, whereas marine taxa that mature in elasmobranchs have been treated in detail by Caira and colleagues (2017). The new order was established only on the basis of molecular data, without any clear morphological or other synapomorphies that would characterize this group (Arredondo et al., 2014).

The first described proteocephalidean was *Taenia percae* Müller, 1780 from a European perch *Perca fluviatilis*, but a number of species were described at the end of the 18th century and in the 19th century, almost exclusively from Europe, with a few taxa described from North America. Because of the presence of 4 spherical suckers resembling those of taeniids infecting humans and mammals, these cestodes were frequently called *Ichthyotaenia* Lönnberg, 1894 (= fish cestode or fish *Taenia*). However, Weinland's (1858) name *Proteocephalus* has taxonomic priority.

The North American scientist George Roger La Rue described several new species, mainly from European and North American freshwater teleosts, and made the first taxonomic revision of the group (La Rue, 1914). The current classification at the subfamily and family level is based on the concept of the British scientist W. N. F. Woodland who published a series of papers on Neotropical fish proteocephalideans and focused on the position of the testes, uterus, and vitelline follicles in relation to the inner longitudinal musculature (Freze, 1965; Rego, 1994). However, the hypothesis of arrangement of species in these groups as families and subfamilies defined as outlined by Freze and Rego is rejected by analysis of newer molecular data that shows that these groups are not derived from a common ancestor (not monophyletic) (de Chambrier et al., 2017b).

Current Classification

Molecular phylogenetic analyses focused on interrelationships of the orders of cestodes (Waeschenbach et al., 2007; 2012; Caira et al., 2014) demonstrated close relationships of some tetraphyllideans with hooks on their scolex and are included in the family Onchobothriidae with proteocephalideans. Based on this close relatedness, Caira and colleagues (2014) proposed the order Onchoproteocephalidea. The Proteocephalidae as now recognized (= former order Proteocephalidea; see Rego, 1994) is pending a new, more natural classification. All 7 subfamilies for which more than a single genus was included in the analyses by de Chambrier and colleagues (2015) were recovered as non-monophyletic. This confirms that a full revision of the subfamilial classification of the group is needed.

Morphology

Proteocephalidean cestodes are polyzoic as are the more common Cyclophyllidea. Their scolex has 4 spherical or elongate suckers, also called acetabulae, which are used to attach the animal to the intestine by sucking onto the intestinal mucosal surface. Some species have 4 single suckers and other species may have doubled or tripled (bi- and triloculate) suckers. The most anterior (apical) part of the scolex may have a structure that resembles a rostellum (as in many cyclophyllideans) and species in the subfamily Gangesiinae have hooks on the rostellar organ (as in many of the cyclophyllideans).

The testes are situated laterally and anterior-posteriorly in each proglottid with the vitelline follicles forming 2 bands lateral to the fields of numerous testes. The uterus which holds the eggs that are produced by the ovary and are fertilized in the ootype, forms lateral diverticulae. In these cestodes, 3 main types of uterus formation have been recognized and have described by de Chambrier and colleagues (2015). Eggs that fill the uterus are usually spherical, with an external hyaline envelope. This envelope increases in size when released into water, causing the eggs to float. Eggs also consist of a 2- or 3-layered spherical embryophore and a spherical hexacanth, which is a larval cestode called an oncosphere containing 3 pairs of embryonic hooks. Some taxa may have eggs of a different shape or the eggs may form capsules. Eggs are released through the uterine pores on the ventral side of the proglottids.

Proglottids or segments are well separated from each other, each containing 2 pairs of excretory canals. In addition, each proglottid is hermaphrodytic containing a bi-lobed ovary which is usually situated near the posterior margin of the proglottid. Both male and female copulatory structures open together into a genital atrium which is always situated on the lateral margin of the segment. As is usual for cestodes, the male intromittent organs consist of the cirrus sac containing a muscular cirrus that can extend from the genital pore into the vaginal canal of another proglottid. The female parts consist of the vaginal canal which opens into the genital atrium and is sometimes surrounded by a vaginal sphincter. As noted, the terminal parts of the male and female genital apparatus open together in a genital atrium on the lateral margin of the proglottids.

Only very few new morphological characters that may be of some taxonomic value or suitable for the assessment of the evolutionary history of the group have been recently defined such as type of development of the uterus (de Chambrier et al., 2004; 2015). Another character, which may help in reconstruction of the evolutionary history of proteocephalideans and their host associations, is the relative size of the ovary (that is, the ratio of the ovarian size in relation to that of the entire proglottid; see de Chambrier et al., 2012). The ovary of species of *Ophiotaenia* parasitic in snakes in the Americas, Africa, Asia, and Australia was found to be considerably smaller than that of congeneric species in Palaearctic reptiles, but also in all species of *Proteocephalus* that are parasitic in teleost fishes throughout the world (de Chambrier et al., 2012). De Chambrier and collegues (2005; 2012; 2015) relatively recently defined morphological characters that are of significant value in species identification as well as being useful for understanding the phylogenetic history of these cestodes. One of these characters is the relative size of the ovary in these and some other related tapeworms (see Figure 1).

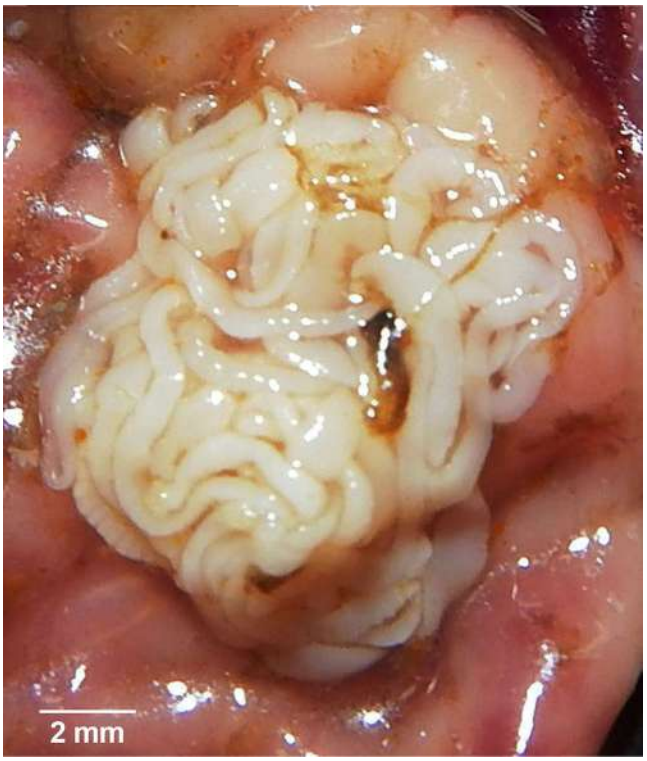

Figure 1. *Proteocephalus perplexus* in the intestine of *Amia calva*, United States. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

Species Diversity

De Chambrier and colleagues (2017b) provided the most recent survey of the whole order, with the complete list of all species recognized as valid (a total of 316 species of 68 genera) with their type hosts and country of origin. However, this number of species is most likely lower than the actual species diversity of the group as indicated by continuous descriptions of new taxa (for examples see de Chambrier et al., 2017a; Scholz et al., 2017). New taxa will undoubtedly

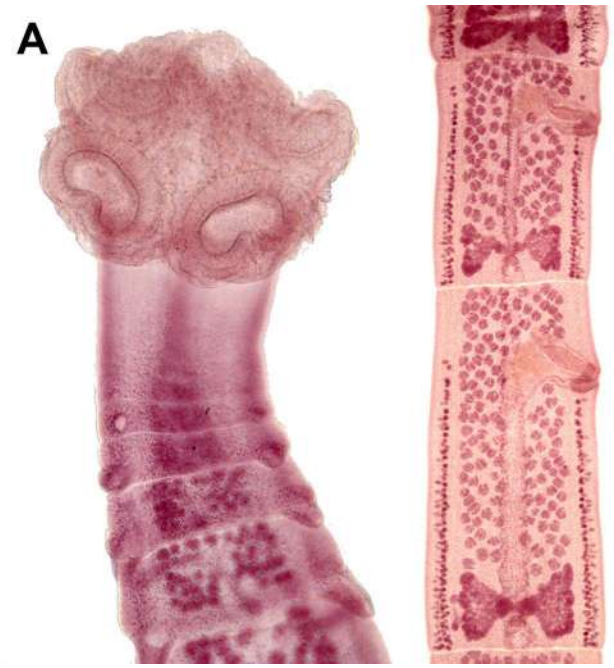

Figure 2. Adults from pimelodid catfishes in Peru (scolex and anterior proglottids of *Pseudocrepidobothrium eirasi* from *Phractocephalus hemioliopterus* and mature proglottids of *Proteocephalus sophiae* from *Paulicea luetkeni*. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

Figure 3. Adults of *Thaumasioscolex didelphidis* from *Didelphis marsupialis*, Mexico. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

be discovered in the near future, especially from Neotropical fishes and reptilian hosts in insufficiently studied regions such as South America and Australia (de Chambrier et al., 2017a; 2018).

Life Cycles

Overall, little attention has been paid to studies of the life cycles of proteocephalidean cestodes (Freze, 1965). Most species from fishes in the temperate zones (Palaearctic and Nearctic regions) for which data on their development are available (see Scholz, 1999 for a review) use only 1 intermediate host—planktonic copepods—in which a larva (metacestode), called a plerocercoid, develops to become infective for the definitive host (Chervy, 2002). Life cycles of species of *Ophiotaenia* from reptiles and frogs as well as that of the bass tapeworm, *Proteocephalus ambloplitis* (Leidy, 1887), include 2 intermediate hosts (Fischer and Freeman, 1969; 1973). Very little is known about the transmission of species maturing in terrestrial hosts (Freze, 1965), including the only species parasitizing mammalian hosts, *Thaumasioscolex didelphidis* Cañeda-Guzmán et al., 2001. Participation of second intermediate or paratenic hosts that live at least temporarily in water seems to be a plausible explanation of transmission of taxa with terrestrial hosts.

Host Associations

Proteocephalideans are intestinal parasites primarily infecting freshwater teleost fishes (194 of 316 species, that is, almost two-thirds), with catfishes (order Siluriformes) representing the most important host group (133 species, that is, 69% of species in fishes). Among the catfishes, pimelodids living in the Neotropical region are definitive hosts for 34% of protocephalideans (Scholz and Kuchta, 2017) (Figure 2). However, proteocephalideans occur in a wide spectrum of teleost fishes, as many as 47 families of phylogenetically distant orders such as Polypteriformes and Osteglossiformes on one side versus Perciformes and Centrarchiformes on the other (Scholz and Kuchta, 2017). Some proteocephalideans occur in amphibians (frogs and salamanders) and reptiles (monitors, lizards, and snakes). One species, *Thaumasioscolex didelphidis*, is a parasite of a mammal (an opossum) in Mexico (de Chambrier et al., 2017b; 2018; see Figure 3).

Scholz and Kuchta (2017) indicate that these cestodes have varied host range with some species of Proteocephalidae occurring in many species of fish and others more restricted. The limits of host-range are probably a combination of both ecological and phylogenetic constraints (Brooks and McLennan, 2002). Intensity of infection varies considerably between individual hosts infected, but it is generally low in all host groups. An extreme case of a heavy parasite load was reported by Ruedi and de Chambrier (2012) who found as many

as 12,228 cestodes representing 7 species in a redtail catfish *Phractocephalus hemioliopterus* from the Amazon River in Brazil (see Figure 2).

Geographical Distribution

Proteocephalidean cestodes have a worldwide distribution, but they are absent in marine ecosystems. Most taxa occur in freshwater habitats of temperate and tropical latitudes. A number of species parasitize terrestrial tetrapods in all zoogeographical regions but 1 (Antarctica), and only very few species live in brackish waters. Scholz and Kuchta (2017) analyzed the distribution of fish proteocephalideans and found that by far the highest number of species occurs in the Neotropical region. Proteocephalideans are also common in the Palaearctic and Nearctic regions. Unlike fish proteocephalideans, those parasitizing reptiles are quite common also in tropical Asia and Australia (de Chambrier et al., 2017b; 2018). In amphibians, most proteocephalideans have been recorded in the Nearctic and Neotropical regions. Species parasitizing reptiles are widely distributed throughout the globe, with the highest number in the Neotropical region, followed by the Indo-Malayan and Ethiopian regions (de Chambrier et al., 2017b).

Figure 4. Two adults and 1 small larvae of *Proteocephalus ambloplitis* from *Micropterus salmoides*, United States. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

Phylogenetic Relationships

Proteocephalidean cestodes hold the privilege as serving as one of the first helminth groups for which a phylogenetic analysis was applied (see Brooks 1978; 1995). Molecular data demonstrate that the previous classification of subfamilies is artificial and does not correspond to the evolutionary history of the group. Species-rich genera such as *Nomimoscolex, Ophiotaenia,* and *Proteocephalus* are not monophyletic and include assemblages of unrelated taxa with similar morphology (de Chambrier et al. 2017b). The most basal proteocephalideans are those of the non-monophyletic family Acanthotaeniinae, which includes parasites of reptiles throughout the world, and the non-monophyletic family Gangesiinae, comprising species parasitizing catfishes (order Siluriformes) in Asia (de Chambrier et al., 2015). Neotropical taxa from fishes do not form a monophyletic clade and their phylogenetic relationships are largely unresolved (de Chambrier et al., 2015; 2017b).

Selected Nearctic Taxa

A total of 49 species of proteocephalidean cestodes have been reported from the Nearctic region, that is, North America and the Neotropical part of Mexico (de Chambrier et al., 2017b). Among them, the following species are selected to document diversity, host associations, life cycles, and phylogenetic affinities in this group of cestodes in North America.

1) The bass tapeworm (*Proteocephalus ambloplitis*) is the only fish proteocephalidean cestode with a 3-host life cycle (Fischer and Freeman, 1973; see Figure 4). This relatively large cestode (total length up to 41 cm) is typified by the presence of 4 deep lobes on the scolex, a large glandular apical organ, a large, thick-walled cirrus sac, and an elongate, thick vaginal sphincter. It has been reported as a pathogen of fishes of the family Centrarchidae, with plerocercoids penetrating into the body cavity and different internal organs, including the gonads, thus causing mortality in heavily infected fish (William and Jones, 1994). This species is more closely related to species from Neotropical teleosts and Holarctic snakes (*Ophiotaenia* spp.) than to congeneric species from fishes in the Nearctic region, such as *P. plecoglossi* from bass or *P. pinguis* from pike (de Chambrier et al., 2017b).

2) *Megathylacoides giganteum* (subfamily Corallobothriidae) is a typical and fairly common parasite of channel catfish which has a large-sized scolex with a metascolex (folds of tissue encircling or hiding the suckers) and the opening of the suckers surrounded by a strong muscular sphincter (Essex, 1928). The life cycle is known to include only 1 intermediate host, a planktonic copepod. Even though this and related species of the genera *Essexiella* (Figure 5) and *Corallotaenia* were placed in the subfamily Corallobothriinae, this placement is erroneous, having been based mainly on a similar shape of the scolex, which is evidently a result of convergent

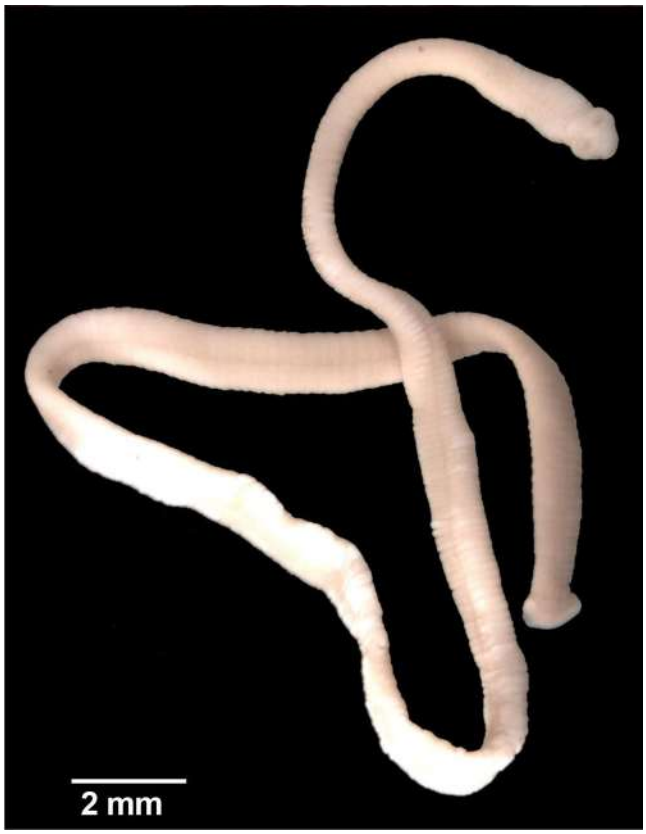

Figure 5. Adult of *Essexiella fimbriatum* from *Ictalurus punctatus*, United States. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

evolution, not close relatedness (Scholz et al., 2011). Therefore, a new subfamily should be proposed to accommodate North American proteocephalideans with a metascolex that parasitize channel catfishes.

3) *Ophiotaenia perspicua* is the type species of the most species-rich genus of the family (La Rue, 1911). This cestode has been reported from several species and genera of water snakes (Colubridae) in North America. Specimens from these hosts differ from each other in their morphology and most likely represent separate species (reptilian proteocephalideans are usually known to have a very narrow host range; see de Chambrier et al., 2018). In phylogenetic analyses, this species was revealed within a large 'Neotropical' clade with unresolved relationships composed mainly of species from Neotropical teleosts. The well-known European *Ophiotaenia europaea* forms a sister taxon of the Nearctic *O. perspicua* (see de Chambrier et al., 2017b).

Conclusions

The current classification is largely artificial and a new arrangement based on phylogenetic relationships is pending. However, a high degree of homoplasy of morphological characters previously used in defining proteocephalidean genera and subfamilies represents a serious obstacle in proposing a new, more natural classification. Defining new boundaries of species-rich genera represents a key, but difficult challenge for future research, similarly as redefinition of proteocephalidean subfamilies that should be in line with the results of phylogenetic analyses. Well-delimited lineages using DNA sequencing data often share morphological traits with not closely related taxa as a result of convergent evolution. Another important challenge for future research is to confirm the validity of the order Onchoproteocephalidea, which was characterized exclusively based on the position of its constituting taxa on the phylogenetic tree (see Arredondo et al., 2014).

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21

Eucestoda

Bothriocephalidea Kuchta et al., 2008 (Order)

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Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Bothriocephalidea

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Chapter 21

Bothriocephalidea Kuchta et al., 2008 (Order)

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Introduction

Members of Bothriocephalidea are included among the bothriate groups of cestodes in which the attachment organs are not separated from the surrounding tissue by a well-demarcated plasma membrane (Kuchta et al., 2008a). The name of this order is derived from the Greek terms **bothrion** (small pit) and **kephalē** (head), which refer to the presence of dorsal and ventral longitudinal grooves, named bothria, along the scolex. What characterizes these organisms is that the strobila are generally segmented completely, and are craspedote and anapolytic; also, the adult worms often are intestinal parasites of Actinopterygii (ray-finned fishes, mainly from marine environments), although they are occasionally found in amphibians, particularly newts (Kuchta et al., 2008a). The species richness of this order is moderate, but the group has a worldwide distribution, with the majority of species described from the Atlantic Ocean (Kuchta et al., 2008a).

Based on morphological and molecular evidence, Bothriocephalidea arises from the suppression of the former order Pseudophyllidea. It was separated from Diphyllobothriidea based on several traits, mainly: 1) The position of the genital pore located dorsally, dorsolaterally, or laterally in the proglottid, posterior to the ventral uterine pore in Bothriocephalidea, versus the ventral position of the genital pore, anterior to the uterine pore in Diphyllobothriidea; 2) the presence of an external seminal vesicle in Diphyllobothriidea, but which is absent in the Bothriocephalidea; 3) the lack of a uterine sac in Diphyllobothriidea, but which is present in Bothriocephalidea; and 4) due to members of both orders parasitizing different groups of hosts. Bothriocephalidea is found mainly in actinopterygians and is never found in homoeothermic vertebrates, while Diphyllobothriidea infects tetrapods, commonly mammals (Kuchta et al., 2008a; Kuchta and Scholz, 2017).

Species of 48 genera included in this order belong to 3 families: Bothriocephalidae (16 genera), Echinophallidae (8 genera), and Triaenophoridae (24 genera). In addition, 1 species (*Dactylobothrium choprai*) is considered to be a species of doubtful indentity also known in Latin as species inquirenda. As of 2017, 132 species of bothriocephalidean cestodes were described (Kuchta and Scholz, 2017) (see, for example, images of *Clestobothrium cristinae* in Figure 1).

Main Morphological Characteristics

Species included in this order of bothriate cestodes are all characterized by having a scolex that is composed of 2 elongated bothria (dorsal and ventral longitudinal grooves) as the attachment organs on the anterior end of the animal.

The shape of the scolex is quite variable among the genera; they can have an apical disc and either have hooks or the hooks may be absent. Hooks, when present, vary in size, shape, and number. In some bothriocephalideans the scolex might be what is called a pseudoscolex or it might be highly modified, sometimes called a deformed scolex, which is sometimes referred to as scolex deformatus. Scanning electron micrographs of the scolexes of species representing several genera have revealed the presence of microtriches and lumpy globular surface structures. A neck may be present or absent.

The strobila of species of cestodes included in this order ranges from small to large. Segmentation may be complete, incomplete, or completely absent in species of some genera while in all species in the order, the proglottids are craspedote, rectangular, and anapolytic. The osmoregulatory canals are paired, with the ventral canals usually being wider than the dorsal pair. Most of the bothriocephalideans have 1 set of reproductive organs per segment, but some may have 2 symmetrical sets. The testes are numerous and usually found in the middle of the segment and, in general, they are distributed in 2 lateral fields (in the middle of the segments). The genital pores are located on the dorsal surface of the proglottids (or segments) and from anterior to posterior, the genital pores can be located submarginally, marginally, or medially, alternating irregularly. A cirrus sac is present, either with or without an internal seminal vesicle. There are coiled sperm ducts and there is no external seminal vesicle. The cirrus is smooth with tegumental bumps (folds) or with spinitriches. The ovary is located in the middle of the segment, posterior and is commonly bi-lobed and may be either compact,

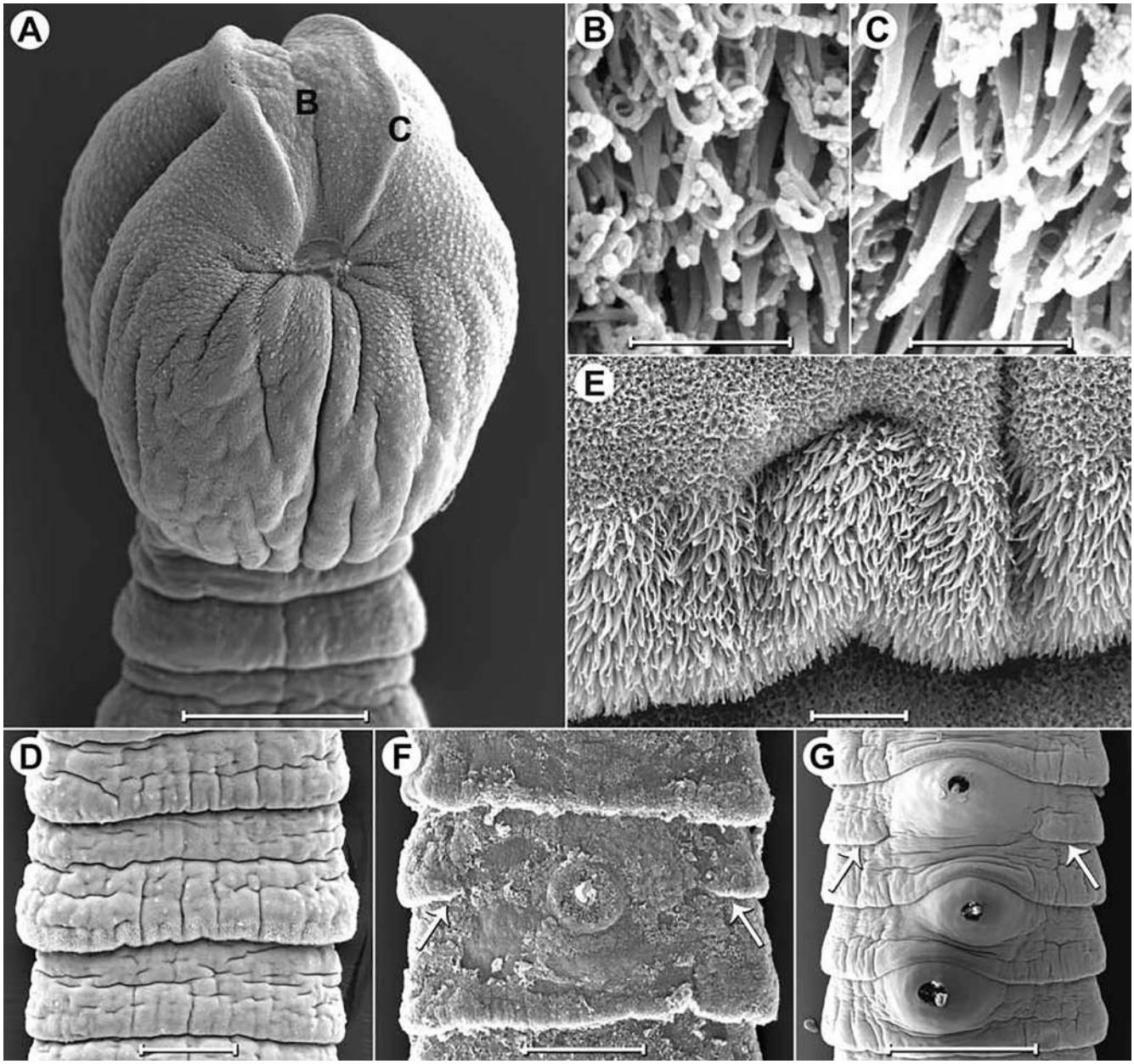

Figure 1. *Clestobothrium cristinae* from *Merluccius hubbsi*, scanning electron micrographs. A) Dorsoventral view of scolex showing tumuli; the surfaces of B and C are shown at high magnification; B) Central surface between lips; C) Marginal surface of lips; D) Piece of immature strobila; E) Surface of middle and posterior part of immature proglottis; F) Piece of mature strobila showing position of genital pore relative to spurious articulation (white arrows), dorsal view; G) Piece of gravid strobila showing uterine pore, eggs, and spurious articulation (white arrows); ventral view. Scale bars: A = 200 µm; B, C = 2 µm; D = 100 µm; E = 10 µm; F, G = 500 µm. Source: Gilde Pertierra et al., 2011. License: CC BY 4.0.

follicular, or dendritic. The vagina may be armed or not, with or without a muscular sphincter and the terminal end of the vaginal canal opening may be posterior, anterior, at the same level, or alternating in relation to the cirrus sac. The vitellarium is follicular and extensive, and there is rarely just a single one. The vitellarium may be cortical, medullary and cortical, or exclusively medullary in cross section. The uterus may be compact or lobed; the uterine duct may be coiled or elongated and the uterine sac may be compact or branched; and a uterine pore may be present or absent, and is ventral, if present. The eggs, whether operculated or not, may contain an intrauterine embryo; in non-embryonated eggs, there may be a free ciliated coracidium (Kuchta et al., 2008b; Kuchta and Scholz, 2017).

Description and Summary of a Representative Species

Note: This work is not intended for the purposes of zoological nomenclature.

***Schyzocotyle acheilognathi* (Yamaguti, 1934) Brabec et al., 2015**

According to Scholz (1997a), this cestode is unusual in its extreme morphological variability. Following is a brief characterization of this species based on the morphological information compiled by Scholz based on material collected from Europe, Asia, and Africa. The scolex is generally heart-shaped with short, deep bothridia, directed anterolaterally, with non-creanulate margins. The terminal disc is weakly developed and unarmed. A neck is absent; the first proglottids are immediately posterior to and narrower than the scolex. The strobila is acraspedote (meaning that the segments connect without a velum or without overlapping parts) and are relatively short (22–32 mm-long) but can reach up to 1,000 mm (= 1 m); both mature and gravid proglottids are elongated (length/width ratios 1:7–8 and 1:3–4.5, respectively). There are 33–100 testes distributed in 2 lateral fields that are located in the middle of the segment (medullary) and are spherical to oval. The cirrus sac is spherical and is situated anterior to the ovary. The cirrus is unarmed. The genital pore is median, opening into a common genital atrium while the ovary is median, and transversally bi-lobed, near the posterior margin of the proglottids. The vagina is tubular, opening posterior to the cirrus sac into the genital atrium. The vitelline follicles are circumcortical. The uterus is spherical, near the anterior part of the proglottids. The uterine pore is median andhe eggs are operculate and unembryonated within the proglottid (Scholz, 1997b; Brabec et al., 2016).

Taxonomic summary.

Type host: Kanehira, *Acheilognathus rhombea* (Cyprinidae).

Site of infection: Intestine.

Type locality: Lake Ogura (37° 24′ 01″ N, 139° 57′ 51″ W), Honshu, Japan; however, Choudhury and Cole (2012) considered the Amur River (eastern Asia) as the original distribution area of this cestode species, and other authors, such as Scholz et al. (2012), suggest that the parasite's origin is Africa.

Type specimens deposited: Unknown.

***Schyzocotyle* Genera**

The genus *Schyzocotyle* currently comprises 2 species, including: *S. acheilognathi* and *S. nayarensis*, both characterized by the possession of a heart-shaped scolex (Brabec et al., 2015). However, the inadequate original description of *S. nayarensis* (Brabec et al., 2016) as well as the extreme morphological variability of *S. acheilognathi* (Scholz, 1997b), makes it difficult to identify. Traits of *S. nayarenis* such as body size (12–27 mm), number of testes (52–78), or egg diameter (10–46 μm) (Malhorta, 1983) are included in the characteristics provided by Scholz (1997b) for *S. acheilognathi* obtained in fish collected from several sites in Europe, Asia, Africa, and the Americas. Interestingly, specimens that are designated as holo- or paratypes are not known to exist in any collections (Brabec et al., 2016), which limits the knowledge of this species. The study of phylogenetic relationships among the members of this group of cestodes has been addressed on several occasions. Bray and colleagues (1999) conducted a preliminary morphological cladistic analysis based on 16 species representing the type-genera, and they considered it reasonable to divide the Pseudophyllidea into 2 suborders: Bothriocephaloidea and Diphyllobothrioidea. Similar conclusions were reached by Mariaux (1998) when studying the molecular phylogeny of the Eucestoda in general, noting that the species included in the Pseudophyllidea at the time were clearly paraphyletic. The suppression of the order Pseudophyllidea with its formal separation into 2 orders (Bothriocephaliidea and Diphyllobothriidea) was inferred by Kuchta and colleagues (2008a) based on molecular evidence. As a result of this study, Kuchta and colleagues (2008a) considered that Bothriocephalidea may be a sister-group to the tetrafossate cestodes, which are generally considered to have derived characters. Brabec and colleagues (2015) confirmed the monophyly of Bothriocephalidae, a family constituted of a single clade of freshwater worms and several marine clades.

Life Cycles

The life cycle of species from the order Bothriocephalidea includes 1, or occasionally 2, intermediate hosts with procercoids in copepods and plerocercoids in fishes that eat the infected copepods. The adult stage is mainly found in the intestine of fishes and a few are found in newts (Kuchta et al., 2008b). For example, the life cycle of *Bothriocephalus claviceps*, a specific parasite of eels (*Anguilla* spp.), was studied by Scholz (1997a) under experimental conditions where he found that the development of the worm takes 4 months to complete (at 22–24 °C). The spontaneous hatching of ciliated, motile coracidia from the eggs occurs in 2 days in experimental conditions.

The zooplanktonic coracidia are ingested by copepods of species in the genera *Macrocyclops*, *Cyclops*, or *Acanthocyclops*. In these crustaceans procercoids develop after 8–12 days at 22–24 °C and the fish definitive hosts become infected through ingestion of infected copepods. Egg production in the fish then begins around 3 months post infection. Some

small fishes, such as *Perca fluviatilis* and *Poecilia reticulata*, can act as paratenic hosts of *B. claviceps*. In *P. reticulata*, the plerocercoid survives up to 14 days after exposure and they develop into adults in the definitive host after the paratenic host is consumed.

Host Range

Host range of bothriocephalideans is usually narrow; however, *Schyzocotyle acheilognathi* is a parasite with a very wide host range and is among the most generalist species of all helminths worldwide. Members of the order Bothriocephalidea are commonly found in perciform fishes, particularly Centrolophidae. Most of the species (65%) parasitize marine fishes, while 32% are found in freshwater fishes. Three species, including: *Eubothrium acipenserium*, *E. crassum*, and *E. salvelini*, may live in both types of environments (see Kuchta et al., 2018).

Zoogeography

The geographic distribution of bothriocephalideans is heterogeneous. Data on the marine species of this order probably do not totally agree with their actual distribution because the sampling effort by scientists looking for species in this group has been relatively low in the marine environment. About 38% of the known species richness has been reported from hosts from the Atlantic Ocean and 29% from the Pacific Ocean, while around 17% of the species were reported from the Indian Ocean. In addition, some species are only found in deep sea teleost fishes. In contrast, freshwater bothriocephalidean species are distributed mainly in Eurasia (27 species) and North America (18 species). On other continents, the representation of this group of cestode is very low, being especially scarce in fishes from South America (Kuchta et al., 2017).

***Schizocotyle acheilognathi* (Asian Fish Tapeworm)**

Prevalence

Schizocotyle acheilognathi, also known as the Asian fish tapeworm (AFT), was described as *Bothriocephalus acheilognathi* (Yamaguti, 1934) from the cyprinid fish *Acheilognathus rhombeus* from Lake Ogura, Japan. Since then, *S. acheilognathi* has become the most successful globally invasive parasite of freshwater fish, infecting a broad spectrum of hosts. According to Kuchta and colleagues (2018), until now the number of fish hosts parasitized by this tapeworm is 312 (belonging to 38 fish families and 14 orders), as well as 11 non-fish vertebrate host species including the amphibians *Ambystoma dumerilii* and *Lithobates megapoda*, and the snake *Thamnophis melanogaster* in Mexico (see Pérez-Ponce de León et al., 2018). This cestode has been found in 74% of Cyprinidae fishes examined (170 species), mainly in the common carp *Cyprinus carpio*. *Schizocotyle acheilognathi* is distributed throughout the world, except in Antarctica, but the highest concentrations are found in North America, Asia, and Europe (Kuchta et al., 2018).

Since the first discovery of AFT in Mexico, parasitizing the grass carp *Ctenopharyngodon idella* (López-Jiménez, 1981), the number of fish species known to be infected by this worm has increased to 110, which represents 22% of the freshwater fish fauna in Mexico; therefore, Mexico probably has the greatest prevalence of this parasite in the world (Pérez-Ponce de León et al., 2018).

The success of *Schizocotyle acheilognathi* as an invasive species was discussed by Kuchta and colleagues (2018), highlighting 3 factors: 1) Synanthropic association (probably this cestode was initially introduced anthropogenically via aquaculture practices and has continued through the natural dispersal of its hosts); 2) efficient resource use and wide environmental/physiological tolerance (note that *S. acheilognathi* is able to exploit important resources, and is capable of invading numerous species of copepods (first intermediate host) and almost any species of fish (final host) through a wide range of water temperatures); 3) life history strategy, reproductive style, capacity, and timing. In the vocabulary of population biology, the AFT is evidently an r-strategist with a reproductive potential that is adapted to produce a huge number of eggs dispersed by water currents before they hatch; hatched larvae are also dispersed by water currents. Kutcha and colleagues (2018) also point out that the existence of a niche available in the most common host groups for *S. acheilognathi* (for example, Ciprododontoides), due to the lack of their own typical cestode adult fauna, perhaps allowed the invasion of this cestode. However, they do not exclude the possibility of primary adaptation to the physiological conditions of the intestine of these fish.

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Eucestoda

Diphyllobothriidea Kuchta et al., 2008 (Order): The Broad Tapeworms

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Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Diphyllobothriidea

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Chapter 22

Diphyllobothriidea Kuchta et al., 2008 (Order): The Broad Tapeworms

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Introduction

The cestode order Diphyllobothriidea Kuchta, Scholz, Brabec & Bray, 2008 includes parasites of frogs in Africa, monitor lizards and snakes in the tropics, and fish-eating birds and mammals (including humans) worldwide (Kuchta and Scholz, 2017). The number of species that infect humans are relatively few and infections are usually asymptomatic or without serious effects on human health in the case of adult cestodes (diphyllobothriosis, and exceptionally spirometrosis). In contrast, larvae (plerocercoids) of species of *Spirometra* may cause a serious disease called sparganosis and plerocercoids of *Ligula intestinalis* can castrate fish intermediate hosts and larvae of another species maturing in birds, *Schistocephalus solidus,* and change the behavior of sticklebacks (Williams and Jones, 1994; Barber et al., 2000; Kuchta et al., 2015). The greatest number of species of diphyllobothriideans occurs in marine mammals, mainly in pinnipeds and cetaceans (Scholz et al., 2019). These parasites, commonly known as broad tapeworms (because they have wide segments), are among the largest helminths on the Earth and species from whales can reach more than 30 m in total length (Yurakhno, 1992). The number of nominal species exceeds 150, but only 60 species in 18 genera are considered to be valid (Kuchta and Scholz, 2017; Scholz et al., 2019).

Similar to bothriocephalideans, broad tapeworms possess paired attachment grooves, bothria (singular = bothrium), and were originally placed together in the order Pseudophyllidea Carus, 1863. However, they differ from each other in several morphological characteristics, such as the position of the gonopores, presence/absence of an external seminal vesicle, and enlarged distal part of the uterus (Kuchta et al., 2008). Broad tapeworms usually possess a robust body with well-developed longitudinal musculature and numerous vitelline follicles scattered in the cortex of the proglottids. Their eggs are shelled and operculate (having an operculum), their outer envelope, usually called the capsule, is tanned (hardened by a polyphenol/quinone tanning process), and the eggs are polylecithal having a large number of vitellocytes per oocyte, and they are usually operculate (Conn and Świderski, 2008).

Taxonomic History

The current order Diphyllobothriidea was established by Kuchta and colleagues (2008) who split the non-monophyletic order Pseudophyllidea into the Bothriocephalidea and the Diphyllobothriidea. This taxonomic proposal has been widely accepted (Waeschenbach et al., 2012; Caira et al., 2014; Caira and Jensen, 2017).

The first described diphyllobothriideans were broad fish tapeworms recognized initially as *Taenia lata* Linnaeus, 1758 (now called *Dibothriocephalus latus*, or sometimes referred to as *Dibothriocephalus latum*) from humans, and larvae (plerocercoids) named *Taenia intestinalis* (= *Ligula intestinalis*) from cyprinid fish (adults occur in birds that eat fishes).

When cestodes (tapeworms) were first studied, early scientists did not know that they, in fact, represented different species, the techniques had not yet been developed to discern much of the morphological characters. They were therefore lumped together as ribbon worms (*Taenia*). Thereafter, a number of diphyllobothriideans were described in the 19th and 20th centuries, with the most intensive research on human-infecting taxa occurring from approximately 1930 to 2000 in the United States, Canada, Scandinavia, and the former Soviet Union (see Wardle and McLeod, 1952; Delyamure et al., 1985). In North America, Justus F. Mueller and Robert L. Rausch were notable for their numerous contributions to knowledge of diphyllobothriideans (see Scholz et al., 2019) and Delyamure and colleagues (1985) provided a synopsis of the Diphyllobothriidae. Kamo (1999) deals with all members of the Diphyllobothriidae, with a focus on human-infecting taxa.

Several problems still remain in the taxonomy of broad tapeworms despite the considerable effort of several generations of cestodologists. Most confusion exists in the systematics of 2 species-rich genera, *Diphyllobothrium* (containing nearly 100 nominal species) and *Spirometra* (containing almost 50 species). The validity of the latter taxon has been questioned and *Spirometra* has been considered to be a junior

Figure 1. Microphotographs of diphyllobothriidean tapeworms. A) Live adults of *Cephalochlamys namaquensis* from *Xenopus laevis*, South Africa (arrow indicates the scolex). B) Live *Bothridium pithonis* in the intestine of *Xenopeltis unicolor*, Vietnam. C) Museum specimens of adults of *Diphyllobothrium cordatum* (larger), *D. lanceolatum*, and *D. schistochilos* (smaller) from *Erignathus barbatus*, Greenland. D) Live larvae (spargana) of *Spirometra erinaceieuropaei* in the muscles (arrow) and after their removal from *Pelophylax nigromaculatus*, China. E) Live larva (plerocercoid) of *Dibothriocephalus latus* in the muscles (arrow) of *Perca fluviatilis*, Italy. F) Fixed plerocercoids of *Dibothriocephalus ditremus* (smaller) and *D. dendriticus* (larger) from the body cavity of *Oncorhynchus mykiss*, United Kingdom. Source for all: R. Kuchta and T. Scholz. License for all: CC BY-NC-SA 4.0.

synonym of *Diphyllobothrium* (for example, see Schmidt, 1986). Nevertheless, molecular data provide convincing evidence that *Spirometra* is a valid genus, which is not closely related to any of several lineages of the *Diphyllobothrium,* which is now recognized to be a polyphyletic assemblage, meaning that species assigned to this genus are not derived from a common ancestor and the group is a mixture of unrelated species (see Waeschenbach et al., 2017).

Current Classification

Molecular phylogenetic analyses have focused on interrelationships of the order. Waeschenbach and colleagues (2017) supported the division of the group into 3 families that differ from each other in their morphology, but also with respect to the spectrum of definitive hosts (see Kuchta and Scholz, 2017). These families are discussed below.

Family Cephalochlamydidae Yamaguti, 1959

Species of cestodes assigned to this family are originally parasites of frogs distributed in sub-Saharan Africa and they have been imported by people to California, United States. The type genus is *Cephalochlamys* Blanchard, 1908 (with 2 species). There is an additional (monotypic) genus, *Paracephalochlamys* Jackson and Tinsley, 2001. Species of cestodes in these genera characteristically have a vas deferens that exits directly to the genital pore without expanding into a cirrus sac and the proglottids are acraspedote, that is, their posterior margin is not wider than the anterior margin of the subsequent proglottid (meaning that they have no velum on the posterior margin). The life cycle includes only 1 intermediate host, a freshwater copepod. The copepod becomes infected when it eats a ciliated free-swimming coracidium larva that hatched from the eggs in water (Thurston, 1967).

Family Solenophoridae Monticelli and Crety, 1891

Animals in this family are parasites mainly of varanid and boid reptiles in the tropics and subtropics. The type genus is *Solenophorus* Creplin, 1839 (which is a synonym of *Bothridium* Blainville, 1824) (with 6 species). Additional genera include *Duthiersia* Perrier, 1873 (2 species) and *Scyphocephalus* Riggenbach, 1898 (1 species). The proglottids are craspedote and the genital atrium is large. The life cycles of these species have not yet been elucidated.

Family Diphyllobothriidae Lühe, 1910

These are typically parasites of mammals, but are also (rarely) found in birds. The type genus is *Diphyllobothrium* Cobbold, 1858 (with 27 species). Additional genera include *Adenocephalus* Nybelin, 1931 (1 species), *Baylisia* Markowski, 1952 (2 species), *Baylisiella* Markowski, 1952 (1 species), *Dibothriocephalus* Lühe, 1899 (7 species), *Flexobothrium* Yurakhno, 1979 (1 species), *Glandicephalus* Fuhrmann, 1921 (2 species), *Ligula* Bloch, 1782 (5 species), *Plicobothrium* Rausch and Margolis, 1969 (1 species), *Pyramicocephalus* Monticelli, 1890 (1 species), *Schistocephalus* Creplin, 1829 (5 species), *Spirometra* Faust, Campbell and Kellogg, 1929 (4 species), and *Tetragonoporus* Skryabin, 1961 (1 species).

Morphology

Diphyllobothriideans are medium-sized to large polyzoic tapeworms, that is, their body—the strobila—consists of a series of proglottids maturing consecutively from the proliferative zone (neck) situated posterior to the scolex, that is, the anterior end with the attachment function (Kuchta and Scholz, 2017). The proglottids are usually wider than long and are anapolytic. The scolex is variable in shape and is always unarmed (with no hooks or other sclerotized structures present), with dorsal and ventral longitudinal grooves (termed bothria; singular: bothrium). There are single reproductive organs in the proglottid in most taxa, and so are rarely double or multiple per proglottid. The testes are numerous and medullary, and usually in a single field. The sperm ducts are convoluted, forming a thick-walled, muscular external seminal vesicle attached to the proximal part of the cirrus sac. The cirrus sac is usually thick-walled and the cirrus is unarmed, that is, it is not covered with spinitriches (see Chervy, 2009 for terminology of microtriches in cestodes). The genital pore is ventral, median, or submedian. The ovary is medullary, usually bi-lobed, and is situated posterior to the proglottids. The vitelline follicles are numerous and are usually cortical and circum-medullary. The uterus is tubular and variable in shape, opening to the exterior through a uterine pore situated posterior to the genital pore. The eggs are operculate and unembryonated in the uterus in most taxa, and a freely swimming ciliated coracidium is present.

Species Diversity

Opinions as to the species diversity of broad tapeworms and their classification have changed considerably and constantly over more than 250 years since description of the first 2 broad tapeworms, including *Dibothriocephalus latus*. Taxonomic and nomenclatural problems still remain in this group due to factors such as their general morphological uniformity coupled with high intraspecific variability, the difficulties in observing the internal anatomy in large-sized worms, the poor quality of specimens obtained from dead or frozen hosts, and the absence of type and voucher specimens of numerous species, among other reasons.

In the 20th century, research on broad tapeworms was quite intensive, especially in North America (for example, pivotal papers by Justus F. Mueller on *Spirometra* and Robert L. Rausch's accounts on species of *Diphyllobothrium* from Alaska) and from the former Soviet Union (Delyamure et al., 1985). Numerous studies from Scandinavia and Japan are also noteworthy. However, attention paid to broad tapeworms including their taxonomy sharply declined in the last decades of the 20th century. Kuchta and Scholz (2017) provided a

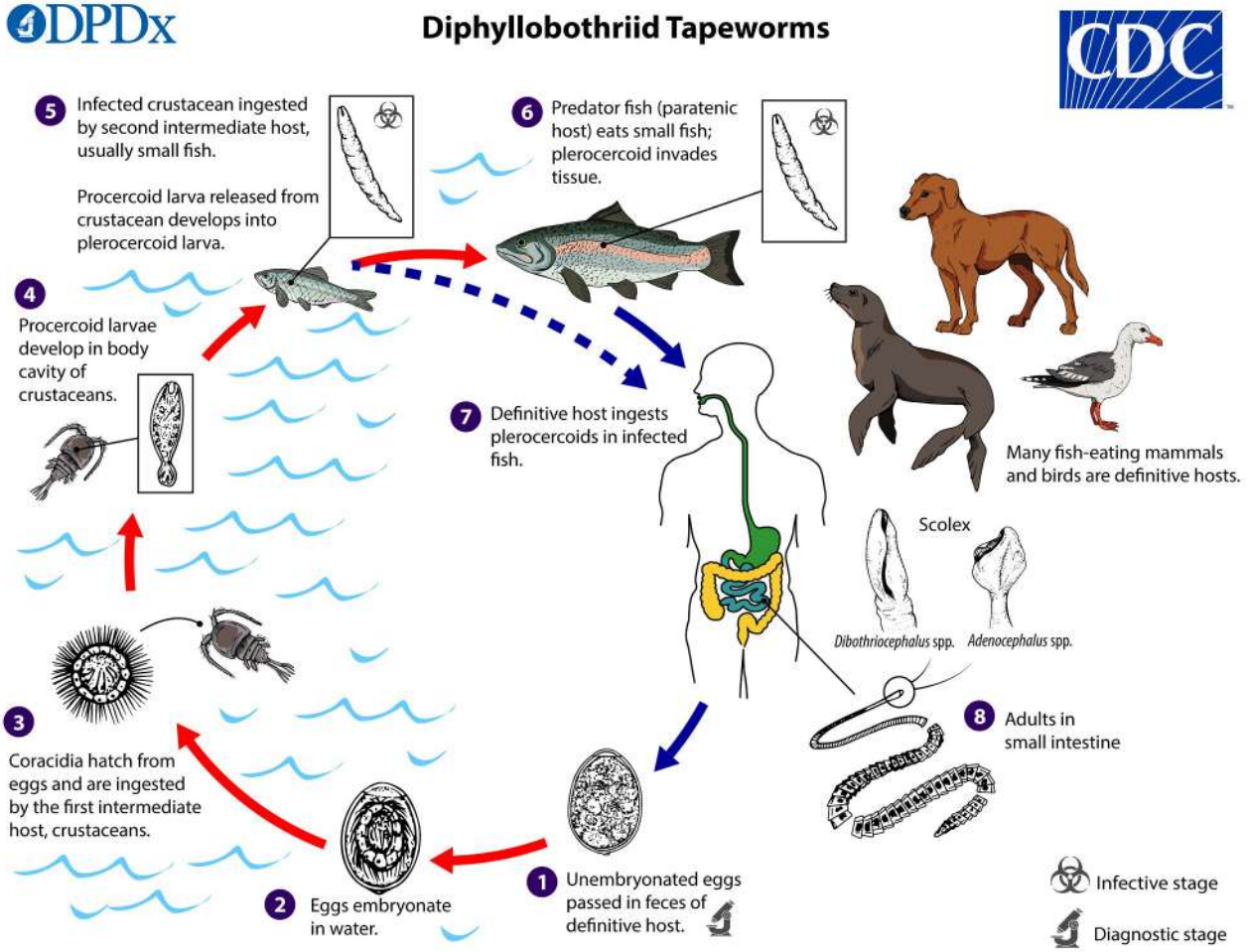

Figure 2.

survey of the whole order, with the complete list of all species recognized as valid (a total of 70 species from 18 genera) with their type hosts. Scholz and colleagues (2019) provided lists of species parasitizing marine mammals and nominal species of *Spirometra*.

Life Cycles

Life cycles of only a limited number of species, especially those infecting humans, are known (see, for example, the life cycle depicted in Figure 2), but planktonic crustaceans (copepods) likely serve as first intermediate hosts of most, if not all broad tapeworms. They are the only intermediate hosts of cephalochlamydids from frogs (Thurston, 1967). Life cycles of species of the Solenophoridae are still unknown, but most likely involve a second intermediate host which is a vertebrate. The life cycles of the Diphyllobothriidae are always connected to an aquatic environment (freshwater or marine), because the first larva (coracidium) swims in the water and is then swallowed by the first aquatic intermediate host, which is a copepod (subphylum Crustacea: subclass Copepoda). Second intermediate hosts are vertebrates, especially freshwater or marine teleost fishes (Dubinina, 1980; Kuchta et al., 2015). The exception is the life cycle of species of *Spirometra*, which includes a wide spectrum of amphibians, reptiles, birds, or small mammals as second intermediate hosts, but not teleosts (Kuchta and Scholz, 2017; Scholz et al. 2019).

Host Associations

Broad tapeworms are peculiar among all but 1 (Cyclophyllidea) of the cestode orders in successful colonizing of all major tetrapod groups. Molecular data reveal that the main lineages, which correspond to 3 families, generally reflect the evolutionary history of their tetrapod definitive hosts. Species of Cephalochlamydidae mature in amphibians (frogs), members of the Solenophoridae in monitors and snakes, and species of the most derived lineage of broad tapeworms, the Diphyllobothriidae, mature in mammals including humans and, to a lesser extent, birds. The earliest diverging groups of

diphyllobothriids (*Spirometra* and *Schistocephalus*) colonized terrestrial ecosystems followed by radiation in marine mammals (pinnipeds and cetaceans: *Diphyllobothrium*, *Adenocephalus*, and so on). Species of the most recently diverging groups (*Ligula* and *Dibothriocephalus*) use terrestrial mammals and fish-eating birds as their definitive hosts (Waeschenbach et al., 2017; Scholz et al., 2019).

Marine mammals are definitive hosts of nearly two-thirds of diphyllobothriids (37 of 58 known species). They infect mainly pinnipeds (reported from 17 and 15 species of the Phocidae and Otariidae families, respectively), but also cetaceans (reported from 8 and 21 species of the Mysticeti and Odonoceti suborders, respectively; see Scholz et al., 2019). The infection rate in seals can be extraordinarily high, with prevalence reaching up to 100%, and extremely high intensity of infections, especially in Antarctic seals. The most heavily infected seal harbored as many as 3,600,000 individuals of *Diphyllobothrium mobile*, but the majority of specimens were juvenile (Yurakhno and Maltsev, 1997).

Broad tapeworms of terrestrial vertebrates are represented by 21 species within 4 genera, *Dibothriocephalus*, *Ligula*, *Schistocephalus*, and *Spirometra* (36% of all known diphyllobothriids). They infect a much wider spectrum of hosts and generally exhibit far lower host range compared to marine species. Members of the genera *Dibothriocephalus* and *Spirometra* are typical parasites of carnivores (several unrelated families, but not pinnipeds), and occasionally of fish-eating birds (some species of *Dibothriocephalus*). Species of *Spirometra* occur in warmer latitudes than species of *Dibothriocephalus* (see Figure 3 in Scholz et al., 2019).

Most species of *Ligula* and *Schistocephalus* are euryxenous at the level of the definitive host, with adults of these genera having been reported from almost 80 species of fish-eating birds (Dubinina, 1980). For example, *S. solidus* has been reported from as many as 42 species of birds across 8 orders (Vik, 1954). In contrast, the 3-spined stickleback, *Gasterosteus aculeatus*, serves as the only second intermediate host species of this cestode. Its plerocercoids continue to grow for an unusually long time (several months), and nearly reach sexual maturity in the fish host. In the definitive host, adults of *S. solidus* survive only few days, producing great numbers of eggs (Dubinina, 1980).

Geographic Distribution

According to Waeschenbach and colleagues (2017), terrestrial and freshwater species of broad tapeworms (Diphyllobothriidea) represent 46% of the total species richness (33 species); they occur in the Palaearctic (17 species; 22%), Nearctic (10 species; 14%), and Ethiopian (8 species; 10%) regions. Most species, especially those of the family Diphyllobothriidae, occur predominately in colder climates between 50–60 °N and 40–70 °S, including 14 species belonging to 4 genera (*Baylisia* Markowski, 1952, *Baylisiella* Markowski, 1952, *Flexobothrium* Yurakhno, 1989, and *Glandicephalus* Fuhrmann, 1921) endemic to Antarctic seals (Delyamure et al., 1985; Scholz et al., 2019). In contrast, species of the early diverging groups, that is, Cephalochlamydidae, Solenophoridae, and *Spirometra* spp., are well adapted to warmer climate zones; and members of the 2 former families occur exclusively in tropical and subtropical latitudes.

Phylogenetic Relationships

Waeschenbach and colleagues (2017) provide the most robust hypothesis to date of interrelations of diphyllobothriidean cestodes, using a phylogenetic framework of 30 species of 11 genera based on large and small nuclear ribosomal RNA subunits (*ssr*DNA and *lsr*DNA), a large subunit of mitochondrial ribosomal RNA (*rrnL*) and cytochrome c oxidase subunit I (*cox1*) sequences. This first multigene family-wide phylogeny of the order provides support for the current classification of the order, recognzing 3 families specific to amphibians (Cephalochlamydidae), reptiles (Solenophoridae), and mammals and birds (Diphyllobothriidae) proposed by Kuchta and colleagues (2008) and Kuchta and Scholz (2017).

Molecular data also reveal the polyphyly of *Diphyllobothrium* and invalidity of *Diplogonoporus*. As a result, a new, more natural classification of broad tapeworms is proposed, including new generic assignment of the most important causative agents of human diphyllobothriosis, namely, *Dibothriocephalus latus* and *D. nihonkaiensis* (see Waeschenbach et al., 2017). Synonymy of *Spirometra* with *Diphyllobothrium* (including the currently resurrected *Dibothriocephalus*) previously proposed by a number of authors is not supported because both genera are not closely related. The former genus is 1 of 2 earliest diverging diphyllobothriid lineages, whereas *Dibothriocephalus* belongs among the most recently diverging clades of broad tapeworms (Waeschenbach et al., 2017).

Selected Taxa from the Nearctic Region

A total of 49 species of diphyllobothriid cestodes have been reported from the Nearctic region and the Neotropical part of Mexico. Of these, the following species are selected to document diversity, host associations, life cycles, and phylogenetic affinities in this group of cestodes in North America.

Dibothriocephalus latus

Dibothriocephalus latus is the most important causative agent of human diphyllobothriosis (about 20 million human cases estimated annually). The species has been known as a

human parasite for long time as evidenced by archaeoparasitological data from mummies and coprolites. These data reveal the presence of eggs of diphyllobothriid cestodes at least since the early Neolithic period (Mitchell, 2013). Several foci of human infections in North America, especially in the Great Lakes region, were reported in the 20th century, but other species such as *D. nihonkaiensis* may have been misidentified as *D. latus*, especially on the Pacific coast of North America.

Dibothriocephalus nihonkaiensis

Dibothriocephalus nihonkaiensis was identified as *D. latus* for long time until Yamane and colleagues (1986) distinguished broad fish tapeworms from Japan from genuine *D. latus*. The Japanese broad tapeworm utilizes different fish intermediate hosts (anadromous Pacific salmon) compared to *D. latus* (freshwater perch, pikeperch, pike, and burbot). Both species also differ from each other in their geographical distribution, with *D. nihonkaiensis* occurring originally on the northern Pacific coast of the United States, whereas the distribution of *D. latus* as a freshwater species is limited to temperate latitudes of Eurasia and North America (Scholz et al., 2019). About 1,000 human cases have been reliably documented, especially in Japan, but human cases have also been reported from northwestern North America (Kuchta et al., 2015; Scholz and Kuchta, 2016).

***Spirometra* spp.**

Several species such as *Spirometra mansonoides* Mueller, 1935 have been reported from North America, but their validity, host range, and distribution are insufficiently known. Adults are reported mainly from felids and canids, whereas larvae (spargana) are known from water snakes. These larvae may also infect humans who consume uncooked infected intermediate hosts. Most of the patients in the United States are from the eastern seaboard and Gulf Coast (Kuchta et al., 2015; Scholz et al., 2019).

Schistocephalus solidus

Plerocercoids of *Schistocephalus solidus*, which matures in fish-eating birds, are very common in the 3-spined stickleback in the Northern Hemisphere. These large larvae (metacestodes) may cause deformation of the body of heavily infected fish, but also change their behavior to facilitate parasite transmission, that is, predation of fish hosts infected with cestode larvae (Barber et al., 2000). The stickleback-*Schistocephalus* model has been successfully used in ecological, behavioral, and evolutionary studies (Heins et al., 2014; Heins, 2017).

Conclusions

Broad tapeworms are among the largest tapeworms on Earth and some species have been known as human parasites for a long time. However, species diversity of these usually large-sized tapeworms is still poorly known, partly because of the existence of numerous unresolved taxonomic problems. They currently get more attention due to the appearance of several human cases in non-endemic areas as a result of importation of unfrozen fish that serves as a significant source of human infection. In contrast, broad tapeworms in wildlife such as marine mammals (pinnipeds and cetaceans) remain largely neglected, even though their actual impact on heavily infected hosts remains to be clarified. These tapeworms may serve as a suitable model group for studies on host-parasite relationships because of their relatively narrow host range, especially taxa in marine mammals. The serious problem that impedes better understanding of the biology, host associations, and epidemiology of these cestodes is a shortage of properly processed material suitable for application of methods of integrative taxonomy and molecular systematics. Molecular data are crucial for reliable diagnosis and species identification of most taxa because of their general morphological uniformity and high intraspecific variability. DNA-based identification using suitable molecular markers (*cox1* sequences) is also inevitable to detect sources of human infection.

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23

Eucestoda

Trypanorhyncha Diesing, 1863 (Order)

Francisco Zaragoza-Tapia and Scott Monks

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Trypanorhyncha

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Chapter 23

Trypanorhyncha Diesing, 1863 (Order)

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Introduction

The members of the order Trypanorhyncha are within the subclass Eucestoda. Trypanorhynchs are common cestode parasites of marine fish, but this group is also among the most enigmatic groups of cestodes. The order Trypanorhyncha was established by Diesing in 1863, and it was then considered to be a putative chaotic order within the phylum Plathyhelminthes. However, taxonomically this order is only a complex group, like many other orders of cestodes.

To date, the species of the order Trypanorhyncha are grouped into 4 superfamilies that currently include 315 species within 81 genera (Beveridge et al., 2017). The adults of these species are typically found infecting the stomach and the spiral intestine of elasmobranchs (sharks and rays) as their definitive hosts. Larval trypanorhynchs infect a wide variety of marine invertebrates and teleost fish (Palm, 2004; 2010; Palm et al., 2009). There are larval trypanorhynchs that have been used for descriptions of species but the morphology of the adults of those species is unknown. In contrast, larvae of other orders of cestodes (for example, Tetraphyllidea) typically have not been identified to the specific level based on morphological criteria because is not possible to do so with the few apparent structures (Jensen and Bullard, 2010).

Morphology of Trypanorhyncha Larva

As described above, trypanorhynchs go through larval developmental stages, including plerocercus, plerocercoid, and merocercoid (Sakanari and Moser, 1989; Palm and Caira, 2008; Palm et al., 2009). These stages all look very different from one another, which makes it difficult to trace out the patterns of life cycles. The morphology of each stage is described briefly below (Table 1).

Morphology of Trypanorhyncha Adults

The body of adult trypanorhynchs consists of 2 main regions, the **scolex** and the **strobila**.

Scolex

Members of the subclass Eucestoda exhibit an amazing variety of forms of the scolex. The scolex is the anterior part of the adult cestode, often highly specialized for adhesion to the host's intestine. The scolex of trypanorhynchs (Figure 1A) is divided into 3 regions: 1) **Pars bothrialis**, anterior end to the hind margin of the bothridia; 2) **Pars vaginalis**, anterior end to the posterior end of the tentacular bulbs; and 3) **Pars bulbosa**, extends the length of the bulbs at the tentacle base.

Trypanorhynchs have 2 or 4 bothridia and the tentacular apparatus consists of 4 retractile tentacles. Each tentacle has hooks and each is attached to a retractor muscle that is within a muscular bulb (Figure 1A and 1B) (Jones et al., 2004; Palm et al., 2009; Jones, 2000; Beveridge et al., 2017).

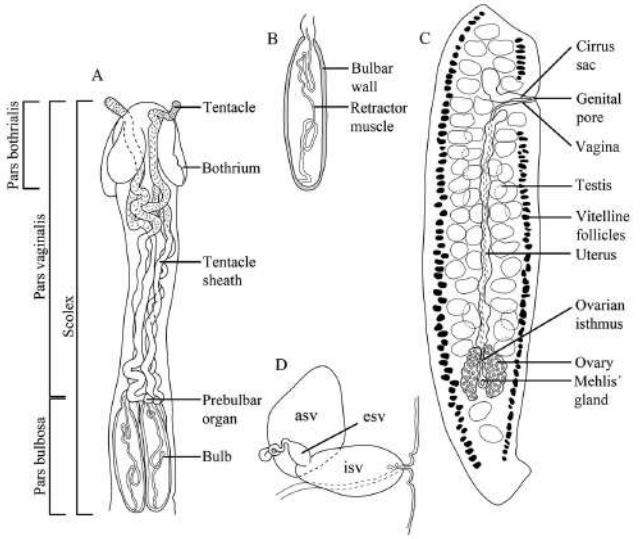

Figure 1. General morphology of a trypanorhynch: A) Scolex; B) Bulb; C) Mature proglottid; D) Terminal genitalia. Abbreviations: asv = accessory seminal vesicle; esv = external seminal vesicle; isv = internal seminal vesicle. Source: Modified from Beveridge and Justine, 2006. License: CC BY-NC-SA 4.0.

Table 1. Types of hooks and patterns of distribution in tentacles of trypanorhynchs.

Armature pattern of the tentacle	Description
Typical	Tentacle without intercalary hooks (Figure 3A).
Atypical	Tentacle with intercalary hooks (Figure 3B).
Convergent	Tentacle in which there are no distinct spaces at the beginning of the rows of principal hooks.
Divergent	Tentacle with distinct space between the beginnings of the rows of principal hooks.
Heteroacanthous	Tentacle with hooks arranged in half spiral rows around the tentacle (Figures 3A–C).
Homeoacanthous	Tentacle with hooks arranged in complete spirals surrounding the tentacle (Figure 3E).
Heteromorphous	Tentacle with hooks of different shape.
Homeomorphous	Tentacle with hooks of similar shape.
Intercalary hook	Tentacle with interpolated microhooks between rows of principal hooks.
Principal hook	Tentacle with enlarged hooks arranged in half spiral rows around the tentacle.
Poeciloacanthous	One tentacle surface bears characteristic hooks arranged in 1–3 longitudinal files along the tentacle, forming a chain of hooks that differs in form and/or size from principal and intercalary hooks (Figure 3D).

Ultrastructures of the tegument of the scolex

Cestodes entirely lack a digestive system and instead absorb nutrients through the tegument. On the tegument of the scolex are **microtriches**, which may help in the absorption of nutrients (Chervy, 2009). Scanning electron microscopy (SEM) reveals different kinds of microtriches across the entire surface of the tegument of the different groups of cestodes (Chervy, 2009; Faliex et al., 2000; Caira et al., 1999). There are different forms of microtriches in the different groups of trypanorhynchs, such as capilliform, papilliform, palmate, filiform, and others (Figure 2) (Whittaker, 1985; Palm, 2008; Caira et al., 2010; Menoret and Ivanov, 2015; Haseli et al., 2016).

Scolex armature

The retractile tentacles have hooks (armature) that are highly variable. The type (size, curvature, etc.) of hooks and the armature pattern is used in the classification of the groups. The armature patterns are classified as described by Palm et al. (2009) (Table 1 and Figure 3).

Strobila

Strobila refers to the set of proglottids located posteriad to the border of the posterior margin of the scolex. The reproductive organs are located in the proglottids or segments. There are various types of proglottids in the strobila of a cestode: 1) Immature proglottids in the anterior part of the strobila; the anlagen (the beginning primordia of the genitalia) are found here; these lack distinct internal structures; these lack distinct internal structures; 2) mature proglottids, in which at least 1 reproductive system is functional (male, female, or both) (Figure 1C); and 3) gravid proglottids, in which fertilization has occurred and the uterus is filled with eggs.

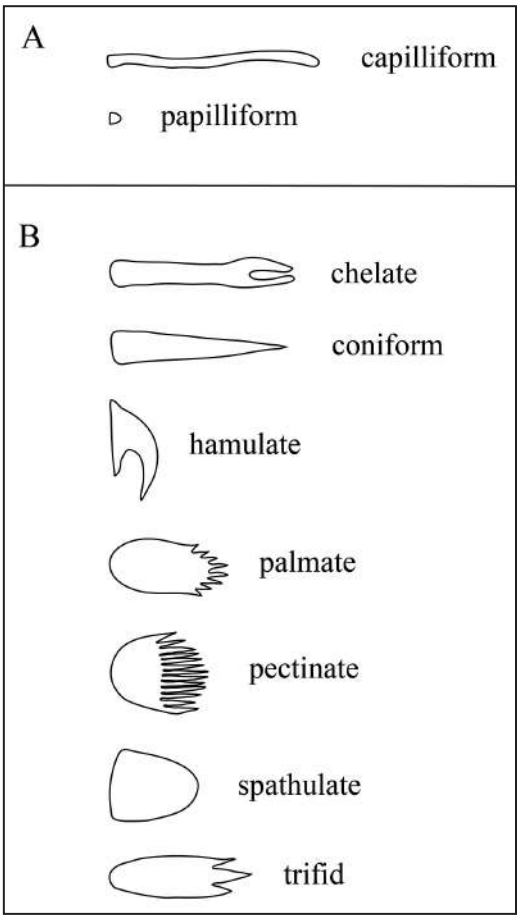

Figure 2. Schematic representation of some ultrastructural features of the scolex reported for trypanorhynchs. **Microtriches** with basal widths ≤ 200 nm are considered to be **filitriches** (A); in contrast, microtriches with basal widths > 200 nm are considered to be **spinitriches** (B). Source: Modified from Chervy, 2009. License: CC BY-NC-SA 4.0.

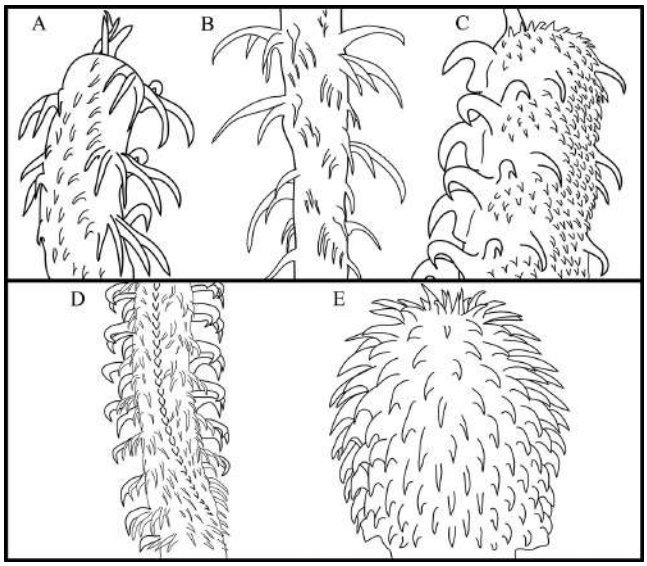

Figure 3. Armature patterns of tentacles of some trypanorhynchs. A) Typical heteroacanthous tentacular armature; B) Atypical heteroacanthous tentacular armature; C) Multiatypical heteroacanthous tentacular armature; D) Poeciloacanthous tentacular armature; E) Homeocanthous tentacular armature. Source: Modified from Beveridge et al., 2017. License: CC BY-NC-SA 4.0.

In all members of the subclass Eucestoda, apolysis refers to the loss of the most-posterior proglottids from the strobila. The terms used to describe the apolysis of the proglottids are as follows. **Hyperapolytic** refers to a strobila that never possesses proglottids that are either mature or gravid. **Euapolytic** refers to a mature terminal proglottid without gravid proglottids. **Apolytic** refers to a strobila that has some gravid proglottids. **Anapolytic** refers to a strobila containing gravid proglottids along with older, degenerated proglottids (Caira et al., 1999; Franzese and Ivanov, 2018).

The anatomy of the proglottid of Trypanorhyncha is similar to other orders of cestodes (for example, Rhinebothriidea, Tetraphyllidea, and Onchoproteocephalidea). The trypanorhynchs are hermaphroditic and the proglottids include both male and female reproductive organs (Figure 1C). There are numerous testes. The ovary is composed of 4 lobes and it is positioned in the posterior part of the proglottid. The vitellarium is follicular with vitelline follicles and, in some species, the vitelline follicles form lateral bands. The genital pores usually alternate irregularly and are located in the lateral part of the proglottid. The uterus is saccate in gravid proglottids. The vagina opens posteriorly to the cirrus sac and it is positioned ventrally (Figure 1C). In addition, the Trypanorhyncha as a whole exhibit remarkable variation in the arrangement of their terminal genitalia, which may include accessory, internal, and external seminal vesicles, and a hermaphroditic duct or vesicle (Palm et al., 2009; Beveridge et al., 2017) (Figure 1D).

Table 2. Genera within the superfamily Eutetrarhynchoidea.

Genera	Number of species
Cetorhinicola Beveridge and Campbell, 1988	1
Didymorhynchus Beveridge and Campbell, 1988	1
Dollfusiella Campbell and Beveridge, 1994	30
Eutetrarhynchus Pintner, 1913	4
Fellicocestus Campbell and Beveridge, 2006	1
Halysiorhynchus Pintner, 1913	1
Hemionchos Campbell and Beveridge, 2006	3
Hispidorhynchus Schaeffner and Beveridge, 2012	3
Mecistobothrium Heinz and Dailey, 1974	6
Mixodigma Dailey and Vogelbein, 1982	1
Mobulocestus Campbell and Beveridge, 2006	3
Nataliella Palm, 2010	1
Oncomegas Dollfus, 1929	4
Parachristianella Dollfus, 1946	10
Paroncomegas Campbell, Marques and Ivanov, 1999	3
Poecilorhynchus Schaeffner and Beveridge, 2013	1
Prochristianella Dollfus, 1946	21
Progrillotia Dollfus, 1946	3
Pseudochristianella Campbell and Beveridge, 1990	3
Rhinoptericola Carvajal and Campbell, 1975	1
Shirleyrhynchus Beveridge and Campbell, 1988	3
Tetrarhynchobothrium Diesing, 1854	5
Trigonolobium Dollfus, 1929	2
Trimacracanthus Beveridge and Campbell, 1987	2
Trygonicola Beveridge and Campbell, 1998	1
Zygorhynchus Beveridge and Campbell, 1988	4

Table 3. Genera within the superfamily Tentacularoidea.

Genera	Number of species
Heteronybelinia Palm, 1999	15
Kotorella Euzet and Radujkovic, 1989	1
Kotorelliella Palm and Beveridge, 2002	1
Mixonybelinia Palm, 1999	6
Nybelinia Poche, 1926	29
Paranybelinia Dollfus, 1966	1
Pseudonybelinia Dollfus, 1966	1
Tentacularia Bosc, 1797	1

Life Cycle of the Trypanorhyncha

In general, the life cycles of species of cestodes that parasitize marine hosts are poorly known. In agreement with Sakanari and Moser (1989), Palm and Caira (2008), and Palm et al.

Table 4. Genera within the superfamily Gymnorhynchoidea.

Genera	Number of species
Aporhynchus Nybelin, 1918	4
Chimaerarhynchus Beveridge and Campbell, 1989	1
Deanicola Beveridge, 1990	2
Gilquinia Guiart, 1927	4
Gymnorhynchus Rudolphi, 1819	2
Hepatoxylon Bosc, 1811	2
Heterosphyriocephalus Palm, 2004	2
Molicola Dollfus, 1935	3
Nakayacestus Caira, Kuchta and Desjardins, 2010	2
Pintneriella Yamaguti, 1934	4
Plesiorhynchus Beveridge, 1990	3
Sagittirhynchus Beveridge and Justine, 2006	1
Sphyriocephalus Pintner, 1913	4
Vittirhynchus Beveridge and Justine, 2006	1

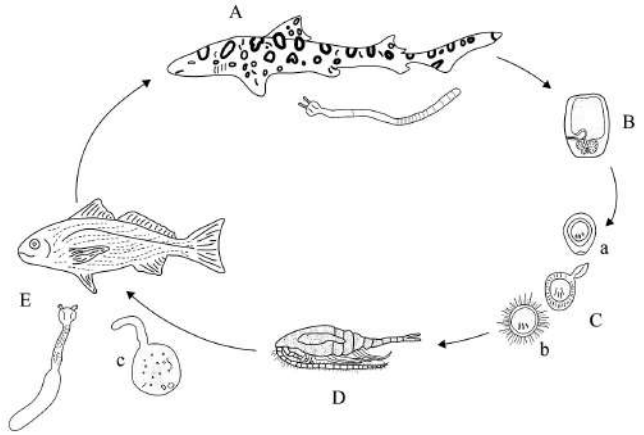

Figure 4. Life cycle of *Lacistorhynchus dollfusi* Beveridge and Sakanari, 1987. A) Adult trypanorhynch in the spiral valve of *Triakis semifasciata* Girard, 1855 (leopard shark); B) Gravid proglottids pass out with the feces and release eggs into the water; C) Coracidium larva (b) hatch from operculate eggs (a); D) The coracidium larvae are eaten by *Tigriopus californicus* (Baker, 1912) (copepod) in which the procercoids are developed; E) The infected copepods are ingested by teleosts such as *Genyonemus lineatus* (Ayres, 1855) (white croaker), in which the plerocercus form inside blastocysts (c). The life cycle is completed when *T. semifasciata* eats an infected *G. lineatus* individual. Source: Modifed from Sakanari and Moser, 1989. License: CC BY-NC-SA 4.0.

(2009; 2017), trypanorhynchs share a general pattern of life cycle. In this general cycle, the first intermediate host (often a copepod) becomes infected when it consumes an oncosphere or a coracidium larva (free-swimming larva). Inside the first intermediate host, the zygote develops into a procercoid larva. The

Table 5. Genera within the superfamily Lacistorhynchoidea.

Genera	Number of species
Ancipirhynchus Schaeffner, Gasser and Beveridge, 2011	1
Bathygrillotia Beveridge and Campbell, 2012	2
Bombycirhynchus Pintner, 1931	1
Callitetrarhynchus Pintner, 1931	2
Campbelliella Palm, 2004	1
Cavearhynchus Schaeffner and Beveridge, 2012	1
Dasyrhynchus Pintner, 1928	5
Diesingium Pintner, 1929	3
Diplootobothrium Chandler, 1942	1
Floriceps Cuvier, 1817	2
Fossobothrium Beveridge and Campbell, 2005	1
Grillotia Guiart, 1927	17
Grillotiella Palm, 2004	1
Hornelliella Yamaguti, 1954	1
Iobothrium Beveridge and Campbell, 2005	1
Lacistorhynchus Pintner, 1913	2
Microbothriorhynchus Yamaguti, 1952	2
Otobothrium Linton, 1890	12
Paragrillotia Dollfus, 1969	3
Parotobothrium Palm, 2004	2
Poeciloacanthum Palm, 1995	1
Poecilancistrium Dollfus, 1929	1
Pristiorhynchus Schaeffner and Beveridge, 2013	1
Proemotobothrium Beveridge and Campbell, 2001	3
Protogrillotia Palm, 2004	2
Pseudogilquinia Bilqees and Khatoon, 1980	5
Pseudogrillotia Dollfus, 1969	6
Pseudolacistorhynchus Palm, 1995	5
Pseudotobothrium Dollfus, 1942	2
Pterobothrioides Campbell and Beveridge, 1997	2
Pterobothrium Diesing, 1850	15
Stragulorhynchus Beveridge and Campbell, 1988	1
Symbothriorhynchus Yamaguti, 1952	2

first intermediate host is consumed by the second intermediate host. These include a wide array of marine animals (invertebrates and teleost fish). The definitive host, an elasmobranch, is infected when it consumes the infected second intermediate host (Palm et al., 2017).

Although the full life cycles are unknown, partial life cycles of the trypanorhynchs were described by Sakanari and Moser (1989) in the 1980s. Figure 4 shows an example of the complete life cycle of a trypanorhynch that was completed by these authors in a laboratory setting.

Unlike most other orders of cestodes, the final stage of the larva of trypanorhynch (namely, plerocercoid,

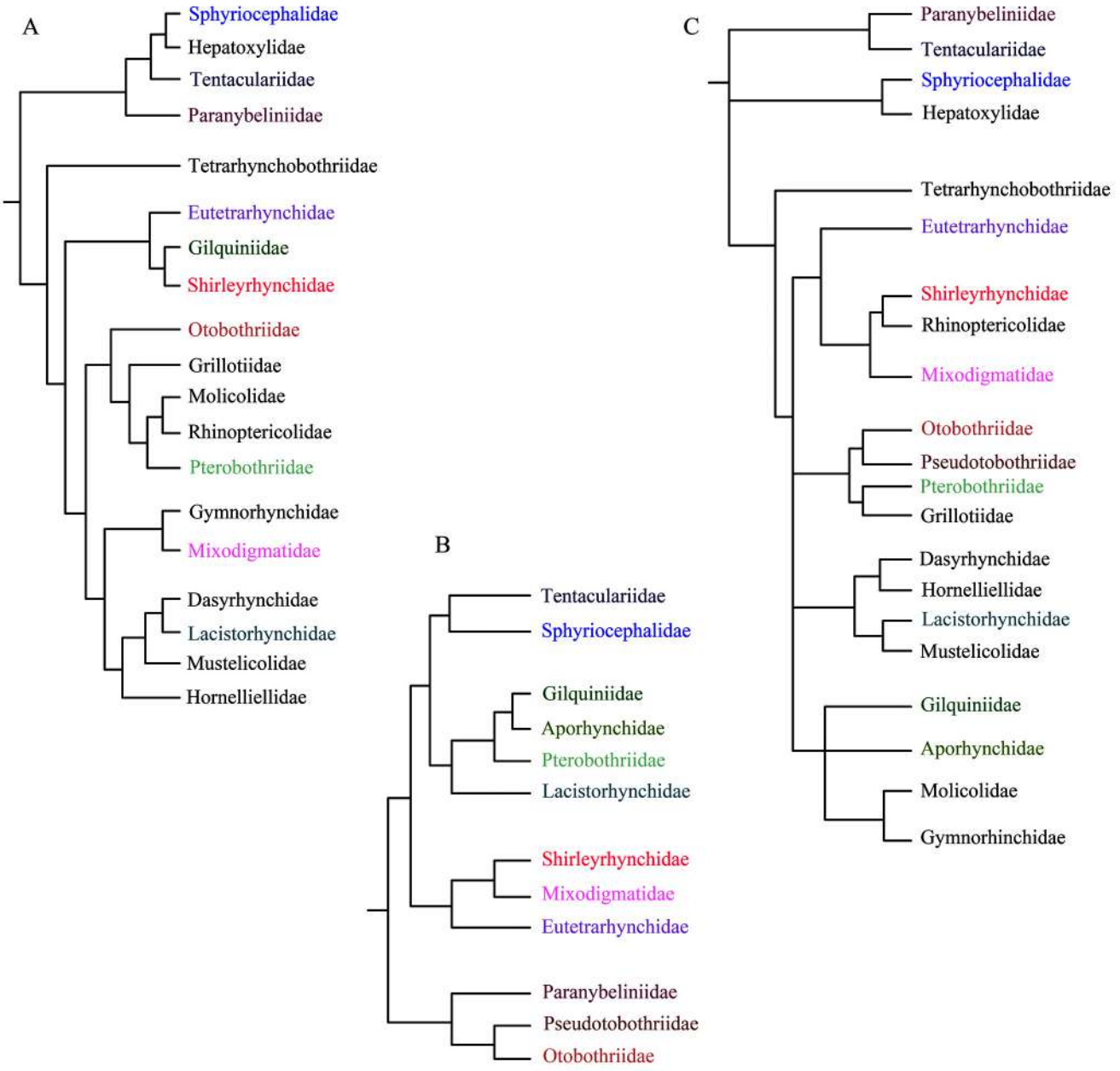

Figure 5. Classification of the order Trypanorhyncha to the family level using morphological characters. A) Hypothesis by Campbell and Beveridge, 1994; B) Hypothesis by Palm, 1997; C) Hypothesis by Beveridge et al., 1999. Source: Modified from Beveridge et al., 1999.

plerocercus, or merocercoid) generally helps identify the species because the adult hook pattern is evident. Some trypanorhynchs use a paratenic host following the final intermediate host; this paratenic host serves to bridge the food web between types of organisms that normally do not come into contact with one another (Palm et al., 2017). As adults, trypanorhynchs parasitize the spiral intestine of sharks and rays (Palm et al., 2017).

General Characteristics of Each Superfamily of the Order Trypanorhyncha

Since its inception, the order Trypanorhyncha has had many changes in its taxonomic classification (Campbell and Beveridge, 1994; Palm, 1995; 1997; 2004; Beveridge et al., 1999). However, Beveridge and colleaagues (2017) can serve as a basis for a summary of the general characteristics of the superfamilies of trypanorhynchs, as follows.

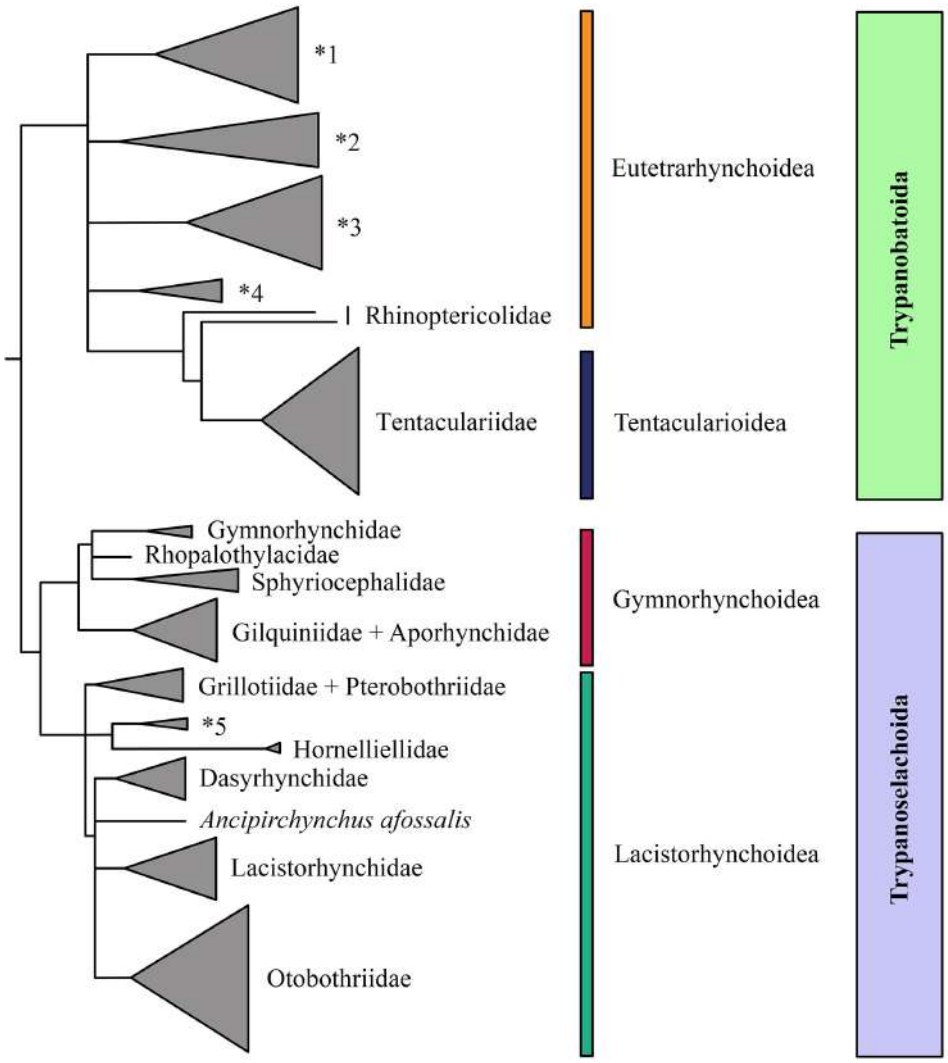

Figure 6. Schematic molecular phylogeny of Trypanorhyncha with recognized suborders, superfamilies, and families. Note: The clades marked with an asterisk * followed by a number are new clades for which there is no existing a name for the family. Source: Modified from Beveridge et al., 2017. License: CC BY-NC-SA 4.0.

Currently, the order Trypanorhyncha is divided into 2 suborders: **Trypanobatoida** and **Trypanoselachoida** (Olson et al., 2010). The suborder Trypanobatoida is divided into 2 superfamilies (**Eutetrarhynchoidea** (6 families) and **Tentacularioidea** (1 family)) and the suborder Trypanoselachoida is also divided into 2 superfamilies (**Gymnorhynchoidea** (5 families) and **Lacistorhynchoidea** (8 families)).

Superfamily Eutetrarhynchoidea

The species within this superfamily are characterized by the presence of prebulbar organs and gland cells within the bulbs. It comprises 118 species within 26 genera (Table 2). Of these genera, only the species within *Prochristianella* do not have prebulbar organ glands and gland cells within the bulbs.

Superfamily Tentacularioidea

The species within this superfamily are characterized by a ventro-submarginal genital pore and a uterus that develops laterally from the end of the uterine duct. There are 55 species within the 8 genera of the superfamily (Table 3).

Superfamily Gymnorhynchoidea

The species within this superfamily are characterized by the retractor muscle originating near the middle of the tentacular bulb (Olson et al., 2010) and, typically, a heteroacanthus armature (Palm, 2004). There have been 35 species reported within 14 genera (Table 4).

Superfamily Lacistorhynchoidea

The species of this family possess a hermaphroditic duct. It is composed of 107 species within 33 genera (Table 5).

Phylogenetic Relationships of the Trypanorhyncha

The relationship between Trypanorhyncha and the other orders of cestodes is not clear (see Brooks et al., 1991; Hoberg et al., 1997; Mariaux, 1998). One of the first to try to relate the species of Trypanorhyncha was Dollfus (1942), who considered the number of bothridia and the tentacular armature to be the most important characters. Succeeding works (Beveridge and Campbell, 1988; Campbell and Beveridge, 1994; Palm, 1995; 1997) have culminated in the preliminary cladistic analysis for the order by Beveridge et al. (1999). However, this provided evidence that conflicted with previous classifications (Figure 5). The hypothesis presented by Beveridge and colleaguea (2017) detailing the relationships of species of Trypanorhyncha uses molecular data from various species from each of the superfamilies (Figure 6). The classification provided in this chapter is based on the hypothesized relationships of that study. These authors (Beveridge et al., 2017) have suggested that this order of cestodes requires a more detailed review using both molecular and morphological characters. To learn more about the phylogenetic hypotheses, ecology, or biogeography of different groups of helminth parasites, the work of Brooks and McLennan (1991; 1993; 2002) covers these aspects in greater detail.

Zoogeography

The distribution of each species of parasite is determined by and limited, at least in part, to the distribution of its host or hosts. The trypanorhynchs are, obviously, restricted to the localities where elasmobranchs are distributed. This linked relationship between the distributions of host and parasite is a continuing area of study (see, for example, Brooks and McLennan, 1991, for further information on host-parasite coevolution and cospeciation). According to Last and colleagues (2016), there are 34 families comprising approximately 516 valid species of sharks and 26 families of rays with 636 valid species. The number of species of trypanorhynchs that have been reported from those species of elasmobranchs is low and this information shows the relative scarcity of known species that parasitize elasmobranchs. This suggests that a very large amount of work with these groups remains to be done. Let's get to it!

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Eucestoda

Cathetocephalidea Schmidt and Beveridge, 1990

(Order)

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Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Cathetocephalidea

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Chapter 24

Cathetocephalidea Schmidt and Beveridge, 1990 (Order)

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Introduction

Species in the order Cathetocephalidea Schmidt and Beveridge, 1990 are segmented worms and are parasites of the spiral intestine (also called the spiral valve) of sharks. Despite their low species richness, they have an almost cosmopolitan distribution. Cathetocephalidea is one of the 19 orders constituting the class Cestoda (Platyhelminthes). Their name is derived from the Greek terms **kathetos** (= perpendicular) and **kephalē** (= head), which refers the position of the fleshy fixation organ (**scolex**) with respect to the body (**strobila**).

This order was proposed by Schmidt and Beveridge (1990) based on the uniqueness of the scolex of the 2 species known of the family Cathetocephalidae Dailey and Overstreet, 1973 although the strobila and proglottids are similar to those in the orders Tetraphyllidea, Trypanorhyncha, and Lecanicephalidea. However, Euzet (1994) maintained it at the family level and included in the Tetraphyllidea. Currently, the validity of the order was verified using molecular data (Caira et al., 2005).

This order contains the families **Cathetocephalidae** and **Disculicepitidae**. Cathetocephalidae comprises the genera *Cathetocephalus* (with 3 species) and *Sanguilevator* (with 1 species). The genus *Disculiceps* (with 2 species) is included in Disculicepitidae. In addition, 5 taxa are descriptions (nomina nuda = nude names), meaning that a species name was published without the designation of type specimens nor were sufficient data given for valid descriptions (Caira et al., 2017).

Main Morphological Characteristics

The body of individuals within the order Cathetocephalidea Schmidt and Beveridge, 1990 are polyzoic (that is, the strobila is composed of more than 1 proglottid) and are of moderate size, 23 mm-long in *Sanguilevator yearsleyi* and up to 134 mm-long in *Cathetocephalus resendezi*, according Caira and colleagues (2005). The scolex is fleshy and simple (meaning, lacking suckers, bothridia, or armature), is perpendicular to the axis of the strobila, and is T-shaped (except in species of *Disculiceps* spp., in which it is round in cross section). The scolex is divided into 2 regions: An apex that is cushioned with a rugose base (and which is referred to as a collar in species of *Disculiceps*). The anterior region of the scolex in *Cathetocephalus* and *Sanguilevator* possesses bands of minute papillae in the middle portion, but which are absent in *Disculiceps* (Nock and Caira, 1988). A distinctive trait of the scolex of *Sanguilevator* is the presence of 3 dorsoventral pairs of spherical chambers and 2 pairs of elongate transverse channels (1 dorsal and 1 ventral, with numerous lateral posterior branches) located in the center of the scolex proper (Caira et al., 2005; 2017).

The strobila may be fixed to the scolex in any position of the bottom surface of the rugose base. It is acraspedote, that is, without velum (see Palm, 2004), except in *Cathetocephalus australis* whose proglottids have velum, that is, they are slightly craspedote (Dailey and Overstreet, 1973; Schmidt and Beveridge, 1990; Euzet, 1994; Caira et al., 2005; 2017). Most of the species are euapolytic, meaning that there is detachment of the mature proglottids when the eggs are infective (Khalil et al., 1994) or anapolytic, meaning that proglottids remain on the strobila until they senesce and eventually degenerate (Caira et al., 2016), although anapolysis is only observed in both species of *Disculiceps* (Nock and Caira, 1988). The mature proglottids are longer than they are wider in *Cathethocephalus* and *Sanguilevator* and are almost square-shaped in *Disculiceps* (Caira et al., 2017).

Specimens within the order Cathetocephalidea Schmidt and Beveridge, 1990 are hermaphroditic, with numerous testes, varying from 77 (in *Sanguilevator yearleyi*) to 500 (in *Cathetocephalus thatcheri*). The cirrus sac is bent anteriorly and the cirrus is armed. The genital pore alternates irregularly, and is marginal, except in *Disculiceps*, and is equatorial, except in *Cathetocephalus* (where it is post-equatorial). The ovary is bi-lobed and the vagina opens anterior to the cirrus sac at the genital atrium. The uterus is medial and is weakly branched, becoming sacciform in some species. In both species of *Disculiceps,* the uterus opens by longitudinal dehiscence (Nock and Caira, 1988). The vitelline follicles are circum-medullary in cross section. The eggs are clustered in cocoons (Nock and Caira, 1988; Schmidt and Beveridge, 1990; Caira et al., 2005).

Description and Summary of a Representative Species

Note: This work is not intended for the purposes of zoological nomenclature.

***Cathetocephalus resendezi* Caira et al., 2005**

The worms are relatively large (29–134 mm-long) with 79–340 proglottids, and they are acraspedote and euapolytic. The body is covered by michrotriches (tegumentary projections with an apical electro-dense portion, following Chervy, 2009). The morphology of the scolex is described for the order, with the rugose base inconspicuous, covered by palmate microthrix. There is a papillate band with a folded base. The papillae are relatively short throughout the anterior one-half to two-thirds. Mature proglottids are longer than they are wider, bearing 128–285 testes arranged in a single layer. The cirrus sac is bent anteriorly, with blade-like spinitriches (which are a type of microthrix with > 200 nm in basal width; see Chervy, 2009). The genital pore is post-equatorial. The ovary is H-shaped in the ventral view. The vagina opens anterior to the cirrus sac at the genital atrium. The uterus is slightly sinusoidal. The vitellaria are follicular and distributed along the entire proglottid (see Caira et al., 2005).

Taxonomic summary.

Type host: Bull shark, *Carcharhinus leucas.*

Site of infection: Spiral intestine.

Type locality: Bahía de Los Ángeles (28° 85′ 50″ N, 113° 83′ 20″ W), Baja California, Gulf of California, Mexico.

Type specimens are listed here and additional details can be found in the original paper where this species was described (that is: Caira et al., 2005): Holotype (CNHE 5300); paratypes (CNHE 5301; USNM 96411; LRP 3717–3722).

Order Cathetocephalidea Schmidt and Beveridge, 1990 in Relation to Each Other

To date, 3 valid species are recognized in the genus *Cathetocephalus*: *Cat. thatcheri*, parasitizing the bull shark *Carcharinus leucas* from the Gulf of Mexico, United States (Dailey and Overstreet, 1973), *Cat. australis*, parasitizing the copper shark *Car. brachyurus* from Goolwa, South Australia (Schmidt and Beveridge, 1990), and *Cat. resendezi*, found in the spiral intestine of the bull shark *Car. leucas* collected in the Gulf of California, Mexico (Caira et al., 2005). The morphological differentiation among the 3 species of the genus is mainly based on features of the scolex: In *Cat. thatcheri* the papillae are slender and elongate, arranged in the distal third of the papillar band (versus the short, thick, and irregular papillae in *Cat. resendezi,* distributed from the distal one-half to two-thirds of the band). In the third species, *Cat. australis*, the papillae are disposed in 2 bands separated by a medial smooth band. In addition, the configuration of the rugose base of the scolex follows a gradient-like pattern, ranging from inconspicuous in *Cat. resendezi*, to slightly rugose in *Cat. thatcheri*, and conspicuous in *Cat. australis*.

Another distinctive feature is the presence of lobulated margins of the ovary of *Cathetocephalus resendezi*, which is unlike the other 2 species, in which continuous margins are evident (Dailey and Overstreet, 1973; Schmidt and Beveridge, 1990; Caira et al., 2005).

Despite the lack of bothridia and the presence of bands of papillae on its scolex, Cathetocephalidae was placed in the order Tetraphyllidea. However, Schmidt and Beveridge (1990) considered that these characteristics warranted the establishment of a new order for this family. Fifteen years later, Cathetocephalidea was the first order formally recognized since the disintegration of Tetraphyllidea, based on molecular evidence using the gene fragments 18S and 28S (Caira et al., 2005). Other closely related groups that derive from Tetraphyllidea are Phyllobothriidea and Onchoproteocephalidea, these being the sister taxa of Cathetocephalidea (Waeschenbach and Littlewood, 2017). Unlike Cathetocephalidea, specimens from both of those other orders have suckers, bothridia, or armature. In the phylogenetic analysis of Caira and colleagues (2014), Cathetocephalidea is closely grouped among the acetabulate orders of cestodes. Based on these results, the authors suggest the derived condition of the non-acetabulate scolex.

Life Cycles

To date, the life cycle of members of this order remains unknown. Notwithstanding, members of this group show a high affinity to Carcharhiniformes sharks, particularly

Carcharhinidae and Sphyrnidae. This host-parasite association seems to suggest the oioxenous (that is, a 1:1 relationship between parasite and host species) nature of these cestodes. According to the original description of the 6 species known for this order, their distribution is almost worldwide. However, Caira and colleagues (2017) pointed out that they have not been recorded from the Arctic and Southern Ocean marine realms as established by Spalding and colleagues (2007).

Unique Features of the Order Cathetocephalidea Schmidt and Beveridge, 1990

The multistrobilization (that is, the formation of multiple strobilae attached to a single scolex) observed by Dailey and Overstreet (1973) in *Cathetocephalus thatcheri* (occasionally with 14 to 24 strobilae per individual) seems to be an exclusive character of this species more than a general feature at the order level, since it has not been found in other members of this group and only reported in 1% of the specimens collected by Dailey and Overstreet (1973). According to these authors, a more detailed examination of this phenomenon must be conducted to determine if it represents a type of asexual multiplication or an abnormal condition of the specimens studied by them.

The accumulation of blood cells in the chambers and channels of the *Sanguilevator yearsleyi* escolex is a feature that distinguishes it within the Cathetocephalidea and the cestodes in general. According to Caira and colleagues (2005), there is no plausible explanation for how the cestodes separate the host's cells as well as what the purpose of this accumulation may be.

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Eucestoda

Diphyllidea van Beneden in Carus, 1863 (Order)

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Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Diphyllidea

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Chapter 25

Diphyllidea van Beneden in Carus, 1863 (Order)

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Introduction

This group of small and polyzoic cestodes inhabiting the spiral valve of elasmobranchs (most commonly, species of Carcharhiniformes, Myliobatiformes, Rajiformes, and Rhinopristiformes, according to Ivanov and Caira, 2013) is distributed worldwide. One of the most remarkable traits of this group is the presence of only 2 bothridia on the scolex (feature from which the name of this order derived: **di** (= 2, Latin) and from **phyllidium** (= leaf, Greek)) and a genital pore at the mid-ventral region. Despite the wide variation in the presence or absence and arrangement of the scolex structures, in general diphyllideans may bear an apical organ armed with hooks, lateral hooklets, a cephalic peduncle that may be armed with spines, and a corona of spines (Caira et al., 2013; 2017).

This order, proposed by van Beneden in Carus (1863), currently is widely accepted, although their validity has been controversial (see Caira et al., 2017). The monophyly of the order has been demonstrated by morphological data by Ivanov and Hoberg (1999) and ratified based on molecular data by Caira and colleagues (1999; 2013) and Waeschenbach and colleagues (2012).

This order contains only 1 family (**Echinobothriidae**) with 6 genera and 59 described species. *Echinobothrium* is the genus with the highest number of species (see Figure 1), with 33, followed by *Halysioncum*, with 16 species, *Coronocestus*, with 6 species, and *Ditrachybothrium*, with 2 species. The genera *Andocadoncum* and *Ahamulina* are monotypic (Caira et al., 2017).

Diphyllideans are cosmopolitan. According to Spalding and colleagues (2007), its members have been recorded in all marine realms.

Main Morphological Characteristics

Diphyllideans are polyzoic worms, relatively small in body size, ranging from 0.46 mm in *Echinobothrium weipaense* (Ivanov and Caira, 2012) to 95.3 mm-long in the largest species (*Ditrachybothridium piliformis*, see Faliex et al., 2000).

The scolex is composed of a pair of sessile bothridia (1 dorsal and 1 ventral), often bearing a corona of spines, a cephalic peduncle armed with spines, and hooks and lateral hooklets on the apical organ (Khalil, 1994; Caira et al., 2017). In contrast, the cephalic peduncle in *Ditrachybothridium* is short and unarmed and lacks apical hooks (Ivanov and Hoberg, 1999). The scolex is covered by spinitriches of different types, distributed in patterns that vary at the species level. The cephalic peduncle lacks spinitriches and filitriches are present in some species (Ivanov and Caira, 2013).

They have acraspedote and apolytic strobila (that is, those that release gravid proglottids). Their mature proglottids are longer than they are wider. A common trait among the species of this order is the arrangement of the hermaphroditic reproductive system with the genital pore placed on the mid-ventral line in the posterior part of the proglottid, a bilobed ovary in cross section located in the posterior margin, as well as an absence of uterine pores (Ivanov and Hoberg, 1999). Other reproductive characteristics shared by diphyllideans are the presence of a vaginal opening posterior to that of the unipartite cirrus sac, a cirrus with spinitriches, testes disposed in 2 columns anterior to the ovary (between 4–6 in *Halysioncum rayallemangi*, according to Tyler (2006) to 43–81 in *Ditrachybothridium piliformis*; see Faliex et al. (2000)), vitellaria in 2 lateral bands or circumcortical in cross section, and a saccular uterus (Caira et al., 2017). Eggs in most species are without filaments, and some have a polar

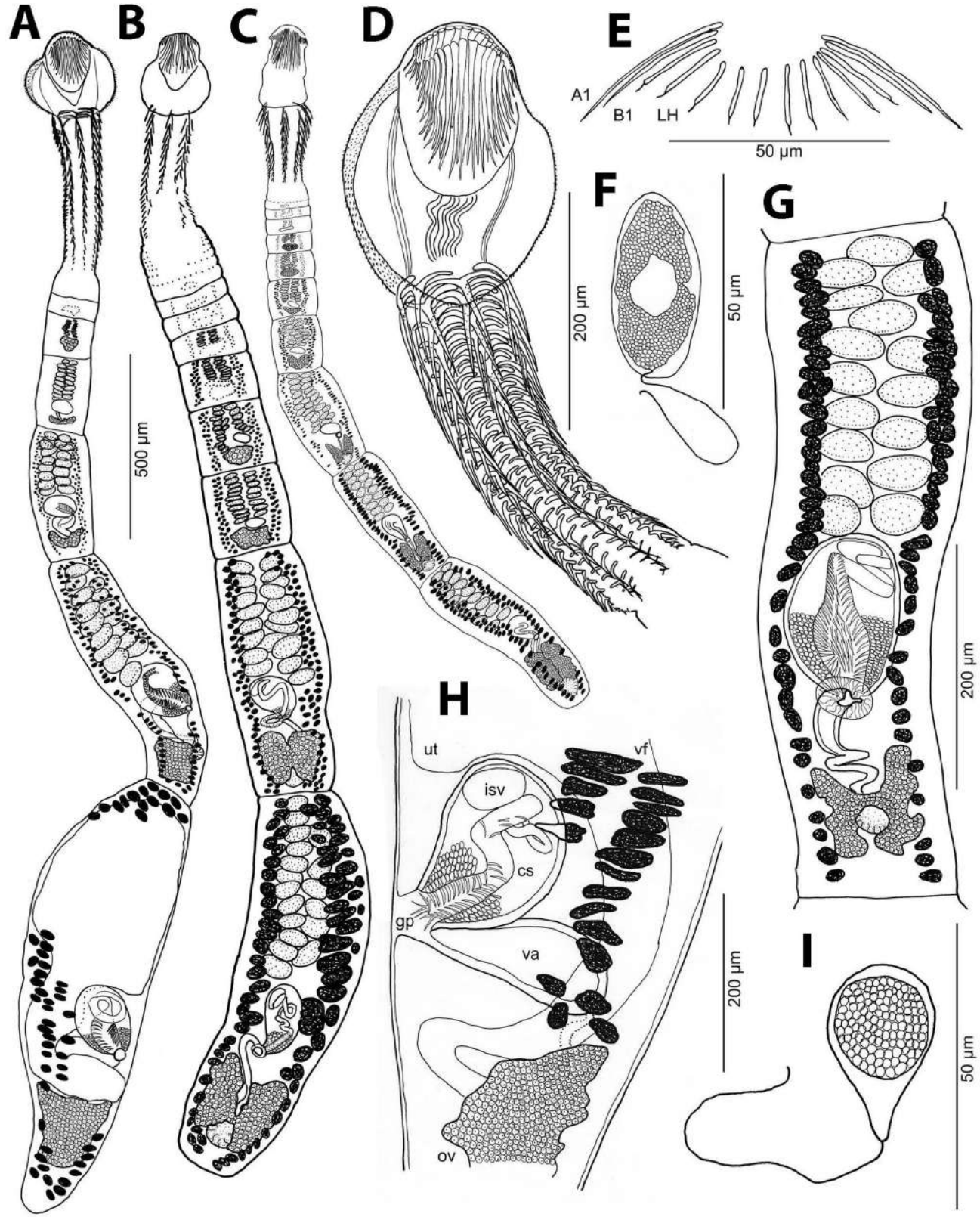

Figure 1. Line drawings. A, D–H) *Echinobothrium nataliae* A) Whole worm; D) scolex; E) L lateral hooklets; F) egg. G) mature proglottid; H) detail of terminal genitalia, lateral view; B) *Echinobothrium reginae*, whole worm; C, I) *Echinobothrium vojtai*; C) whole worm; I) egg. Abbreviations: A1) First A (anterior) hook; B1) fFirst B (posterior) hook; cs) cirrus sac; gp) genital pore; isv) internal seminal vesicle; LH) lateral hooklets; ov) ovary; ut) uterus; va) vagina; vf) vitelline follicles. Source: Kuchta and Caira, 2010. License: CC BY.

projection, such as *Echinobothrium harfordi*, and some have a polar filament, *Echinobothrium affine* (Ivanov and Hoberg, 1999; Tyler, 2006).

Description and Summary of a Representative Species

Note: This is not intended for the purposes of zoological nomenclature.

***Halysioncum mexicanum* (Tyler & Caira, 1999) Caira et al., 2013**

These are short-bodied worms (1.16–0.27 mm length), consisting of 4–10 segments, and are longer than they are wide as they reach maturity. Generally, the last segment is the widest portion of the body (0.11–0.44 mm). The scolex is constituted of 2 large oval bothridia (1 ventral and 1 dorsal) and an apical rostellum armed with 2 groups of 23 large apical hooks, 1 dorsal and 1 ventral. The hooks are flanked by 1 continuous row of 10–13 small lateral hooklets on each side. The surface of the bothridia is covered with palmate microtriches, with the number of digits varying along bothridia (3 or 4 at the anterior-most proximal area and 6 at the posterior region). They include short filiform microtriches spread along the proximal surfaces. The microtriches change from palmate to slender filiform abruptly, limiting the border between the distal and proximal surfaces of the bothridia. The cephalic peduncle is large and wide at the middle point, armed with 8 longitudinal columns of 23–40 spines with a triradiate base. The base length decreases from the anterior to the posterior region of the cephalic peduncle, which is covered by short filiform microtriches. The strobila is acraspedote, formed by 1–3 mature segments and 1 gravid proglottid. The testes (10–20 in number) are arranged in 2 or 3 columns at the anterior half of each segment. The cirrus sac is expanded, and is armed along its length with robust curved spines. The ovary is bi-lobed and H-shaped. The ovarian isthmus is stout. Mehlis' gland is prominent. The vagina is robust, ventral, and positioned immediately adjacent to the genital atrium. The genital pore is situated mid-ventrally. The uterus is dorsal, extending from the ovarian isthmus to the anterior margin of a gravid segment. A uterine pore is absent. The follicular vitellaria are arranged in 2 wide lateral bands uninterrupted along the proglottid, and are joined posterior to the ovary. There are small filamented eggs. The excretory ducts are lateral.

Taxonomic summary.

Type host: Snouted eagle ray, *Myliobatis longirostris*.

Site of infection: Spiral intestine.

Type locality: Bahía de Los Ángeles, Gulf of California, Mexico (28º 55′ N, 110º 25′ W).

Type specimens are listed here and additional details can be found in the original paper where this species was described: Holotype (CNHE 3343); paratypes (CNHE 3344–3345; USNPC 88220–88221; HWML 39912–39914).

Members of the Order Diphyllidea van Beneden in Carus, 1863 in Relation to Each Other

Of the 15 additional species described for the genus *Halysioncum*, *H. mexicanum* has 23 apical hooks on its scolex. Therefore, *H. mexicanum* can be distinguished from 6 of the other species because these have a smaller number of hooks: *H. fautleyae* (11 hooks), *H. pigmentatum* (20 hooks), *H. bonasum* (11 hooks), *H. hoffmanorum* (19–21 hooks), *H. californiense* (21 hooks), and *H. kishiense* (10–11 hooks), and from an additional 6 species because they have a greater number of hooks (between 25 and 29): *H. nataliae* (27–29 hooks), *H. reginae* (29 hooks), *H. vojtai* (29 hooks), *H. euzeti* (25 hooks), *H. megacanthum* (27 hooks), and *H. gibsoni* (27 hooks). Finally, the number of apical hooks on the scolex of *H. mexicanum* is similar to that of *H. raschii* (23–25 hooks) and identical to the number of hooks contained within *H. arafuerense* and *H. rayallemangi*. However, the length of the strobilus in *H. raschii* is considerably greater (8.6–21.5 mm) than that of *H. mexicanum* (1.16–5.27 mm) and the number of digits in the microtriches of *H. raschii* can be up to 15 while in the Mexican species its number ranges from 3–6. The number of spines of the cephalic peduncle is another trait that makes it possible to differentiate *H. arafuerense* and *H. rayallemangi* from *H. mexicanum*, since this number ranges from 20 to 24, 2 to 5 and 23 to 40, respectively. Additionally, the number of testes of *H. rayallemangi* is considerably lower (4–6) than that of *H. mexicanum* (10–20) (see Tyler, 2006; Kuchta and Caira, 2010; Ivanov and Caira, 2013; Moghadam and Haseli, 2014).

In the first phylogenetic study about the intrageneric relationships of Diphyllidea, based on morphological traits, Ivanov and Hoberg (1999) recognized monophyly of the order. However, the results suggested that 2 of the 3 formerly recognized genera (*Macrobothridium* and *Echinobothrium*) could be considered synonyms, validating the independence of *Ditrachibothridium*, a proposal ratified by Tyler (2006). The molecular confirmation of this hypothesis was made by Caira and colleagues (2013), who also erected 2 new genera (*Halysioncum* and *Coronocestus*) based on species previously included in *Echinobothrium*. In the same work, the authors identified a new genus of parasite provisionally termed *Leucoraja*, which was formally described a year later and named *Andocandoncum* (Abbott and Caira, 2014).

Life Cycle

The complete life cycle of species of Diphyllidea is poorly known (Tyler, 2001; Bray and Olson, 2004). According to Caira and Reyda (2005) the diphyllidean cestodes follow the same pattern of life cycles as other elasmobranch cestodes. In this pattern the life cycle appears to involve 2 intermediate hosts and 1 elasmobranch as the definitive host. The intermediate hosts are species of Mollusca, Arthopoda, and Actinopterygii. There are many records of larvae in teleost fishes and invertebrates such as crustaceans and molluscs (Bray and Olson, 2004). For example, Cake (1976) reports larvae from 1 species of *Echinobothrium* from the gastropod *Cantharus cancellaerius* and *Narrasius vibex* from the northern Gulf of Mexico; Jones and Beveridge (2001) collected a single plerocercoid of *Echinobothrium chisholmae* from the decapod *Penaeus longistylus* from Heron Island, Queensland, Australia, and Muñoz and colleagues (2001) found larvae in the intestine of the fish *Notothenia* c.f. *angustata* in the Gulf of Arauco, Chile. The adults of this order parasitize mainly batoids (skates and stingrays) (Tyler, 2001) although some species of *Coronocestus* have been recorded in sharks of the genera *Mustelus* and *Iago* (Ivanov, 1997; Haseli and Azad, 2015). Finally, Tyler (2006) suggests that diphyllidean species follow this pattern of life cycle: Eggs are shed with the feces of the definitive host, and posteriorly ingested by a first intermediate host (an invertebrate) such as an amphipod. In the intestine, the eggs hatch, releasing a hexacanth larva which then develops a procercoid. Then, the amphipod is ingested by the second intermediate host (a crab or a shrimp). Into this host the procercoid encysts in the liver and develops a plerocercoid. Finally, this stage is eaten by a definitive host (a shark or batoid) in which the cestodes reach sexual maturity. In some cases, the plerocercoid can be ingested by another type of host (a teleost fish), acting as a paratenic host (which is an organism that carries the immature stage of parasites).

Additional Notes about the Morphology

Diphyllidea and Trypanorhyncha are the only 2 orders of parasites of elasmobranchs in which metacestodes harbored by the last intermediate host bear the diagnostic taxonomic characters of the adult scolex (Beveridge et al., 2017; Caira et al., 2017). This allows identification of the metacestodes to the species level using morphology only.

The morphology of the scolex in Diphyllidea shows a wide range of modification in terms of the presence or absence and arrangement of structures. These variations oscillate from the total absence of spines in the cephalic peduncle and hooks in the scolex-proper of *Ditrachibothridium* (Faliex et al., 2000), to the lack of spines on the cephalic peduncle in some species of *Echinobothrium* and *Ahamulina* (Tyler, 2006; Marques et al., 2012), or may have between 100 and 107 spines along each of 8 longitudinal rows disposed in the cephalic peduncle, as is found in *Halysioncum euzeti* (Campbell and Carvajal, 1980).

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26

Eucestoda

Lecanicephalidea Hyman, 1951 (Order)

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Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Lecanicephalidea

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 26

Lecanicephalidea Hyman, 1951 (Order)

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Introduction

Lecanicephalidea (the name derived from Greek, **lekane** = dish or pot and **kephalē** = head) is an order of cestodes remarkably diverse in its morphology. They are mainly parasites of the spiral intestine of batoid elasmobranchs distributed around the world (Jensen et al., 2016). The main diagnostic trait of this group is the presence of an apical structure on the scolex, called a myzorhynchus or, more recently, termed the apical organ, which is found in a wide variety of forms. Other important characteristics of this group include: The presence of 4 suckers (also termed bothridia), proglottids with the vagina opening posterior from the cirrus sac into the genital atrium (Jensen et al., 2017), and a sizeable vas deferens often expanded into a sacciform external seminal vesicle that extends from the level of the ovarian isthmus to the cirrus sac (Jensen et al., 2016).

They were discovered in the 1890s. The first valid species described for this order was *Polypocephalus radiatus* Braun, 1897; however, the ordinal status of Lecanicephalidea has been questioned (their elevation to this level was even invalidated by Butler (1987)) and its species were often included in the order Tetraphyllidea (Jensen et al., 2017). Currently, based on molecular data analyses, Lecanicephalidea is considered the earliest diverging lineage among the acetabluate cestode orders (Jensen et al., 2017).

According to Jensen and colleagues (2017), Lecanicephalidea contains 8 families with 29 genera and 90 described species, as well as 7 incertae sedis species and 66 species inquirendae. *Polypocephalus* is the genus with the highest number of species (16 species; see Figure 1), while *Adelobothrium*, *Cephalobothrium*, *Collicocephalus*, *Rexapex*, *Anthemobothrium*, *Corrugatocephalum*, and *Quadcuspibothrium* are monotypic.

Main Morphological Characteristics

The strobila of this group of polyzoic cestodes is relatively small since the smallest worm measures less than 500 mm (Jensen, 2005) and only a few species have strobila measuring up to 6 cm, according to Butler (1987). Lecanicephalideans are generally euapolytic, but some species can be anapolytic, apolytic, and hyperapolytic. The proglottids

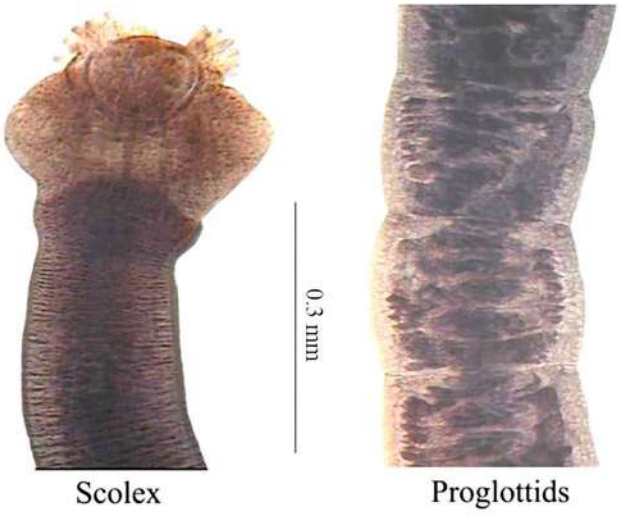

Figure 1. Scolex and proglottids of *Polypocephalus moretonensis* Butler, 1987, holotype specimen from the Queensland Museum, South Brisbane, Queensland, Australia. See https://www.gbif.org/occurrence/1066763010 for more information about this specimen. Source: Queensland Museum, 2023. License: CC BY.

tend to be craspedote (or may rarely be acraspedote) and may be laciniated (fringed in the posterior end) or not (Jensen et al., 2016).

With the exception of *Aberrapex* and *Paraberrapex*, which lack an apical structure in the scolex, the remaining lecanicephalideans are distinguished from most other orders of cestode parasites of elasmobranchs by having this structure (Jensen, 2005). The apical organ can be external or entirely internal; its morphology varies from a foldable sheet to an oval muscular pad or may present as an inverted cone with papilliform projections. In families such as Cephalobothriidae, there can be a glandular sphere. In others, such as Polypocephalidae, the apical organ is divided into tentacles. The tentacles can be retractable (or not) and some are invaginable. The scolex is also characterized by having 4 uniloculate acetabula or bothridia (and are biloculate only in Zanobatocestidae and diamond-shaped only in *Quadcuspibothrium*). Immature proglottids may be laterally expanded or not, and may form a trough (although only in Eniochobothriidae) (Jensen et al., 2016).

Reproductive Structures

Lecanicephalideans are hermaphroditic.

The female reproductive system is markedly heterogeneous; it consists of the following structures. It contains an ovary that is variable in form (it may be H-shaped, bi-lobed, tetra-lobed in cross section, digitiform, irregularly lobed with each lobe divided in 3 sub-lobes, etc.). It includes a vagina, which may be positioned medially, laterally, or sub-laterally (or may even be absent), opening into a genital atrium posterior to the cirrus sac. It includes a follicular vitellarium, generally arranged in 2 lateral bands. The vitellarium may reach the posterior end of the proglottids or only the anterior border of the ovary, and they do not exceed the anterior limit of the testicular field. The vitellarium may be distributed in 3 fields (1 posterior to the ovary, 1 between the genital atrium and the anterior margin of the ovary, and a field consisting of 2 lateral bands before the cirrus sac) or may present in 2 lateral bands from the middle of the cirrus sac to the level of the ovarian isthmus. It includes a uterus that is medial, saccate, or bisaccate (and constricted to the level of the genital atrium), and is variable in extent, from the anterior of the ovary to the genital pore, or almost occupying the entire length of the proglottid (Jensen, 2005; Jensen et al., 2016).

In contrast, the morphology of the male reproductive system is more homogeneous: The number of testes varies from 4 (in *Seusapex karybares*) to more than 40 (in *Tetragonocephalus kazemii*) that are distributed commonly in 1 to 2 columns, located anteriorly to the genital pore, ovary, or cirrus sac (Russell and Jensen, 2014; Jensen et al., 2016; Roohi and Malek, 2017). Internal and external seminal vesicles may be present or absent. The cirrus sac is pyriform (or elliptical in some Polycephalidae). The cirrus is unarmed (although it is armed in Tetragonocephalidae and Eniochobothriidae and rarely in Polycephalidae and Lecanicephalidae). The genital pore is lateral (or sub-lateral in Polycephalidae), alternating irregularly (Jensen, 2005; Jensen et al., 2016).

Description and Summary of a Representative Species

Note: This work is not intended for the purposes of zoological nomenclature.

***Aberrapex senticosum* Jensen, 2001**

These are small, euapolytic worms, 1.48–6.33 mm-long, with a maximum width of 31–38 mm at the ends of the strobila. The scolex consists of 4 bothridiated acetabula. There is apical modification of the scolex proper and an apical organ is absent. The acetabula and scolex proper are partially covered with large blade-like spiniform microtriches and long filiform microtriches. A cephalic peduncle is absent. The strobila has long filiform microtriches, becoming wider toward the posterior margins of the proglottids. The proglottids are craspedote and laciniate. There are 29–36 immature proglottids with 1 or 2 proglottids containing 20–40 testes arranged in a single field from the anterior margin of the proglottid to the anterior limit of the ovarian isthmus. The external seminal vesicle is wide and saccate, while an internal seminal vesicle is absent. The cirrus sac is pyriform and the cirrus is unarmed. The ovary is H-shaped in the dorsoventral view and tetra-lobed in cross section. It is also lobulated and symmetrical. The vagina runs laterally from the ootype zone to the genital pore; it is open posterior to the cirrus sac into the genital atrium. The genital pore is lateral, pre-equatorial, and alternates irregularly. The uterus is saccate, extending along the midline of the proglottid, almost reaching the anterior margin of the proglottid. A uterine pore is absent. The vitellaria are follicular, medullar, and lateral. The follicles are distributed along the entire length of the proglottid, only interrupted by the ovary (Jensen, 2001).

Taxonomic summary.

Type host: Bat eagle ray *Myliobatis californica* Gill, 1865 (Rajiformes, Myliobatidae).

Type locality: Santa Rosalía (27° 81′ 99″ N, 112° 81′ 79″ W), Baja California, Mexico.

Site of infection: Spiral intestine.

Type specimens are listed here and additional details can be found in the original paper where this species was described: Holotype (CNHE 4188) and 2 paratypes (CNHE 4189); 3 paratypes (USNPC 91208); 2 paratypes (HWML 16374); 7 paratypes (LRP 2152–2158).

Lecanicephalidea Hyman, 1951 Taxonomy

In addition to *Aberrapex senticosum*, 6 more species of the genus parasitizing myliobatiform batoids from tropical and temperate waters have been described to date: *A. arrhynchum* (Brooks, Mayes, and Thorson, 1981) Jensen, 2001; *A. ludmilae* Menoret, Mutti & Ivanov, 2017; *A. manjajiae* Jensen, 2006; *A. sanmartini* Menoret, Mutti & Ivanov, 2017; *A. vitalemuttiorum* Menoret, Mutti & Ivanov, 2017; and *A. weipaensis* Koch, Jensen & Caira, 2012 (Menoret et al., 2017). *Aberrapex senticosus* can be distinguished from the other species included in the genus since it has the highest number of testes (20–40 versus 18–25, 24–31, 10–19, 11–16, 15–21, and 10–17, respectively). In addition, *A. ludmilae* and *A. arrhynchum* lack an external seminal vesicle (while it is present in *A. senticosum*). In the remaining species, hastate spinitriches are entirely absent in the acetabular surface (*A. weipaensis*), restricted to the central region of the acetabula (*A. manjiajae*) or cover only two-thirds of the distal acetabular surface (*A. sanmartini* and *A. vitalemuttiorum*) while in *A. senticosus* hastate spinitriches cover the entire distal acetabular surface (Jensen, 2001; 2006; Koch et al., 2012; Menoret et al., 2017).

The first phylogenetic studies on lecanicephalids were based on morphological data (Caira et al., 1999; 2001; Jensen, 2005). In such studies, this group of cestodes was generally nested as a clade by the presence of an apical structure in the adult stage. When authors such as Jensen (2005) included some species lacking apical structure, they were positioned as the first divergent lineages of the order. Relative to its relationship with other orders of cestodes, Caira and colleagues (1999; 2001) detected possible affinities with cyclophyllideans.

Almost simultaneously, several works based on molecular evidence established Lecanicephalidea as the earliest lineage among the acetabulate cestode orders (Olson and Caira 1999; Olson et al., 2001; Caira et al., 2005; Waeschenbach et al., 2007).

The most recent and comprehensive analyses on the relationship among lecanicephalidean cestodes was conducted by Jensen and colleagues (2016); these authors confirmed the monophyletic nature of the order and recognized 8 major groups as independent families: 4 previously existing (Lecanicephalidae, Polypocephalidae, Tetragonocephalidae, and Cephalobothriidae) and 4 new families (Aberrapecidae, Eniochobothriidae, Paraberrapecidae, and Zanobatocestidae).

Life Cycles

Life cycles of cestodes of the order Lecanicephalidea are poorly known; however, according to Caira and Reyda (2005) larvae of these cestodes have been registered in some groups of invertebrates, mainly bivalves (molluscs) and crustaceans, as well as in few actinopterygians. Based on the scarce available information on the developmental stages of lecanicephalideans, Caira and Reyda (2005) suggested that they lack a coracidium (that is, a hexacanth embryo is inside the egg); plerocerci have been found in bivalves and gastropod molluscs and plerocercus, their terminal larval stage, in actinopterygians such as *Scomberoides commersonnianus* from the Arabian Gulf (Bannai et al., 2014).

Lecanicephalideans have circumglobal distribution; currently, members of this cestode order have been described from 8 of the 12 marine biogeographic realms, with the greatest concentration of species (69%) recorded in the central Indo-Pacific (Jensen et al., 2017).

Additional Notes about the Morphology

As noted above, Lecanicephalidea is an order of cestodes remarkably diverse in its morphology. For example, many lecanicephalideans possess additional features of proglottid anatomy that are unusual for other cestodes hosted by elasmobranchs (Jensen et al., 2017). For example, the genus *Hexacanalis* was erected by Perrenoud (1931) based on the presence of 6 excretory vessels, while the most common condition in the cestodes is the presence of 2 dorsal and 2 ventral excretory vessels. Jensen and colleagues (2016) pointed out that the different number of pairs of excretory vessels (1, 3, or more) is so particular, that it can be considered a diagnostic trait of the family Lecanicephalidae. In the same way, 1 species included in this genus (*Hexacanalis folifer*) is unique among lecanicephalideans by having a U-shaped ovary in cross section and proglottids with prominent posterior dorsoventral processes in the form of large lappets (Cielocha and Jensen, 2011).

On the other hand, despite the scarce knowledge about the gravid proglottids of the members of this order, it has been determined that the morphology of the eggs shows drastic variations, even among the congeneric species: In *Anteropora comica*, the eggs are covered with numerous small, regularly-spaced surface protuberances without polar filaments, while in *A. klosmamorphis*, the eggs have a corrugated surface and bipolar filaments (Jensen et al., 2011). Something similar occurs with the cocoons, since in some species (for example, *Zanobatocestus major*), cocoons contain only 2 eggs while in others (such as *Z. minor*), these are arranged in cocoons with hundreds of eggs (Jensen et al., 2014).

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Eucestoda

Litobothriidea Dailey, 1969 (Order)

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Brenda Atziri García-García, and Omar Lagunas-Calvo

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Litobothriidea

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Chapter 27

Litobothriidea Dailey, 1969 (Order)

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Introduction

The order Litobothriidea was established by Dailey (1969) to accommodate 2 new species recovered from the bigeye thresher shark *Alopias superciliosus*, from the California coast. This proposal was based on the unique holdfast features; according to Dailey (1969), the scolex consists of an apical sucker with an auxiliary holdfast modification of the anterior segments of the strobila. The name Litobothriidea, derived from the Greek word **lito** (= simple) and **bothros** (= trench), reflects the simplicity of the scolex. Caira and colleagues (1999) pointed out that the region posterior to the apical sucker can be constituted of up to 5 pseudosegments, a subset of which is cruciform in cross section.

Considering the 9 orders of cestodes parasitizing elasmobranchs, Litobothriidea is the second-least speciose group after Cathetocephalidea (constituting 6 species) (Caira et al., 2017b). The 9 species included in the order, all belonging to the genus *Litobothrium*, infect the spiral intestine of Lamniformes sharks from Mexico and Taiwan (Caira et al., 2017a) in the tropical eastern Pacific to the central Indo-Pacific marine ecoregions, according to Spalding and colleagues (2007).

Main Morphological Characteristics

They are medium-sized worms with a body length ranging from 1.65 mm (as in *Litobothrium alopias*) to 32.8 mm (as in *L. aenigmaticum*). The scolex comprises a single and well-developed apical sucker and 3–5 cruciform pseudosegments (but which is dome-shaped in *L. aenigmaticum* with an extensive cephalic peduncle and special tissue composition). Bothridia and a neck are absent. They have dorsoventrally flattened strobila with numerous craspedote proglottids (13–88 in number) that may be laciniated or not. They are apolytic, anapolytic, euapolytic, or extremely hyperapolytic (the latter a feature only of *L. aenigmaticum*). They are hermaphroditic with a single set of reproductive organs by segment, medullary located. The genital pores are lateral and alternate irregularly. The cirrus sac is pyriform, and the cirrus may be armed or not. There are numerous testes (15–84) that are medullary and preovarian, in general, arranged in 2 columns. They extend from the anterior end of the proglottid to the anterior margin of the ovary, rarely overpassing it. The vagina opens into the genital atrium anterior to or at the level of the cirrus sac. The ovary is usually an inverted U-shape and is medial and posterior. The vitellaria are follicular, encircling a medullary parenchyma, with the exception of *L. amsichensis*, in which it is circumcortical. The uterus commonly reaches the posterior margin of the cirrus sac and is armed at the base in *L. amsichensis*. The eggs do not reach the oncosphere stage while in the uterus (Dailey, 1969; 1971; Kurochkin and Slankis, 1973; Caira and Runkle, 1993; Olson and Caira, 2001; Caira et al., 2014a). The structure of the reproductive organs of *L. aenigmaticum* remain unknown because mature and gravid proglottids have not been found in specimens from that group (Caira et al., 2014a).

Description and Summary of a Representative Species

Note: This work is not intended for the purposes of zoological nomenclature.

Litobothrium amplificum (Kurochkin and Slankis, 1973) Euzet, 1994

These are cestodes with a short body (3.3–6.8 mm). The scolex consists of a cup-shaped apical and muscular sucker

and 4 cruciform pseudosegments. The first pseudosegment has inconspicuous dorsomedial and ventromedial projections; pseudosegments 2 and 3 have well-developed projections, and in the last pseudosegment, the projections are highly modified. The lateral margins are divided into 3 projections: 1 small central, 1 large dorsal, and 1 large ventral, and the last 2 are recurved medially. The first 2 pseudosegments are armed with a single row of spine-like structures that are embedded in its posterior margins.

The first 3 segments of the strobila are highly laciniated, with the laciniations of the first reaching the posterior end of the third segment. The body is covered with filitriches, which are longer in reproductive segments than those in the immature proglottids.

The strobila is euapolytic and consists of 13–19 craspedote segments, 12–19 immature segments that gradually become longer than they are wide and with 0–2 mature segments that are longer than they are wide. There are 53–84 oval to round testes. The cirrus sac is pyriform and extends approximately to the median line of the segment. The cirrus is highly coiled and is armed with spiniform microtriches. The vas deferens is anterior to the cirrus sac and is bifurcated prior to the ovary. The ovary is inverted, U-shaped, posterior, and bi-lobed in cross section.

The genital pore is located at 60–78% of the segment length from the posterior end and alternates irregularly. Mehlis' gland is posterior to the ovary in the segment. The uterus extends from the ovarian isthmus to the posterior margin of the cirrus sac. The vitellarium is follicular and is positioned across the length of the segment, interrupted by the ovary and cirrus sac.

Taxonomic summary.

Host: Pelagic thresher shark *Alopias pelagicus* Nakamura, 1935.

Site of infection: Spiral intestine.

Type locality: Gulf of Tehuantepec, Oaxaca, Mexico.

Additional localities: Bahía de los Ángeles (28° 55′ N, 113° 32′ W) and Santa Rosalía (27° 19′ N, 112° 17′ W), Gulf of California, Mexico.

Type specimens: Unknown.

This species was described by Kurochkin and Slankis (1973) as *Renyxa amplifica* from 2 specimens of *Alopias superciliosus* from the Gulf of Tehuantepec in Oaxaca, Mexico (but according to Olson and Caira (2001), this shark was misidentified and probably belongs to *A. pelagicus*). Subsequently, *Litobothrium amplificum* was redescribed by Olson and Caira (2001) based on 17 worms obtained from *A. pelagicus* from the Gulf of California. This new record extends the geographic distribution of this cestode.

Litobothriidea Dailey, 1969 Taxonomy

Litobothrium amplificum was originally described as a member of *Renyxa* by Kurochkin and Slankis (1973). However, Euzet (1994) considered this genus to be a synonym of *Litobothrium*.

Litobothrium amplificum can be distinguished from 5 of the 8 remaining species included in the genus by having 4 cruciform pseudosegments in the scolex while *L. amsichensis* (see Figure 1), *L. daileyi*, and *L. nickoli* each have 5, and *L. coniformis* and *L. gracile* each have 3 pseudosegments. *Litobothrium alopias* and *L. janovyi* share the same number of pseudosegments with *L. amplificum*; however, the fourth cruciform pseudosegment of *L. amplificum* has recurved laciniations and medial projections that are absent in the other 2 species (Olson and Caira, 2001). *Litobothrium aenigmaticum*, the most recently described species for the genus, differs from all the other species because it has a dome-shaped, grooved scolex, while in the other species, the scolex is constituted of an apical sucker and several cruciform pseudosegments without glandular tissue (Olson and Caira, 2001; Caira et al., 2014a).

The establishment of this order was strongly supported by molecular phylogenetic analyses that included broad sampling of cestodes belonging to several orders (Waeschenbach et al., 2012; Caira et al., 2014b). In both studies, litobothriideans were recovered as the sister taxon of the clade that includes the acetabulate cestode orders and as a monophyletic order. Intraorder relationships show that the clade formed

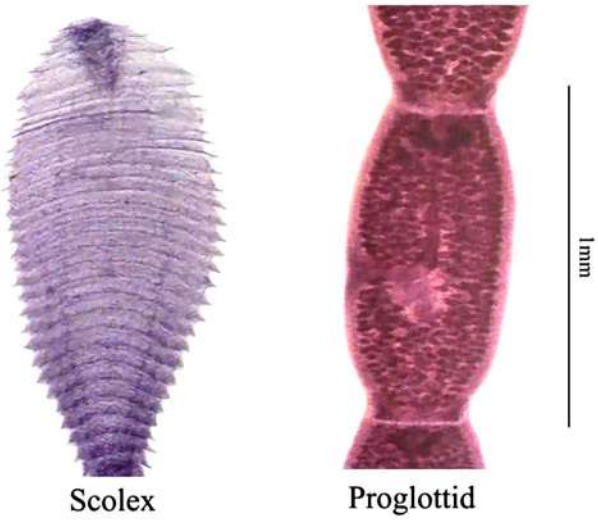

Figure 1. Scolex and proglottids of *Litobothrium amischensis* Caira & Runkle, 1993, holotype specimen from the Queensland Museum, South Brisbane, Queensland, Australia. See https://www.gbif.org/occurrence/1066761304 for more information about this specimen. Source: Queensland Museum, 2023. License: CC BY.

by *Litobothrium aenigmaticum* + *L. amplificum* was robustly supported as the sister taxon of *L. nickoli*. This is interesting because all the members of this clade parasitize pelagic thresher sharks and have *L. janovyi* as a sister taxon, whose host is a different species (the bigeye thresher shark). In this context, future molecular phylogenetic studies could reveal that *L. alopias*, *L. daileyi*, and *L. coniformis* are closely related to *L. janovyi* since they share the same host species. On the other hand, *L. gracile* (hosted by the sand shark) and *L. amsichensis* (a parasite of the goblin shark) could constitute independent groups of the other 7 species.

It is important to mention that the sequences of partial 28S rDNA (D1–D3) obtained from *Litobothrium aenigmaticum* and *L. amplificum* by Caira and colleagues (2014a) were identical; so, inclusion of other molecular markers is necessary for future studies.

Life Cycles

The life cycles of elasmobranch cestodes are practically unknown (Caira and Jensen, 2014); however, authors such as Caira and Reyda (2005) suggested that the life cycle of this group follows a pattern similar to other elasmobranch cestodes. The life cycle can include 2 or 3 intermediate hosts and larvae are trophically transmitted. In some cases, they can infect paratenic hosts (Caira and Jensen, 2014). Particularly, litobothriidean species only have been found parasitizing 4 species of lamniform sharks, among them members of Alopiidae (thresher sharks), Mitsukurinidae (goblin shark), and Odontaspididae (sand tiger sharks) (Caira and Jensen, 2014).

Additional Notes about the Morphology

The litobothriidean scolex consists of an apical sucker followed by a series of pseudosegments, a subset of which are cruciform (Caira and Jensen, 2014). However, these features are not present in most recently described species for the genus, namely, *Litobothrium aenigmaticum*. In contrast, this species exhibits a scolex consisting of a dome-shaped, grooved scolex proper and an extensive cephalic peduncle. In addition, the analysis of histological sections has revealed 4 distinct tissue types not seen in other litobothriideans.

When Caira and colleagues (2014a) described *Litobothrium aenigmaticum*, they pointed out that this species was the only hyperapolytic one so far in the order; nevertheless, these authors suggested that this material could represent a larval stage due to the lack of mature proglottids. The correspondence of microtriche distribution between adults and early juveniles corroborates that type specimens truly represent adult stages and ratify the hyperapolysis in this group for the first time (Caira et al., 2017a).

In spite of the remarkable morphological differences between *Litobothrium aenigmaticum* and the remaining 8 species included in this genus, molecular data robustly place it among the species in this order (Caira et al., 2014a).

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Eucestoda

Phyllobothriidea Caira et al., 2014 (Order)

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Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Phyllobothriidea

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Chapter 28

Phyllobothriidea Caira et al., 2014 (Order)

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Introduction

These acetabulate cestodes are parasites of the spiral valve or spiral intestine of sharks and occasionally batoid rays. Previously included in the Tetraphyllidea (Caira and Jensen, 2014), this order is named after the genus *Phyllobothrium* (from the Greek **phyllon** = leaf-shaped and **bothros** = trench) and was not formally recognized until the phylogenetic analysis with molecular data conducted by Caira and colleagues (2014). These worms are characterized by unarmed bothridia harboring apical suckers, their body size (which can be from small to medium), and their spectacular ornamentation on the scolex (Caira and Jensen, 2014; Ruhnke et al., 2017). Only the members of the former Phyllobothriidae are included in this order, bearing 73 species in 24 described and valid genera, plus 3 genera yet to be described. *Paraorygmatobothrium* is the most speciose and geographically widespread genus, with 25 formally described species and 4 still-undescribed taxa (Cutmore et al., 2017; Ruhnke et al., 2017). In general, these cestodes exhibit a cosmopolitan distribution, but the records are less common at higher latitudes (Caira and Jensen, 2014).

Main Morphological Characteristics

Phyllobothriidea Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014 are polyzoic worms of small to medium size. They are hermaphroditic. Most of the species are euapolytic or anapolytic; just a few exceptions are hyperapolytic. They may be craspedote or acraspedote with spinitriches restricted to the bothridial surfaces, often being serrate or gongylate. The neck and the strobilar surfaces are filled with filitriches distributed in scutes or in leaf-like structures. The scolex has 4 unarmed muscular bothridia and an anterior accessory sucker. Stalks are absent and an accessory sucker lacks lateral muscular projections. They do not include facial loculi, although some can show marginal loculi. Some species can present laciniated proglottids. There is 1 set of reproductive organs on each proglottid. They have 2 pairs of lateral osmoregulatory canals; in general, the ventral canals are wider than the dorsal ones. There are numerous testes, and a post-poral field is almost always present. The vas deferens is convoluted. An external seminal vesicle may be present or absent. The cirrus is armed with spinitriches. The genital pore is lateral and alternates irregularly, and is mainly located in the anterior half of the proglottid. The vagina opens anterior to the cirrus sac into the genital atrium. The vitellarium is follicular and the follicles are usually arranged in lateral fields, occasionally circumcortically or circummedullarly. The uterus lacks lateral diverticula (Ruhnke, 2010; Caira et al., 2014; Ruhnke et al., 2017).

Description and Summary of a Representative Species

Note: This work is not intended for the purposes of zoological nomenclature.

Paraorygmatobothrium prionacis (Yamaguti, 1934) Ruhnke, 1993

These are tetrabothridiated worms of medium body size (7.2–19.3 mm-long with a maximum width of 400–750 mm at the level of the scolex), and they are craspedote and apolytic. The number of segments is variable, from 11 to 29. The scolex measures from 430 to 620 mm-long and the apical area is covered with filitriches. The bothridia measure from 420 to 620 mm-long and 270 to 440 mm-wide. Each bothridium bears a single loculus and a round, anterior apical sucker (80–118 mm in diameter). The proximal surfaces of the bothridia

are covered with serrated spinitriches and filitriches. On the distal locular surface and distal surface of the apical sucker, the serrated spinitriches are slender and filitriches are also present. The neck varies from 1.8 to 4.6 mm-long; its dorsal and ventral surfaces are scutellated with small (< 500 nm) overlapping triangular structures covering the surface.

The mature segments are longer than they are wide (generally 3 times), with dorsal and ventral pairs of excretory ducts. A pair of nerve chords is situated laterally. The testes are arranged in 2 irregular longitudinal rows, from 2–4 in number in a horizontal row above the genital pore and 2–3 in number in a horizontal row below the genital pore; they are medullar, 1 row deep in cross section. The genital pores are lateral and alternate irregularly. The vagina is median, extending anteriorly the from ovary to the mid-level of the segment, then laterally along the anterior margin of the cirrus sac to the genital pore. The ovary is near the posterior end of the proglottid and is H-shaped in the frontal view and tetralobed in cross section. The uterus is ventral to the vagina and extends from the anterior margin of the ovary to the posterior margin of the cirrus sac in mature proglottids. A uterine duct is present, median, parallel, and dorsal to the uterus. The vitellarium is follicular and arranged in 2 lateral fields, each constituting 1–2 dorsal and 1–2 ventral columns, interrupted by the ovary and the cirrus sac. The eggs are spindle-shaped (Ruhnke, 2010).

Taxonomic summary.

Type host: Blue shark *Prionace glauca* Linnaeus, 1758 (Carcharhiniformes).

Site of infection: Spiral intestine.

Type locality: Pacific coast, Japan.

Type specimens deposited: Unknown.

Phyllobothriidea Caira et al., 2014 Taxonomy

The taxonomic history of this group has been difficult to ascertain since some of the genera and species were originally only loosely defined (Ruhnke, 2010). Morphologically, the genus *Paraorygmatobothrium* is defined by the possession of bothridia with a single apical sucker and an undivided oval posterior loculus; likewise, this genus has serrate gladiate spinitriches on the proximal bothridial surface, the subterminal and terminal proglottids are longer than they are wide, they possess post-vaginal testes, and have vitelline follicles that are distributed in 2 lateral fields (Cutmore et al., 2017). Among the 25 species that belong to the genus, *Paraorygmatobothrium prionacis* has an apical sucker of 80 to 118 μm in diameter, a range that is similar to those registered from *P. exiguum*, *P. janinae*, *P. triacis*, *P. sinclairtaylori*, and *P. ullmanni* (Ruhnke, 2010; Cutmore et al., 2017). From these 5 species, *P. prionacis* can be distinguished due to its body size (7.2–19.3 versus 35–46 mm in *P. triacis*); from *P. janinae*¸ because this species has more proglottids (59–104 versus 11–29). Two other species have remarkable differences in relation to the number of testes: *P. prionacis* only has from 34 to 62 testes, while the number of testes is higher (57–152 and 86–116 testes, respectively) in *P. sinclairtaylori* and *P. ullmanni*. Finally, it can be separated from *P. exiguum* by the position of the genital pore along the proglottids (from 74–83% in this species versus 48–59% in *P. prionacis*) (Ruhnke, 2010; Cutmore et al., 2017). According to Cutmore and colleagues (2017), it is essential to analyze the molecular data for the *Paraorygmatobothrium* genus in order to understand their relationships; the description of species on the basis of morphological data alone is considerably problematic. However, the last analysis published by them using molecular evidence, does not show this genus as a monophyletic group (see Cutmore et al., 2017).

Despite the fact that the molecular data analysis is derived from the elevation of this family to the order level, the phylogenetic relationships among the Phyllobothriidea with respect to the other acetabulated clades such as Onchoproteocephalidea, the residual tetraphilideans, and the clade that comprises mainly cestodes of terrestrial hosts (Cyclophyllidea, Tetrabothriidea, Nippotaeniidea, and *Mesocestoides*) requires further investigation and expansion of the collecting sites as well as an increase in the number of species examined because fewer than 30% of the valid taxa have been put into a molecular phylogenetic context (Caira and Jensen, 2014; Caira et al., 2014; Ruhnke et al., 2017; Waeschenbach et al., 2017).

Life Cycles

The Carcharhiniformes sharks harbor the majority of species of this cestode group, but they can also parasitize species of the Pristiophoriformes, Squaliformes, Orectolobiformes, and Lamniformes. Just a few phyllobothriideans have been found inhabiting the spiral valve of batoids (Myliobatiformes, Torpediniformes, and Rajiformes). Some authors have considered that this occurrence underlies a host-capture event, particularly since *Chimaerocestos* has been found to parasitize Chimaeriformes. In spite of the great richness of hosts, these worms are considered to be oioxenous (Ruhnke and Workman, 2013; Caira and Jensen, 2014; Caira et al., 2014; Ruhnke et al., 2017). The recent discovery of species of this group from the Southern African marine realm by Ruhnke and colleagues (2017) expanded the distribution to all 12 marine realms considered by Spalding and colleagues (2007), making this group of parasites cosmopolitan.

According to Caira and Reyda (2005), the life cycle of the marine cestodes lacks free-living stages and the transmission

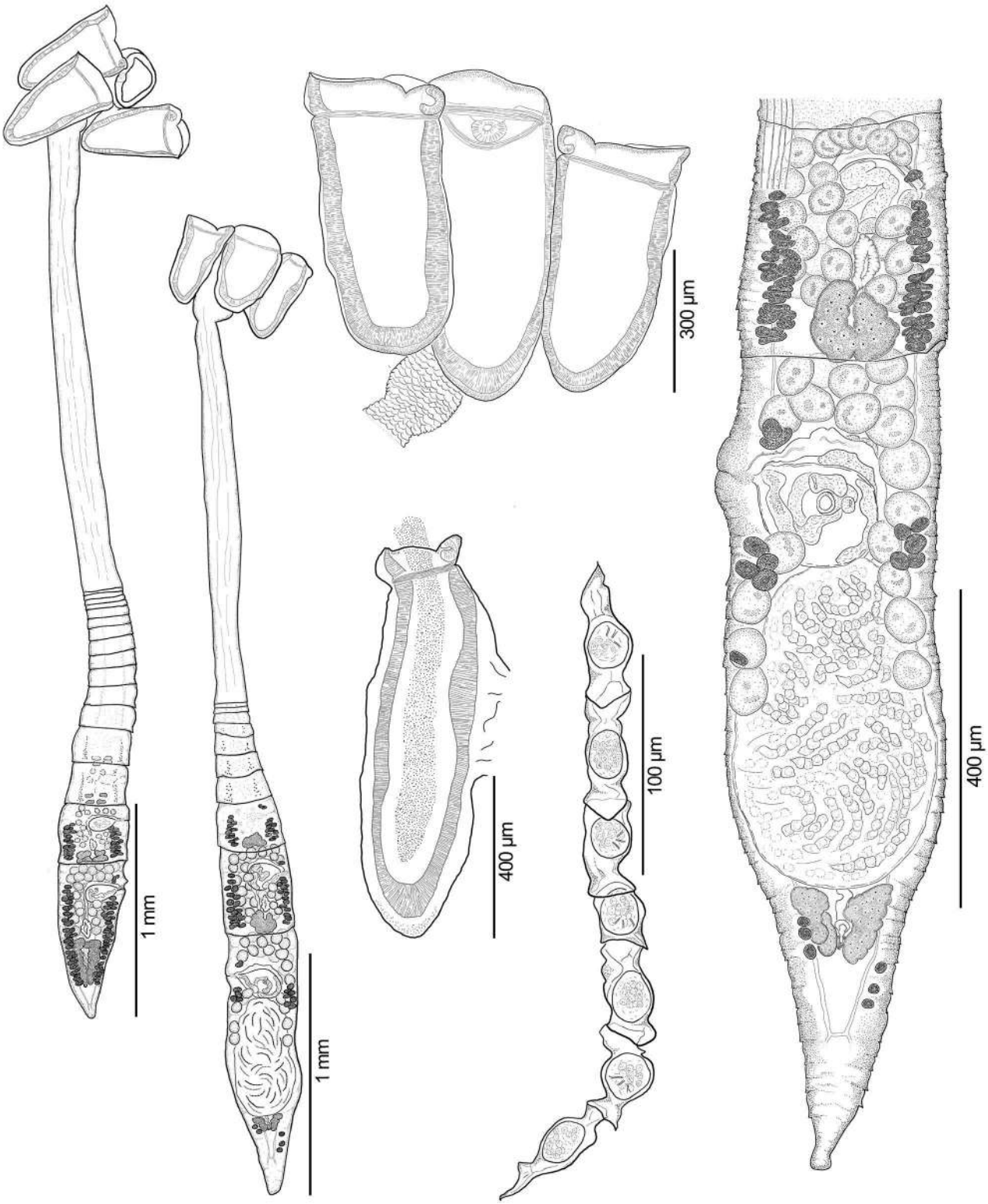

Figure 1. *Guidus francoi* sp. n. from *Bathyraja magellanica* (Philippi), line drawings. A) Entire mature worm (holotype MACN-Pa No. 739); B) entire gravid worm (paratype MACN-Pa No. 746/6); C) scolex (paratype MACN-Pa No. 743); D) bothridium attached to host tissue, muscular bothridial sphinchter contracted (paratype MACN-Pa No. 741/1); E) terminal portion of gravid strobila, ventral view (paratype MACN-Pa No. 746/6), longitudinal muscles partially drawn to allow the view of internal organs; F) cocoon. Source: Menoret and Ivanov, 2021. License: CC BY 4.0.

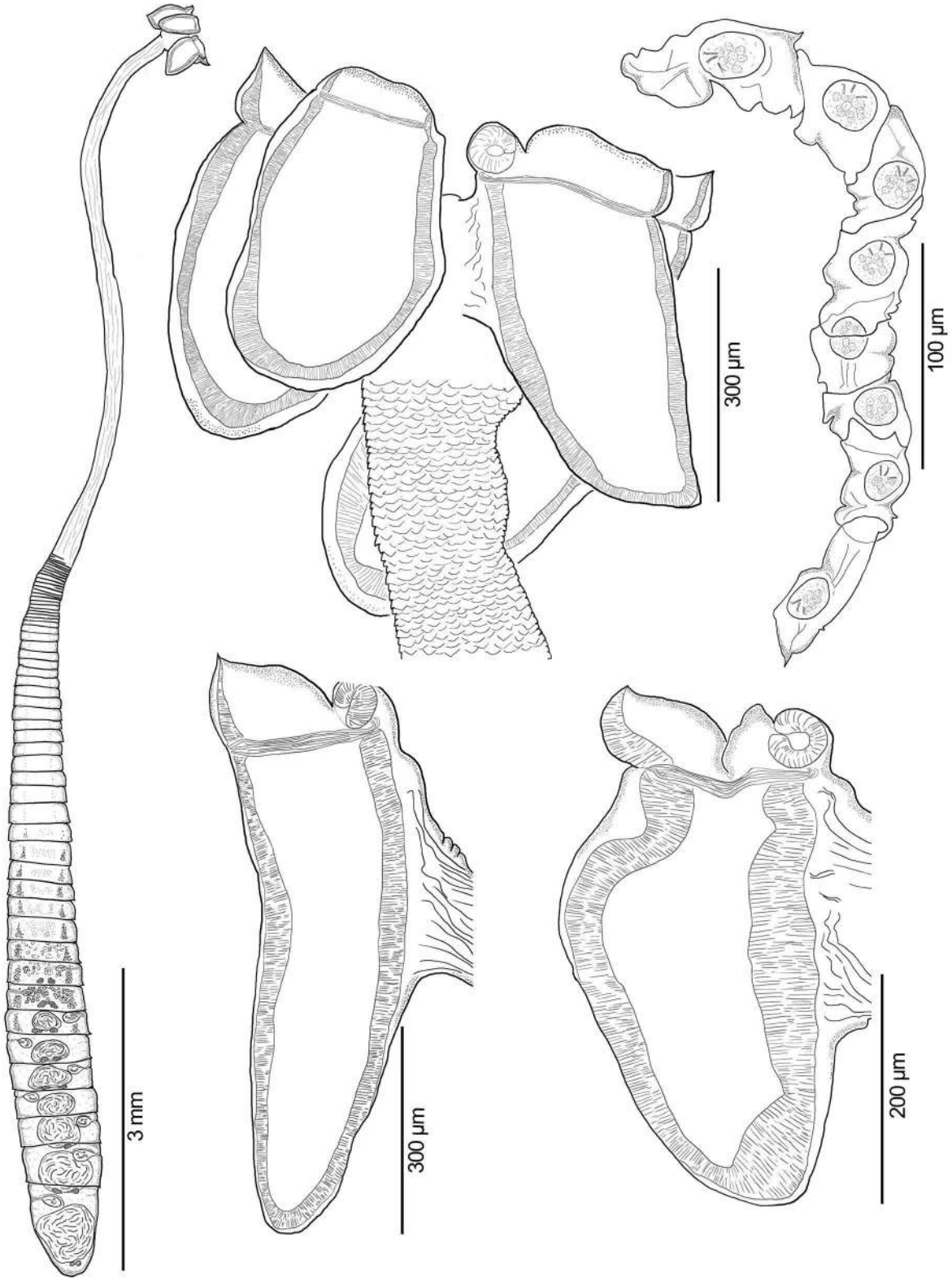

Figure 2. *Guidus magellanicus* from *Bathyraja magellanica* (Philippi), line drawings. A) Entire gravid worm (holotype MACN-Pa No. 747); B) scolex (holotype MACN-Pa No. 747); C) cocoon; D) bothridium, muscular sphincter relaxed (paratype MACN-Pa No. 748/2); E) bothridium, muscular sphincter contracted (paratype IPCAS No. C-888). Source: Menoret and Ivanov, 2021. License: CC BY 4.0.

between hosts depends on the particular food web dynamics. However, it is known that at least 2 or 3 intermediate hosts are involved. The work conducted by Jensen and Bullard (2010) allowed the identification of larval forms that could be assigned to what are now recognized as phyllobothriidean genera; these larvae were found only on teleost fishes that they considered to be acting as intermediate hosts involved in the life cycle of these parasites. Notwithstanding, recent observations have suggested the possibility that pinnipeds and cetaceans also serve as intermediate hosts of some species of this group, particularly in geographical regions where they represent the preferred prey of adult sharks (Klotz et al., 2018). This may help ratify the proposal about the high complexity of the web of intermediate-definitive hosts in the life cycle of these cestodes (Jensen and Bullard, 2010).

Additional Comments on the Taxonomy of the Group

There is no doubt about the position of this group as an order of elasmobranch-hosted cestode. As mentioned before, this group was named after *Phyllobothrium*, the type genus of Phyllobothriidae. Curiously, the taxonomic status of this genus remains problematic: The majority of species (21) are considered incertae sedis, and only 5 species are considered to be valid (including the type species *P. lactuca*) (Ruhnke, 2010; Ruhnke et al., 2017) (see Figures 1 and 2). Further investigation should be conducted on these species to understand their relationships inside the order.

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29

Eucestoda

Rhinebothriidea Healy et al., 2009 (Order)

Omar Lagunas-Calvo, Brenda Atziri García-García,

Berenice Adán-Torres, and Luis García-Prieto

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Rhinebothriidea

doi:10.32873/unl.dc.ciap029
2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 29

Rhinebothriidea Healy et al., 2009 (Order)

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Introduction

Species allocated to this order comprise small cestodes that occur in the spiral intestine (valve) of rays (Batoidea) recorded in marine and freshwaters around the world. Rhinebothriidea was historically included in the order "Tetraphyllidea" despite evidence that the members of this order represent an independent clade; however, the formalization of this order did not take place until the first decade of the 2000s (Healy et al., 2017).

Rhinebothriidea was created by Healy and colleagues (2009) based on molecular evidence. This analysis fully supports the monophyly of the rhinebothriideans, which was corroborated in subsequent works (Caira et al., 2014; Ruhnke et al., 2015; Marques and Caira, 2016). Currently, the presence of stalked bothridia is the only morphological synapomorphy of this group. Other morphological characters such as the presence of a cirrus armed with spinitriches, a follicular vitellarium, and a posterior ovary, have sometimes been considered to be important features to identify members of Rhinebothriidea; however, these traits are also found in other elasmobranch-hosted cestodes (Ruhnke et al., 2017) (for example, see Figure 1).

This order is composed of 4 families: **Anthocephaliidae**, **Echeneibothriidae**, **Escherbothriidae**, and **Rhinebothriidae**. The first family includes the genera *Anthocephalum* (with 22 species), *Barbeaucestus* (4 species), *Cairaeanthus* and *Divaricobothrium* (2 species each), and *Sungaicestus* (which is monotypic). The second family is composed of the genera *Clydonobothrium*, *Echeneibothrium, Notomegarhynchus*, *Pseudanthobothrium* (with 2, 50, 2 and 5 species, respectively) as well as the monotypic *Tritaphros*. Escherbothriidae is formed by the monotypic genus *Escherbothrium* and *Stillabothrium* (7 species). The last family contains 8 genera, 2 of them monotypic: *Biotobothrium* and *Crassuseptum*; *Rhabdotobothrium* and *Spongiobothrium* (with 2 species each); and *Rhodobothrium* and *Scalithrium* (including 7 species each). *Rhinebothrium* and *Rhinebothroides* are the most diverse genera of the family, with 49 and 8 species, respectively (Ruhnke and Seaman, 2009; Kornyushin and Polyakoya, 2012; Ruhnke et al., 2015; Reyda et al., 2016; Caira et al., 2017; Herzog and Jensen, 2018).

Main Morphological Characteristics

The body is composed of 2 or more proglottids (making it polyzoic). The proglottids are hermaphroditic, with the posterior margin overlapping the next proglottid (craspedote) or not overlapping (acraspedote). Most species are euapolytic, but some are apolytic or hyperapolytic. Each segment contains 1 set of male and female reproductive organs. There are lateral osmoregulatory canals which are arranged in 2 pairs; the ventral canals are generally wider than the dorsal canals. A neck is absent. The scolex is armed with 4 muscular simple bothridia. The bothridia are stalked, mostly lacking differentiable apical suckers, and may either include marginal and/or facial septa (as in *Anthocephalum and Echeneibothrium*) or not (for example, in *Stillabothrium cadenati*). A myzorhynchus is present (for example, in *Clydonobothrium*, *Echeneibothrium*, *Notomegarhynchus*, *Phormobothrium*, *Pseudanthobothrium*, and *Tritaphros*) or absent (for example, in *Barbeaucestus*, *Divaricobothrium*, and *Sungaicestus*). The male reproductive system usually contains numerous testes or (rarely) just 2 testes (for example, as in some members of the genus *Rhinebothrium*, such as *R. asymmetrovarium*, *R. biorchidum*, and *R. ditesticulum*). Post-poral testes are usually lacking. The vas

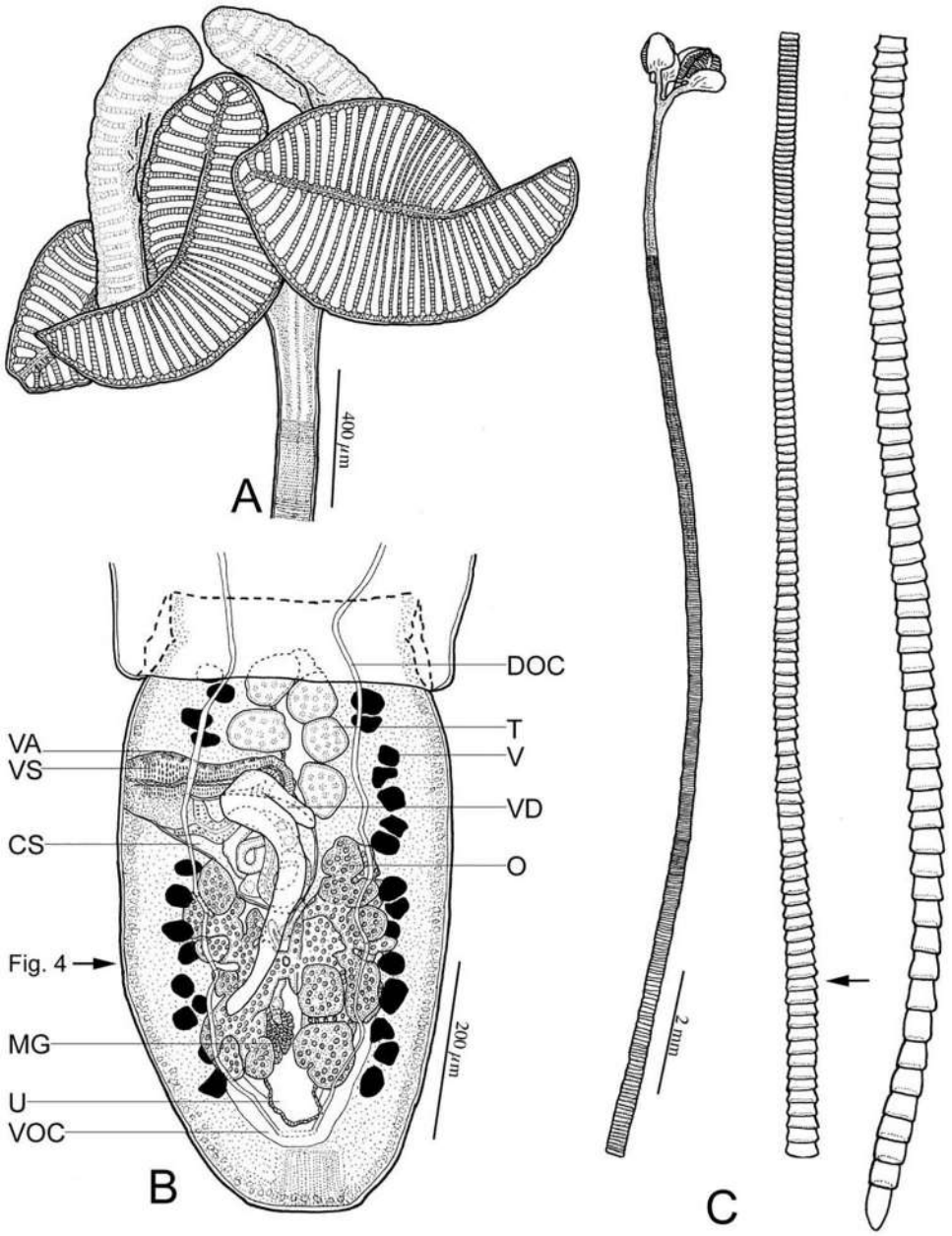

Figure 1. Line drawings of *Rhinebothrium paratrygoni* Rego & Dias, 1976 collected from the type locality. A) Scolex of voucher (MZUSP 6214); B) terminal mature proglottid of voucher (MZUSP 6214). The vas deferens is above the cirrus sac. The arrow indicates the location of the section shown in the portion labeled Fig. 4; C) whole worm of voucher (MZUSP 6260k), illustrated in 3 fragments, from left to right: Anterior, middle, and posterior. The arrow indicates the anterior most mature proglottid. Abbreviations: CS) Cirrus sac; DOC) dorsal osmoregulatory canal; MG) Mehlis' gland; O) ovary; T) testes; U) uterus; V) vitellaria; VA) vagina; VD) vas deferens; VS) vaginal sphincter; VOC) ventral osmoregulatory canal. Source: Reyda and Marques, 2011. License: CC BY.

deferens is convoluted. An internal seminal vesicle is absent, while an external one may be present or not. The cirrus has spinitriches. The genital pore is lateral, and alternates irregularly. The vaginal opening is anterior to the cirrus sac opening into a genital atrium. The ovary is posterior and bilobed in cross section (as in *Notomegarhynchus navonae*) or tetra-lobed in cross section (as in *Anthocephalum currani*). The vitellarium is follicular and the follicles are arranged in lateral fields, sometimes displaced towards the median line of proglottids. The uterus is tubular and lateral diverticula may be present or absent, without pre-formed uterine pores (Healy, 2006; Healy et al., 2009; 2017; Ruhnke et al., 2015) See Figures 2–4 illustrating some of the characteristics of an example of *Rhinebothrium* sp.

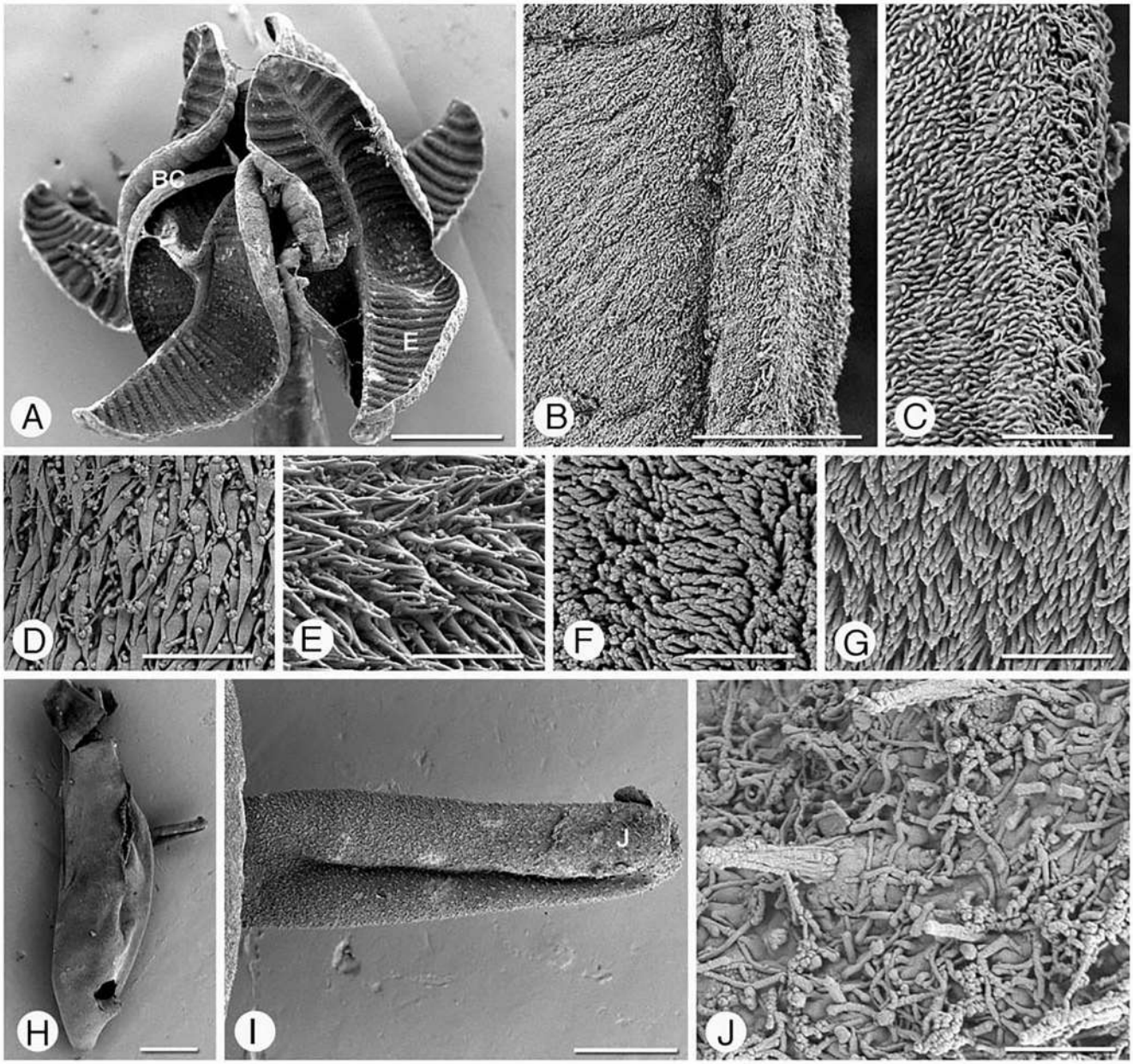

Figure 2. Scanning electron micrographs of *Rhinebothrium paratrygoni*. Scolex, Figures A–G. A) Scolex; B) small letter indicates the locations of details shown in B–C and E. Proximal surface of the rim of the bothridium; C) proximal bothridial surface adjacent to the bothridial rim; D) proximal bothridial surface; E) transverse septum on the distal bothridial surface; F) stalk surface; G) strobila surface. Cirrus, Figures H–J. H) Free proglottid with everted cirrus; I) everted cirrus. Small letter indicates location of detail shown in J; J) coniform spinitriches and capilliform filitriches on the distal portion of the cirrus. Scale bars: A = 200 mm; B = 10 mm; C–G = 2 mm; H = 200 mm; I = 50 mm; J = 2 mm. Source: Reyda and Marques, 2011. License: CC BY.

Species in this order can be distinguished from Amphilinidea and Gyrocotylidea by the shape of the scolex and due to the presence of a polyzoic body. Of the remaining 16 orders, Rhinebothriidea is separated by scolex conformation, since in this order, it bears 4 acetabulated and stalked bothridia (Healy et al., 2009).

Description and Summary of a Representative Species

Note: This work is not intended for the purposes of zoological nomenclature.

***Anthocephalum currani* Ruhnke and Seaman 2009**

These comprise small-bodied worms (6.6–14.4 mm-long)

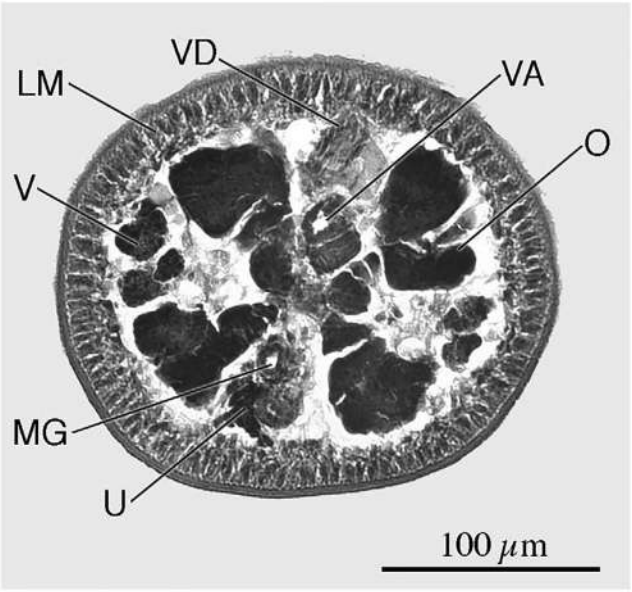

Figure 3. Cross section through a mature proglottid of *Rhinebothrium paratrygoni* at the level of the ovarian isthmus. Abbreviations: LM) Longitudinal muscles; MG) Mehlis' gland; O) ovary; U) uterus; V) vitellaria; VA) vagina; VD) vas deferens. Source: Reyda and Marques, 2011. License: CC BY.

composed of 35–70 proglottids that are slightly craspedote and apolytic. The scolex has 4 bothridia, 430–940 mm-wide. The bothridia are folded and pedicellate, each with 81–110 marginal loculi and a round anterior accessory sucker. The proximal surfaces of the loculi, bothridia, and bothridial rim are covered with spinitriches. Filitriches are present in the loculi and in the strobilar surface; the distal surfaces of the bothridia and accessory suckers covered also with slender spinitriches. The proglottids have the following measures: Immature (67–570 mm × 101–330 mm; length/width ratio 0.3–1.9:1), terminal and subterminal (580–1,700 mm × 134–410 mm; length/width ratio 1.9–9.4:1). The testes are oblong and are 37–50 in number at the terminal and subterminal proglottids, arranged in 2–4 irregular columns, completely anterior to the cirrus sac. The cirrus sac is posteriorly recurved with a coiled cirrus armed with spinitriches. The genital pores are lateral and alternate irregularly. The vagina is sinuous and anteriorly extends to the Mehlis' gland, then ventrally and laterally to the cirrus sac, and opens into the genital atrium anterior to the cirrus sac. The ovary is H-shaped in the frontal view and is tetra-lobed in cross section, and is located near the posterior end of the proglottid. The aporal lobe of the ovary is slightly longer than the poral lobe. The oviduct is spread out posteriorly to the level of Mehlis' gland and is ventral to it. The oviduct extends posteriorly to the level of Mehlis' gland and is ventral to it. The

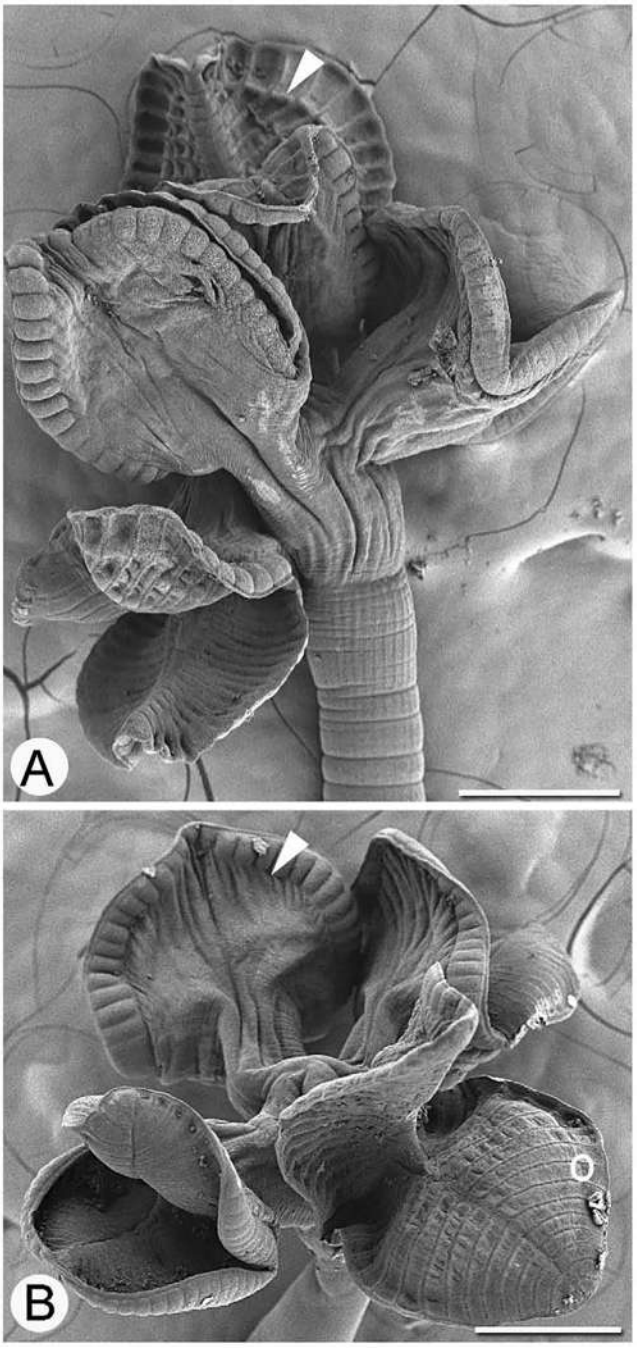

Figure 4. Scoleces of *Rhinebothrium copianullum*. A) Scolex in which marginal longitudinal septa are visible, indicated by the white arrow; B) scolex in which marginal longitudinal septa are visible on the proximal bothridial surface, indicated by the white arrow. The white circle indicates the position of the marginal longitudinal septum on the distal surface. Scale bar: A–B = 200 mm. Source: Reyda and Marques, 2011. License: CC BY.

ovicapt is ventral at the posterior margin of the ovarian isthmus. The uterus extends from the anterior of the cirrus sac to the anterior end of the mature proglottids and is ventral

to it. Two lateral bands of vitelline follicles are distributed from the anterior to the posterior end of the proglottid and the follicles are interrupted by the ovary. Each band consists of 3–5 dorsal and 3–5 ventral irregular columns of follicles (Ruhnke and Seaman, 2009).

Taxonomic summary.

Type host: Bullseye stingray *Dasyatis brevis.*

Site of infection: Spiral intestine.

Type locality: Punta Arena (24° 04′ N, 109° 50′ W), Baja California Sur, Mexico.

Type specimens are listed here and additional details can be found in the original paper where this species was described: Holotype (CNHE 6234); paratypes (CNHE 6235; USNM 100993–100994; LRP 4241–4244).

Rhinebothriidea Healy et al., 2009 Taxonomy

To date, the genus *Anthocephalum* includes 22 valid species and *A. currani* differs from the rest of members based on the presence or absence of a number of features. For example, it differs from *A. blairi*, *A. duszynskii*, *A. gravisi*, *A. hobergi*, *A. mounseyi*, and *A. odonnellae* in total length (6.6–14.4 versus 2.5–4.9, 18–31, 1.8–3.7, 28, 2.6–3.4, and 11.6–20.1 mm, respectively); from *A. alicae*, *A. blairi*, *A. cairae*, *A. centrurum*, *A. gravisi*, *A. haroldsoni*, *A. lukei*, *A. odonnellae*, *A. papefayei*, and *A. philruschi* in the number of marginal loculi (81–110 versus 57–80, 65–73, 197–198, 71–80, 43–52, 41–57, 107–138, 135–159, 45–60, and 200–219, respectively). *Anthocephalum currani* can be distinguished from 12 other species based on the number of proglottids, since *A. currani* specimens have between 35–70 proglottids while *A. alicae* have 9–15, *A. blairi* have 13–21, *A. cairae* have 80–100, *A. decrisantisorum* have 20–33, *A. duszynskii* have 120–160, *A. gravisi* have 368–831, *A. haroldsoni* have 17–29, *A. healyae* have 150–171, *A. mounseyi* have 7–10, *A. odonnellae* have 86–120, *A. papefayei* have 106–177, and *A. ruhnkei* have 11–30. In the same way, the great number of testes of *A. centrurum* (47–78) allows separating it from *A. currani*, which has 37–50 testes. Eleven other species have a smaller number of testes per proglottid than *A. currani*: *A. blairi* (10–15 testes), *A. decrisantisorum* (17–24 testes), *A. gravisi* (23–38 testes), *A. haroldsoni* (25–32 testes), *A. jensenae* (14–20 testes), *A. kingae* (30–37 testes), *A. meadowsi* (15–25 testes), *A. mounseyi* (24–34 testes), *A. papefayei* (6–9 testes), *A. philruschi* (17–25 testes), and *A. ruhnkei* (22–34 testes). It also differs from *A. centrurum* and *A. kingae* in ovarian length (161–360 mm versus 390–710 mm and 376–440 mm, respectively). Testes in *A. mattisi* and *A. michaeli* are arranged in 2 regular columns while in *A. currani* they are grouped in 2–4 irregular columns (Ruhnke, 1994; 2011; Zamparo et al. 1999; Ruhnke et al., 2015; Marques and Caira, 2016; Herzog and Jensen, 2018).

Anthocephalum is now included within the Rhinebothriidea since the order was established, along with the genera *Rhabdotobothrium*, *Rhinebothrium*, *Rhinebothroides*, *Scalithrium*, *Spongiobothrium*, *Echeneibothrium*, and *Rhodobothrium*. Although the monophyly of rhinebothriideans in relation to the other acetabular cestode orders was strongly supported by 3 types of phylogenetic analyzes and 3 data partitions, Healy and colleagues (2009) refrained from establishing relationships at the family level until such time as the analyses included a large sample of taxa to provide a more accurate assessment of intraordinary relationships (Ruhnke et al., 2015). The work of Ruhnke and colleagues (2015) not only includes the description of 8 new species for *Anthocephalum*, but also designated to the family each of the clades that resulted from its analysis based on molecular data.

The subfamily Echeneibothriidae was elevated to the family level to include the genera *Echeneibothrium* and *Pseudanthobothrium*. This clade is unique because the apical organ (myzorhynchus) is retained in the adult stage. Rhinebothriidae was elevated from the subfamily to the family level to group the genera *Rhabdotobothrium*, *Rhinebothrium*, *Rhinebothroides*, *Rhodobothrium*, *Scalithrium*, and *Spongiobothrium*. The lack of apical suckers and lack of a definitive anterior/posterior orientation of the bothridia distinguishes this family from the remaining families. Anthocephaliidae was erected to include the genus *Anthocephalum* along with 4 other genera not described before. Members of this family exhibit a conspicuous anterior/posterior orientation signaled by the presence of an apical sucker in the bothridia and they have marginal loculi or 1 or more rows of facial loculi, and have vitelline follicles that are, in general, interrupted by the ovary. Escherbothriidae is characterized by facial loculi arranged in columns anteriorly and rows posteriorly rather than arranged in multiple rows, or may be entirely lacking, such as in members of Anthocephaliidae. Escherbothriidae was proposed to include the genus *Escherbothrium* and 1 undescribed taxon.

Life Cycle

Cestodes included in Rhinebothriidea exclusively parasitize batoid elasmobranchs. Most of the species described have been recovered from Myliobatiformes (stingrays and eagle rays), and in a smaller number of Rajiformes (skates), Rhinopristiformes (sawfishes), and Torpediniformes (electric rays) (Rhunke et al., 2017). The life cycle of species in this order of cestodes is poorly known because the identification of larvae at the species level (using morphology) is practically

impossible as in other orders of elasmobranch-hosted cestodes. However, the results obtained by Jensen and Bullard (2010) using molecular and morphological data suggest that rhinebothriideans use some teleosts (members of Gadidae, Lobotidae, Paralichthyidae, Serranidae, and Sparidae) and molluscs (such as *Donax variabilis*) as intermediate hosts. Once these hosts are eaten by the definitive hosts (rays), the parasites reach sexual maturity in the spiral intestine and reproduce.

Zoogeography

The species in this order have a cosmopolitan geographical distribution. At the family level, the pattern of distribution seems to be related to the temperature of the waters: Echeneibothriidae seem to be restricted to temperate waters and Echeneibothriidae are restricted to tropical waters, while Anthocephaliidae and the Rhinebothriidae are found in both (Healy et al., 2017).

Species of rhinebothriideans can inhabit freshwater systems despite being predominately marine. In marine environments, the relationship between these parasites and their definitive hosts seems to be very strict and usually oioxenous. Notwithstanding, in freshwater systems, host range tends to be rather broad, and 1 species of cestode can parasite more than 1 host species. The relatively broad host range of some cestodes associated with freshwater rays may be due to the uniqueness of this relationship or to a recent event of colonization, but this hypothesis needs to be tested (Reyda and Marques, 2011). These authors provided an example of how this relationship appears in freshwater environments: *Rhinebothrium*, as *R. copianullum* and *R. paratrygoni*, each parasitize 8 and 7 potamotrygonid species, respectively. Another singular case is *Stillabothrium davidcynthiaorum*, which was registered from 4 genera of dasyatids as *Brevitrygon*, *Himantura*, and *Maculabatis* (Reyda et al., 2016). Reyda and colleagues (2016) also recorded the most extreme case of a non-oioxenous pattern, the species *Stillabothrium cadenati* was recovered from *Rhinobatos rhinobatos* (Rhinobatidae) and *Zanobatus schoenleini* (Zanobatidae), 2 species of hosts belonging to 2 different families. This is unusual because most members of this order have a 1:1 relationship with their hosts (that is, a very narrow host range), so that some species of cestodes can only be found in 1 species of host. The uniqueness of the exceptions to oioxeny is worth noting; and the questions related to the rupture of this pattern open a new perspective for further studies related to the ecology and evolution of the host-parasite relationship.

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Eucestoda

Relics of "Tetraphyllidea" van Beneden, 1850 (Order)

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Brenda Atziri García-García, and Luis García-Prieto

Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order "Tetraphyllidea"

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Chapter 30

Relics of "Tetraphyllidea" van Beneden, 1850 (Order)

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Introduction

The cestodes referred to the "Tetraphyllidea" (from the Greek **tetra** = 4, and **phyllon** = leaf-shaped) are so allocated because of the morphological characteristics of the scolex (the attachment organs) found in the spiral intestine and occasionally the stomach of species representing all orders of elasmobranch fishes. This group was proposed by van Beneden in 1850 to accommodate the family "Tetraphyllidés." This family included cestode parasites with 4 lobes of the scolex that live in elasmobranchs. Under this name, van Beneden established 3 subgroups: Phyllobothriens (now Phyllobothriidea), phyllacanthiens (now Onchoproteocephalidea), and phyllorhynchiens (now Trypanorhyncha) (Euzet, 1994). Although van Beneden (1850a; 1850b) made the first taxonomic analysis of the group, he never considered it to be an order (Euzet, 1994). Subsequent to van Beneden, Braun (1894–1900) was the first author that considered Tetraphyllidea to be an order consisting of 4 families (Onchobothriidae, Lecanicephalidae, Phyllobothriidae, and Ichthyotaeniidae). Since then, the "Tetraphyllidea" has included cestode species that lack exclusive diagnostic characteristic as the other cestode orders hosted by elasmobranchs (Caira et al., 2017). For this reason, orders such as Onchoproteocephalidea, Trypanorhyncha, Cathetocephalidea, Lecanicephalidea, Litobothriidea, Phyllobothriidea, and Rhinebothriidea have been derived from this group (Braun, 1894–1900; Olson and Caira, 2001; Caira et al., 2005; 2014; Healy et al., 2009). Despite all these changes, "Tretraphyllidea" remains the most problematic order of Cestoda, because it is not a monophyletic group and contains cestodes with morphology that varies remarkably from one another. Consequentially, the remaining taxa of "Tetraphyllidea" require a phylogenetic analysis to establish accurate relationships (Caira and Jensen, 2014; Caira et al., 2014).

Main Morphological Characteristics

"Tetraphyllidea" are polyzoic cestodes. The scolex of all species of "Tetrapyllidea" has 4 sessile or pedunculated bothridia, but present in a great variety of forms. Some species of this order have hooks, loculi, or combinations of these. For example, species of *Pedibothrium* have 1 pair of anterior hooks that are bipronged, while in *Yorkeria*, *Pachybothrium*, and *Spiniloculus* the pair of hooks is unipronged (Caira and Pritchard, 1986; Caira et al., 2007; Desjardins and Caira, 2011). In contrast, species of *Calliobothrium, Symcallio, Erudituncus*, and *Biloculuncus* have 2 pairs of hooks; other species of "Tetraphyllidea" lack hooks. In genera such as *Erudituncus* and *Biloculuncus*, each bothridium is divided into 2 loculi, while in *Calliobothrium* and *Symcallio*, each bothridium is divided into 3 loculi (Nasin et al., 1997; Healy and Caira, 2001; Bernot et al., 2015). Bothridia of *Dioecotaenia cancellata* and *D. campbelli* have 3 columns of facial loculi (Schmidt, 1969; Mayes and Brooks, 1980; Caira et al., 2017). This feature is also present in *Glyptobothrium zwerneri* in which bothridia are divided into 3 longitudinal rows of loculi and separated into 3 parallel longitudinal rows of 10–12 loculi (Pulido-Flores and Monks, 2014). Members of other genera, such as *Ceratobothrium* and *Dinobothrium*, possess an apical pad (Caira et al., 2017); in species of Rhoptrobothriidae, the cephalic peduncle bears 4 stalked extensions, termed remi by Jensen and Caira (2006); this feature is unique to this family.

The morphology of the strobila is very similar to members of Trypanorhyncha and Lecanicephalidea (Schmidt, 1986). Some species are euapolytic (such as *Yorkeria hilli*,

Y. kelleyae, *Caulopatera pagei*, and *Pedibothrium cabrali*) (Caira and Tracy, 2002; Caira et al., 2004; Cutmore et al., 2010), hyperapolytic (such as *Calliobothrium australis* (Ivanov and Brooks, 2002), or apolytic (such as *Symcallio barbarae*) (Ivanov and Brooks, 2002). The proglottids can be acraspedote (Cutmore et al., 2010; 2018) (specifically, *Yorkeria*, *Caulopatera*, and *Carpobothrium*, according to Caira and Tracy (2002); Koontz and Caira (2016)) or craspedote (specifically, *Calliobothrium* and *Symcallio*; see Ivanov and Brooks, 2002; Bernot et al., 2016). The genital pores are lateral or sublateral (as in *Duplicibothrium*; Williams and Campbell, 1978; Ruhnke et al., 2000) and alternate irregularly. There are numerous testes and the vagina opens anterior to the cirrus sac. The ovary is posterior and bi-lobed or tetra-lobed in cross section. The vitelline follicles are arranged in 2 lateral bands.

Currently, "Tetraphyllidea" includes 6 families and 4 clades, as recognized by Caira et al. (2014) and described by Caira et al. (2017): Balanobothriidae is the family with the most species with 38, distributed in 5 genera, followed by Calliobothriidae (26 species and 4 genera), Clade 4 (9 and 3, respectively), Clade 2 (8 and 1, respectively); Rhoptrobothriidae and Serendipidae (both with 6 species and 3 genera); Clade 3 (with 3 species of *Carpobothrium* and the monotypic *Caulopatera*; Gastrolecithidae with the genera *Cerabothrium* (1 species) and *Dinobothrium* (3 species); Clade 1 (with 3 monotypic genera), and, finally, Dioecotaeniidae with the genus *Dioecotaenia*, constituting 2 species. Up until the latest classifications, "Tetraphyllidea" included 106 species and 27 genera (Caira et al., 2017).

Description and Summary of a Representative Species

Note: This work is not intended for the purposes of zoological nomenclature.

Duplicibothrium cairae Ruhnke et al., 2000

The worms are slightly craspedote and euapolytic. The scolex of this species has 4 pyriform bothridia. The dorsal and ventral bothridia are paired and fused. The bothridia each have 27–33 loculi arranged in 5 or 7 anterior rows of 3, 1 posterior row of 5, and the last posterior row with 7. The scolex is covered with round microtriches; the cephalic peduncle is covered with dense microtriches.

There are 20 to 35 proglottids per strobila, progressively becoming longer than wider. The last segments have dorsal and ventral pairs of excretory ducts. The mature segments have 28–43 testes distributed in a post-ovarian field. In cross section, there are 4–10 medullary testes in 2 irregular deep rows. The cirrus is armed with spiniform microtriches. The cirrus sac is oval. The genital pore is positioned within 80–96% of the proglottid length, irregularly alternating and sublateral. The vagina is weakly developed in the mature proglottids. The ovary is digitiform in cross section. The uterus is median and poorly developed in the terminal proglottids. There are 8–12 vitelline follicles that are convergent in a dorsal field and are not found at the level of the ovary and cirrus sac (Ruhnke et al., 2000).

Taxonomic summary.

Type host: Pacific cownose ray *Rhinoptera steindacheneri* Evermann and Jenkins, 1891.

Site of infection: Spiral intestine.

Type locality: Puertecitos (28° 85′ 50″ N, 113° 83′ 20″ W), Baja California, Gulf of California, Mexico.

Type specimens are listed here and additional details can be found in the original paper where this species was described: Holotype (CNHE 3846); paratypes (CNHE 3847; USNM (USNPC) 89726, 89727; HWML (15275, 15276).

"Tetraphyllidea" van Beneden, 1850 Taxonomy

To date, *Duplicibothrium* contains 3 species: *D. cairae*, *D. minutum*, and *D. paulum*, all of them parasites of rays of the genus *Rhinoptera* (Caira et al., 2017). *Duplicibothrium* is characterized by the possession of 4 bothridia, the dorsal and ventral fused lengthwise into 2 pairs; the bothridial surfaces are divided into loculi by muscular septa or horizontal and longitudinal septa, showing a digitiform ovary and sublateral genital pore (Williams and Campbell, 1978; Ruhnke et al., 2000). *Duplicibothrium cairae* possesses a pair of longitudinal septa on each bothridium, while in *D. minutum* and *D. paulum* this feature is absent. Each septum is bifurcated in the posterior third of the bothridia, forming 5 or 7 anterior horizontal rows and ending with 1 row of 5 loculi and 1 more-posterior row of 7 loculi. *Duplicibothrium cairae* differs from the other 2 species by the number of segments: *D. paulum* has 3–11 proglottids, *D. cairae* has 20–35 proglottids, and *D. minutum* has 6–14 proglottids. In addition, *D. cairae* can be distinguished from *D. paulum* and *D. minutum* by the number of loculi in the bothridia; *D. paulum* has 57–63 loculi per bothridia, and *D. minutum* has 6–8 loculi per bothridia versus 27–33 per bothridia in *D. cairae* (Williams and Campbell, 1978; Ruhnke et al., 2000).

In the latest phylogenetic analysis of *Duplicibothrium*, the represented species nested with *Glypthobothrium* and *Serendip*, which are included in Serendipidae (Caira et al., 2017). According to this, the phylogenetic position of *Duplicibothrium* is strongly supported by morphological and molecular evidence, due the 3 genera of Serendipidae being characterized by the presence of facial loculi in the bothridia (Ruhnke et al., 2000).

"Tetraphyllidea" does not represent a monophyletic group. All phylogenetic analyses, both with morphological or molecular data that included species of this order, conducted since 1981 by Euzet and colleagues (1981) through Caira and colleagues (2014), indicate that this order is paraphyletic (Olson and Caira, 1999; Caira et al., 1999; 2001; Waeschenbach et al., 2007; 2012). The resolution of this paraphyly is essential to understand cestode evolution and describe the phylogenetic relations of species currently included in "Tetraphyllidea" (Caira et al., 2014). The last analysis with molecular data of "Tetraphyllidea" shows that this group is non-monophyletic since its species were distributed across trees in different clades (Caira et al., 2014; 2017). For this reason, Caira and colleagues (2014; 2017) retained these species as members of "Tetrapyllidea" and suggested that more exhaustive studies should be conducted.

Finally, according to Caira and colleagues (2014; 2017), "Tetraphyllidea" contains 10 independent groups (see above). Interestingly, Clade 1 of this analysis is the sister taxon of Rhinetobothriidea and Clade 3 of Cyclophyllidea.

Life Cycle

The life cycle of tetraphyllidean cestodes is poorly known. Caira and Reyda (2005) and Caira and Jensen (2014) have suggested that species of "Tetraphyllidea" likely parasitize 2 or 3 intermediate hosts and 1 species of elasmobranch as definitive host. The adults of "Tetraphyllidea" have been reported as hosts of all orders (8 of sharks and 4 of batoids) of Elasmobranchii. At the family level, tetrapyllideans are parasites of 23 families (Caira et al., 2017). The larval stages have been recorded in crustaceans, molluscs, and fishes (Jensen and Bullard, 2010). To date, only the life cycle of the tetraphyllidean *Calliobothrium verticillatum* has yet been described; as an adult, it is a parasite of the spiral valve of the smooth dogfish *Mustelus canis* (Cherry et al., 1991). The plerocercoid larvae have been found parasitizing the lumina of the anterior and midgut ceca of the hermit crab *Pagurus pollicaris* (Cherry et al., 1991). This crab is an important component of the dogfish's diet (Montemarano et al., 2016). In general, the life cycle of *C. verticillatum* begins when worms reach maturity in the spiral valve of *M. canis.* These cestodes produce hexacanth embryos that are released from gravid proglottids and are eaten by the hermit crab, where the procercoids and plerocercoids are developed. Finally, the hermit crabs are ingested by sharks that act as definitive hosts (McDermott et al., 2010).

According to Jensen and Bullard (2010), one factor that contributes to the scarcity of information on life cycles is that the larval stages lack the morphological characteristics of adults, which makes taxonomic identification difficult. Although molecular data have been used to match the larval stages with the adult forms, analyses are scarce. For this reason, there are many records of "Tetraphyllidea" larvae without specific identifications (Álvarez et al., 2002; Palm and Klimpel, 2008; Klimpel et al., 2010; Carballo et al., 2011; Montoya-Mendoza et al., 2014; Centeno-Chalé et al., 2015; Constela et al., 2015; Dallarés et al., 2017; Morales-Serna et al., 2017). The most complete analysis using molecular characters for taxonomic identification of larvae was conducted by Jensen and Bullard (2010). In this analysis the authors identified larvae of *Duplicibothrium minutum*, *Anthobothrium* spp., and possibly *Pedibothrium* spp. The larval stages of *D. minutum* were collected from bivalves and gastropods (that is, *Melongena corona* and *Angulus versicolor*); larvae of *Pedibothrium* spp., were found in the fishes *Opsanus beta* and *Lutjanus campechanus*, and the larval stages of *Anthobothrium* spp. were found in fish such as *Ariposis felis, Trichiurus lepturus, Peprilus burti*, and *Diplectrum formosum*. According to Jensen and Bullard (2010), these organisms act as intermediate hosts for this group of cestodes since they are an important component of the diet of sharks. In addition, some species exhibit heteroxenous associations which allows them to parasitize more than 1 species of host (for example, *Calliobothrium verticillatum*), while other species of "Tetraphyllidea" exhibit oxioenus associations with their hosts. For example, some species of the genus *Symcallio* only parasitize sharks of the genus *Mustelus* (Bernot et al., 2015).

Caira and colleagues (2017) pointed out that the geographical distribution of members in this order is determined by the geographical distribution of their hosts, although these cestodes only have been recorded between 60° N and 60° S latitudes, mainly in tropical localities, such as the Gulf of California (specifically, *Duplicibothrium cairae* (Runhke et al., 2000)).

Additional Relevant Details about the Order "Tetraphyllidea" van Beneden, 1850

Species of *Calliobothrium* and *Symcallio* present different site specificity along the spiral intestine (Bernot et al., 2015). Cislo and Caira (1993) analyzed the parasites of *Mustelus canis* and observed that *S. lintoni* and *C. verticillatum* each have a different site of attachment along the spiral intestine. *Symcallio lintoni* was found in the anterior of the spiral intestine whereas *C, verticillatum* was found in the posterior region.

The majority of species of Cestoda are hermaphroditic; however, there are few exceptions, such as species of *Dioecotaenia*. These species are the unique dioecious cestodes of "Tetraphyllidea," and in both species, the strobila has separate sexes (the proglottids only have male genital organs or only female genital organs) (Schmidt, 1969; Mayes and Brooks,

1981). This feature is also present in some Cyclophyllidea as members of Dioecocestidae, parasites of charadriiform birds, and in the progynotaeiid *Gynandrotaenia*, which are parasites of flamingos (Olson and Caira, 1999; Mariaux et al., 2017).

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31

Amphilinidea

Amphilinidea Poche, 1922 (Order)

Klaus Rohde

Phylum Platyhelminthes

Class Cestoda

Subclass Cestodaria

Order Amphilinidea

doi:10.32873/unl.dc.ciap031
2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 31

Amphilinidea Poche, 1922 (Order)

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Introduction

The cestodes (tapeworms) are a large group of endoparasitic worms infecting various vertebrates. Most species are included in the Eucestoda (true tapeworms), characterized (with few exceptions) by a number of segments (proglottids). Examples are *Taenia* (the pig and cattle tapeworms, of which the adults live in humans) and *Diphyllobothrium* (the broad fish tapeworm) infecting humans. Two groups of cestodes, the Gyrocotylidea and Amphilinidea, do not possess proglottids. The Amphilinidea are discussed here. Only 8 species included in 3 genera are known. They have little economic significance, although 1 species was shown to adversely affect sturgeon, the producers of caviar. Amphilinids are of considerable interest to biologists because they may cast light on the phylogeny of tapeworms and of related forms.

They are large (several cm-long), dorsoventrally flattened worms infecting the body cavity of freshwater and marine teleost (bony) fishes and freshwater turtles. Larvae are **ciliated** and possess 10 posterior **hooks**, which are retained in the adult. A well-known species is *Austramphilina* (= *Gigantolina*) *elongata* from Australia, with freshwater crustaceans as intermediate hosts and freshwater turtles as final (definitive) hosts.

A considerable number of studies deal with its morphology, electron microscopy, and life cycle (Rohde and Georgi, 1983; Rohde and Garlick, 1985a; 1985b; 1985c; 1985d; Rohde, 1986; 1987; 1994; Rohde et al., 1986; Rohde and Watson, 1986; 1987; 1988; 1989; 1990a; 1990b). Brief overviews of the Amphilinidea are by Rohde (2005) and Read (2007). The Tree of Life webpage by Rohde (1998) (available at http://tolweb.org/Amphilinidea) contains an account of all aspects of Amphilinidea and an extensive bibliography. Older references can be found in Dubinina (1982). Important papers on some aspects of *Amphilina foliacea* are by Bisserova et al. (2000) and Dudicheva and Bisserova (2000). *Austramphilina elongata* is also discussed in greater detail.

Structure of the Adult *Austramphilina elongata*

The adult worm reaches a length of about 150 or more mm, with a width of about 14 or more mm (Figure 1). As in all amphilinids, the **uterus** forms 3 loops in the body; it extends from the posteriorly located **ovary** to the anterior end, turns back and forward again, opening through a **uterine pore** at the anterior end. The **vagina** opens at the posterior

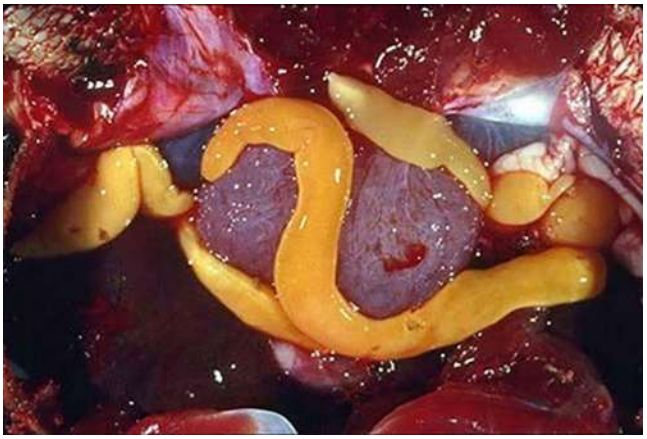

Figure 1. *Austramphilina elongata*. Several worms in the body cavity of the freshwater turtle *Chelodina longicollis*. Source: K. Rohde. License: CC BY-NC-SA 4.0.

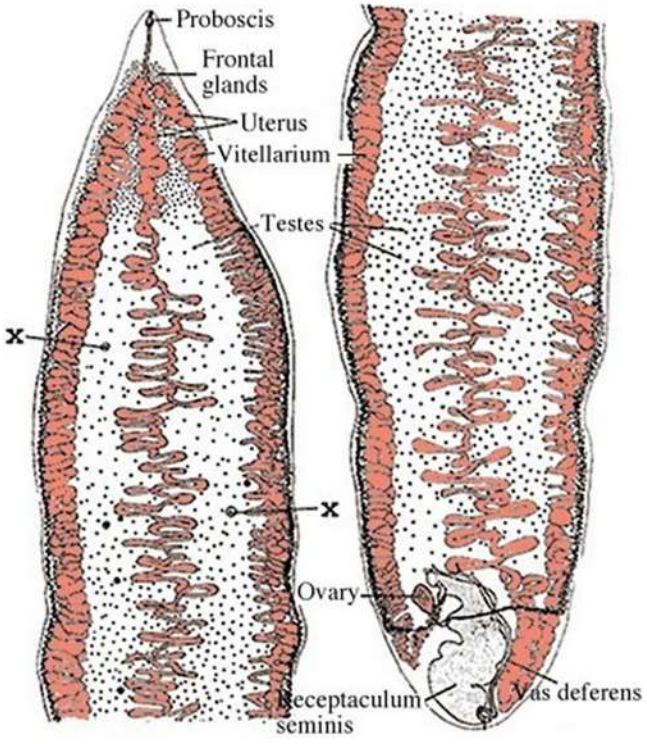

Figure 2. *Austramphilina elongata*, whole mount. X = bodies of unknown function. Source: K. Rohde. License: CC BY-NC-SA 4.0.

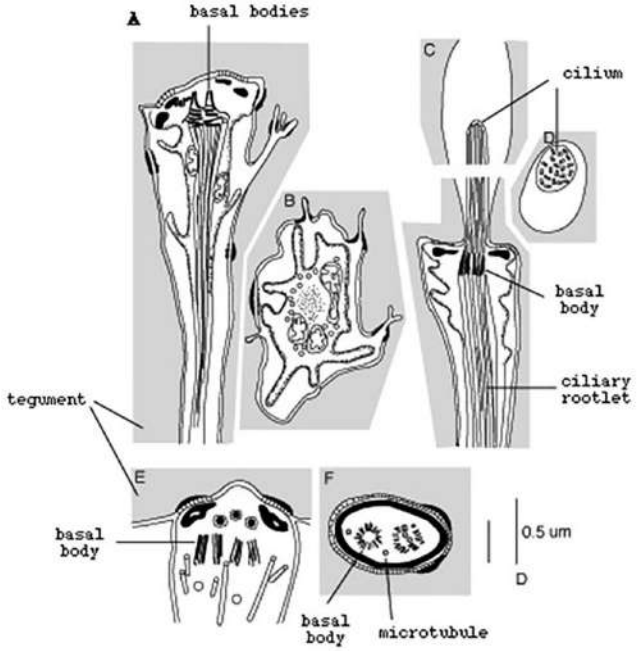

Figure 3. *Austramphilina elongata*, receptors of adult. Source: Adapted from Rohde and Watson, 1990b. License: CC BY-NC-SA 4.0.

end. **Vitellaria** extend in the lateral parts of the body from the anterior to the posterior ends of the body. **Testes** are scattered throughout the body and the male **gonopore** is located near the female one at the posterior end (Figure 2). Electron microscope studies have shown several types of **sensory receptors** (Figure 3).

Structure of Larval *Austramphilina elongata*

The larvae are **ciliated** and possess 10 posterior **hooks** of 3 different kinds. Two pairs are serrate, the others are sickle-shaped (Figures 4 and 5). Ducts of clusters of **gland cells** open near the anterior end. The **protonephridial** (excretory/osmoregulatory) system consists of 3 **flame cells** or **bulbs** on each side of the body, with paired **excretory pores** located near the posterior end (Figure 4). A large number of transverse **muscle bands** extend below the **tegument** (surface layer) of the larva. There are several clusters of **sensilla** (**sensory receptors**) (Figures 5 and 6).

The larvae possess a ciliated **epidermis** located on an underlying tegument which becomes the surface layer (**neodermis**) once the epidermis is shed by the invading larva (Figure 7).

The larva possesses a considerable number of sensory receptor types differing with respect to the presence or absence of cilia, the number and shape of the cilia, and the shape of the basal bodies/ciliary **rootles** (Figure 8).

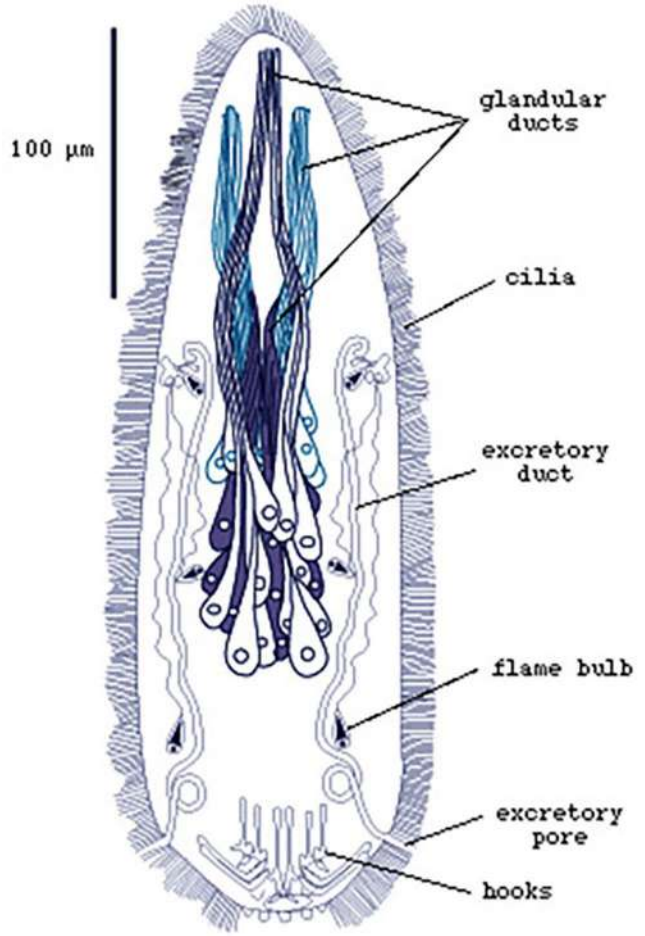

Figure 4. *Austramphilina elongata* larva. Note the bundles of secretory glands opening near the anterior end, the protonephridial system with 3 flame bulbs on each side opening near the posterior end, and the 10 posterior hooks. Source: Adapted from K. Rohde, 1986. License: CC BY-NC-SA 4.0.

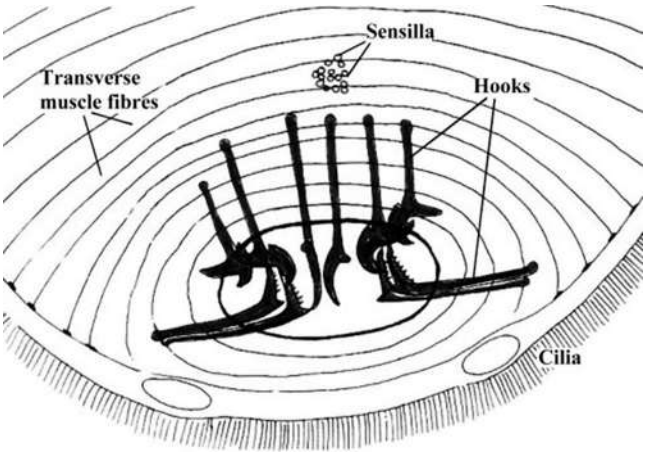

Figure 5. Posterior end of a larval *Austramphilina elongata*. Note the cluster of sensilla, transverse muscle bands, ciliated epidermis, and 5 pairs of hooks of 3 types. Source: K. Rohde. License: CC BY-NC-SA 4.0.

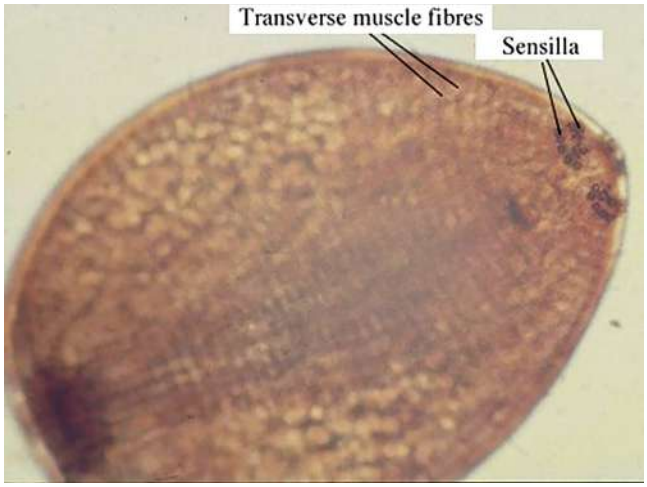

Figure 6. Larva of *Austramphilina elongata* impregnated with silver. Note the transverse muscle bands and receptors (sensilla). Source: K. Rohde. License: CC BY-NC-SA 4.0.

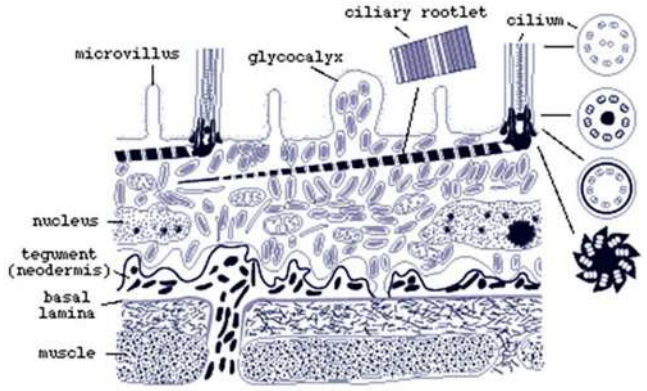

Figure 7. Larval *Austramphilina elongata*, diagram of electron-microscopic structure of surface layers. Note larval syncytial and ciliated epidermis at the surface, based on the tegument (neodermis) that has insunk (below the surface) nuclei (only the process leading to 1 nucleus is illustrated). Source: K. Rohde. License: CC BY-NC-SA 4.0.

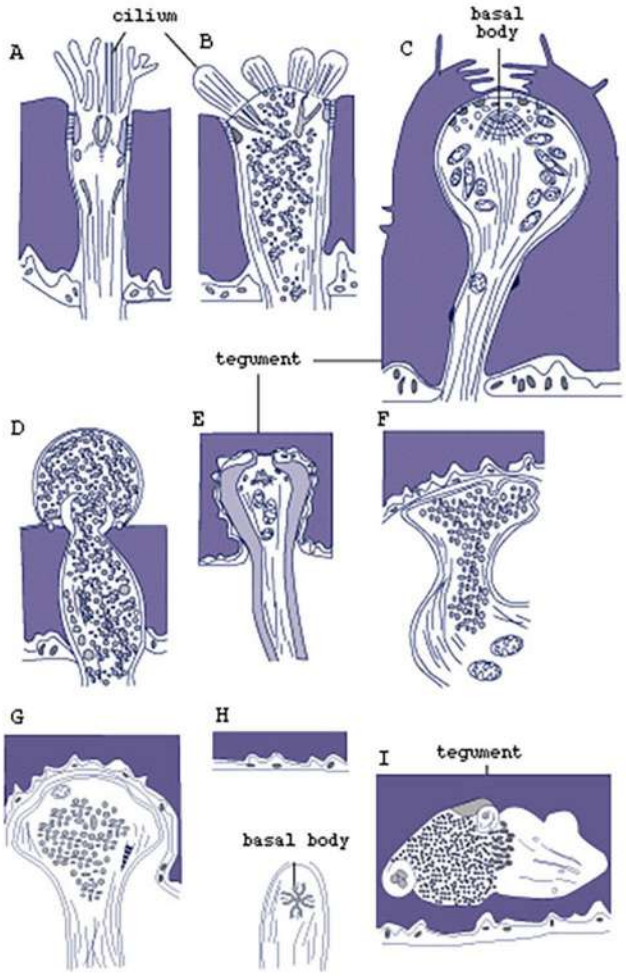

Figure 8. *Austramphilina elongata,* diagrams of larval receptors as seen under the transmission electron microscope. Source: Adapted from Rohde et al., 1986a. License: CC BY-NC-SA 4.0.

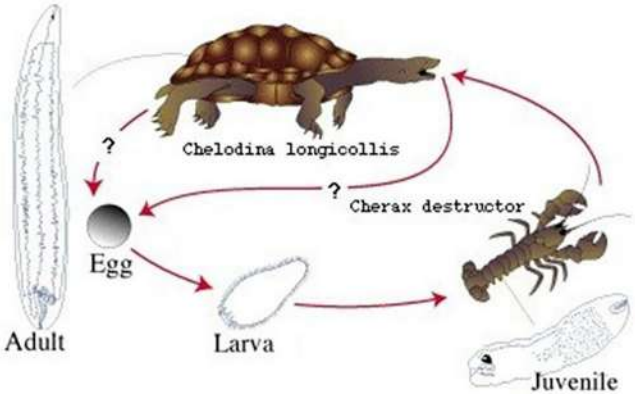

Figure 9. Life cycle of *Austramphilina elongata*. Note: Escape route of egg from turtle is unknown. Source: K. Rohde. License: CC BY-NC-SA 4.0.

Life Cycle of *Austramphilina elongata*

The eggs of *Austramphilina elongata* have to get into freshwater for further development (Figure 9). The escape route from the host is unknown. Larvae hatch in freshwater. They swim around in water until they get into contact with a crayfish (phylum Arthropoda: class Crustacea: order Decapoda). On the crayfish, the larva bends in such a way that both the anterior and posterior ends are located close together on the cuticle of the host. The sickle-shaped hooks pierce into the cuticle, the serrate ones perform sawing movements, cutting through the cuticle. The 3 types of anterior

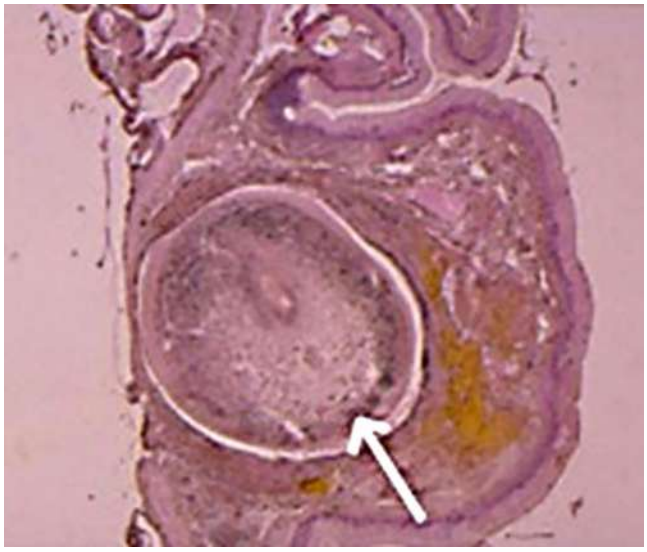

Figure 10. Section though the esophageal wall of a turtle, *Chelodina longicollis,* showing a penetrating *Austramphilina* juvenile (arrow). Source: K. Rohde. License: CC BY-NC-SA 4.0.

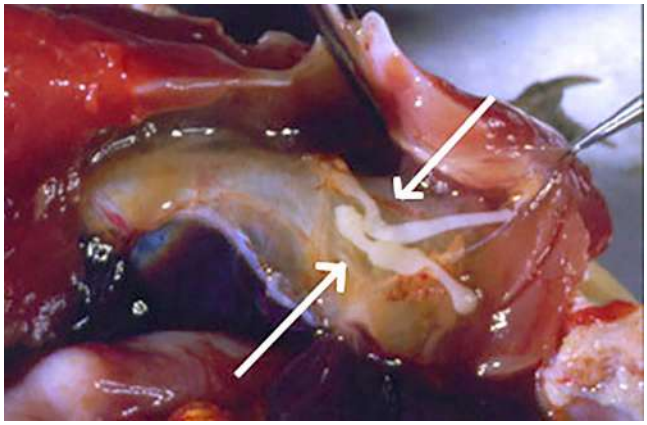

Figure 11. Two juvenile *Austramphilina* specimens (arrows) migrating along the trachea towards the body cavity of a turtle. Source: K. Rohde. License: CC BY-NC-SA 4.0.

glands apparently produce a secretion (which, however, has not been identified) dissolving the surface layer. The larva penetrates into the host's tissue, shedding the ciliated epidermis in the process. Penetration is observed to occur through the gills, and through the thin junctions between the crayfish's segments within 30 minutes after first contact. Larvae infective to turtles are several mm long and may be observed in the abdomen of crayfish. Turtles become infected by eating crayfish. Juvenile worms penetrate through the wall of the esophagus (Figure 10), migrate along the trachea (Figure 11), and through the septum into the body cavity where they mature. Adult worms are seen mainly in the body cavity, but occasionally also in the lungs. This suggests that eggs may

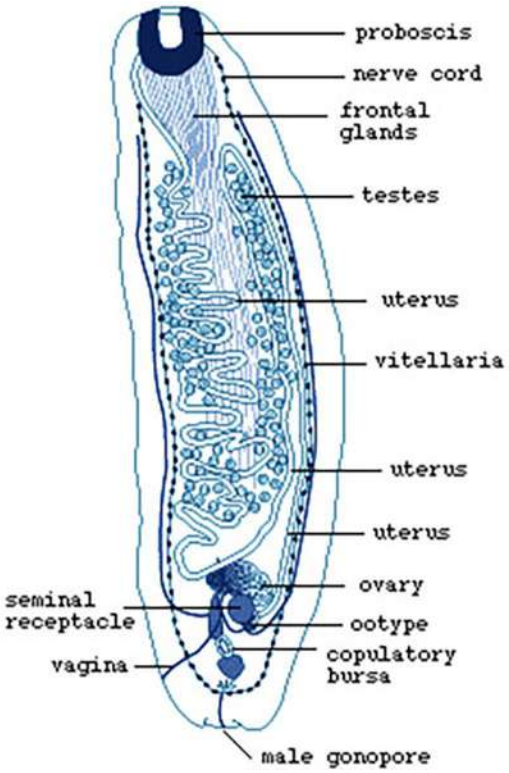

Figure 12. *Amphilina foliacea,* adult. Source: Adapted from Dubinina, 1982. License: CC BY-NC-SA 4.0.

leave the host via the trachea and mouth cavity from where they are spit out into water. Once, an adult was also seen in the urinary bladder, and once in the oviduct of a turtle, suggesting that eggs may be shed through the cloaca. Freshwater shrimps could also be infected experimentally, but larvae did not reach a size infective to turtles in them.

Other Species

Only 1 other species has been studied in detail, namely, *Amphilina foliacea*. It differs from *Austramphilina* in a number of morphological features (Figure 12). Its protonephridial system forms a network of canals, differing from that of other species, for example, *Gephyrolina paragonopora* (Figure 13).

Amphilina foliacea uses freshwater amphipods (class Crustacea: order Amphipoda) as intermediate hosts and *Accipenser* (sturgeon) as final hosts. It inhabits the body cavity of the final host and eggs escape through the coelomic pore which connects the body cavity to the outside (it is not present in turtles!). Eggs containing infective larvae are ingested by the amphipods, whose mouthparts break the eggshell allowing the larva to escape and penetrate into the host.

Adult *Nesolecithus africanus* infect African freshwater fish. Juveniles have been recovered from freshwater prawns (class Crustacea: order Decapoda).

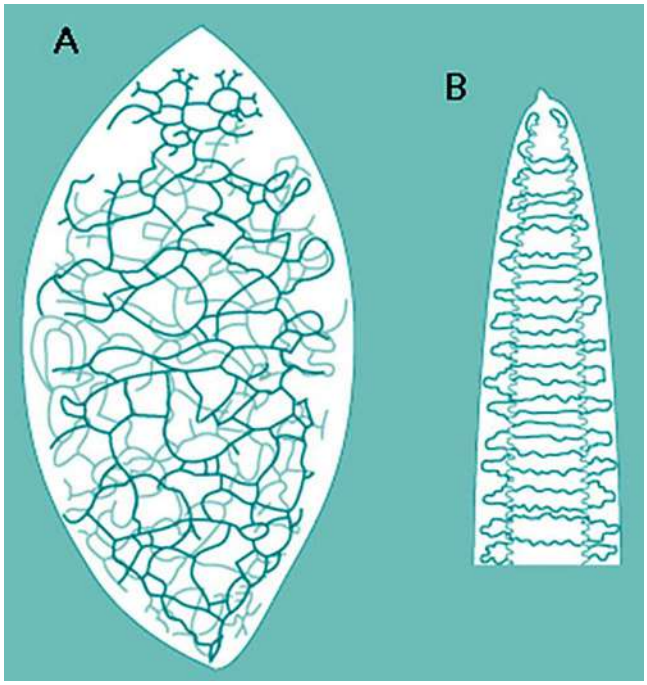

Figure 13. Protonephridial canal system of *Amphilina foliacea* (A) and of *Gephyrolina paragonopora* (B). Adapted from Dubinina, 1982. License: CC BY-NC-SA 4.0.

Taxonomy and Phylogeny

Gibson (1994) has provided a key to the species (see also Schmidt, 1986) and Dubinina (1982), in a detailed monograph of the Amphilinidea, has discussed the position of the group in the phylum Platyhelminthes (see also Galkin, 1999). Eight species have been described:

1) *Amphilina foliacea*
   synonyms *Monostomum foliaceum*, *A. neritina*
2) *Am. japonica*
   synonyms *Am. bipunctata*, *A. foliacea*
3) *Gephyrolina paragonopora*
   synonyms *Am. paragonopora*, *Hunteroides mystel*, *Schizochoerus paragonopora*
4) *Schizochoerus liguloideus*
   synonyms *M. liguloideum*, *Am. liguloidea*
5) *Nesolecithus janickii*
   synonyms *Am. liguloidea*, *M. liguloideum*, *S. janickii*
6) *N. africanus*
   synonym *S. africanus*
7) *Austramphilina elongata*
   synonyms *Kosterina Kuiperi*, *Gigantolina elongata*
8) *Gigantolina magna*
   synonyms *Am. magna*, *Gyrometra albotaenia*, *Gy. kunduchi*

The Gyrocotylidea have often been considered to be the sister group of the amphilinids, both comprising the Cestodaria (non-segmented tapeworms) (Bandoni and Brooks, 1987). However, later studies do not support a monophyletic group, Cestodaria. Instead, gyrocotylids appear to be the earliest divergent lineage within the cestodes followed by the amphilinids and then the eucestodes (true cestodes) (Waeschenbach et al., 2012; Littlewood et al., 2015; Waeschenbach and Littlewood, 2017). The Cestoda must be considered to be the sister group of the Trematoda (see, for example, Park et al., 2007) and all the large groups of parasitic flatworms Polyopisthocotylea and Monopisthocotylea (= "Monogenea"), Trematoda, and Cestoda (including the Eucestoda, Amphilinidea, and Gyrocotylidea) are monophyletic comprising the Neodermata, as first proposed by Ehlers (1985) and later confirmed by numerous electron microscope and DNA studies (for example, Egger et al., 2015). Various hypotheses of these relationships are currently being tested using deep sequencing of DNA at the genome level.

Acknowledgement

Based on the author Rohde's online articles available at
https://krohde.wordpress.com/2009/08/03/the-amphilinidea-a-small-group-of-xk923bc3gp4-21/ and
https://krohde.wordpress.com/2009/08/03/die-amphilinidea-eine-kleine-gruppe-xk923bc3gp4-22/

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32

Gyrocotylidea

Gyrocotylidea (Order): The Most Primitive Group of Tapeworms

Willi E. R. Xylander and Klaus Rohde

Phylum Platyhelminthes

Class Cestoda

Subclass Cestodaria

Order Gyrocotylidea

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 32

Gyrocotylidea (Order): The Most Primitive Group of Tapeworms

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Background

Gyrocotylidea is an order of parasitic flatworms comprising about 10 known species belonging to 2 genera, *Gyrocotyle* and *Gyrocotyloides* (although there has been much confusion about species identities; see, for example, Bristow, 1992). They are about 2–10 cm in length and are exclusively found in the spiral valve (spiral intestine) of Holocephali, a group of marine chondrichthyan fishes called chimaeras or ratfishes, which live in both the deep sea and cold surface marine waters. Like all tapeworms, species of Gyrocotylidea lack an intestinal tract in all developmental stages, have a neodermis with regularly shaped microtriches which are small villi-like protrusions on the external part of the tegument that probably serves to increase the absorptive surface area of the animal (see Poddubnaya et al., 2006), and a reticulate excretory system. Like the Amphilinidea, individuals have 10 posterior hooks (present only in the larvae), and a single set of reproductive organs but no proglottids (no segmentation) characteristic of almost all eucestodes (which are the genuine, or the true, tapeworms). Together with the Eucestoda and Amphilinidea, they form 1 monophyletic group (derived from a common ancestor), the Cestoda (tapeworms) (Ehlers, 1985; see also Littlewood et al., 1999; Xylander, 2001). Recent molecular studies have confirmed morphological indications of the monophyly of the Neodermata and the sister group relationship of the Gyrocotylidea to all other Cestoda (Park et al., 2007; Waeschenbach et al., 2012; Egger et al., 2015; Littlewood et al., 2015; Waeschenbach and Littlewood, 2017; list of morphological characters in Xylander, 2001).

Interestingly, these animals are not of any economic importance but have baffled biologists because of some unique morphological and biological features (Simmons, 1974). For a brief overview of the group see Rohde (2007) and Kuchta and colleagues (2017), and for a more detailed account, see Xylander (2001; 2006a).

Structure of the Adult

The outer surface layer of adult gyrocotylideans is a **neodermis**, that is, a syncytial non-ciliated body covering which replaces the ciliated epidermis of the larva after start of their life as parasites (Xylander, 2001). Larvae and adults lack an intestine. The **attachment organ** is located at the posterior end; in species of the genus *Gyrocotyle* (Figures 1–3) it increases in size and shape from a primitive cup-like structure in earliest intestinal stages to a ruffled structure, the so-called **rosette** (Halvorsen and Williams, 1968). With this structure the worms attach to the intestinal microvilli of their hosts. In the genus *Gyrocotyloides*, the **holdfast** is cup-like and located on a **caudal stalk**. A so-called **funnel** in the posterior part of the body opens dorsally through a pore; its function is unknown (it possibly contributes to attachment). The protonephridial system of the adult consists of **flame bulbs** (also called **flame cells**) and a **network of capillaries and ducts** which have ciliated tufts for transporting the excretory fluid and potentially nutrients (Xylander, 1992a). The paired **excretory pores** open not far from the anterior end.

Figure 1. *Gyrocotyle urna*, rosette left. Source: W. E. R. Xylander. License: CC BY-NC-SA 4.0.

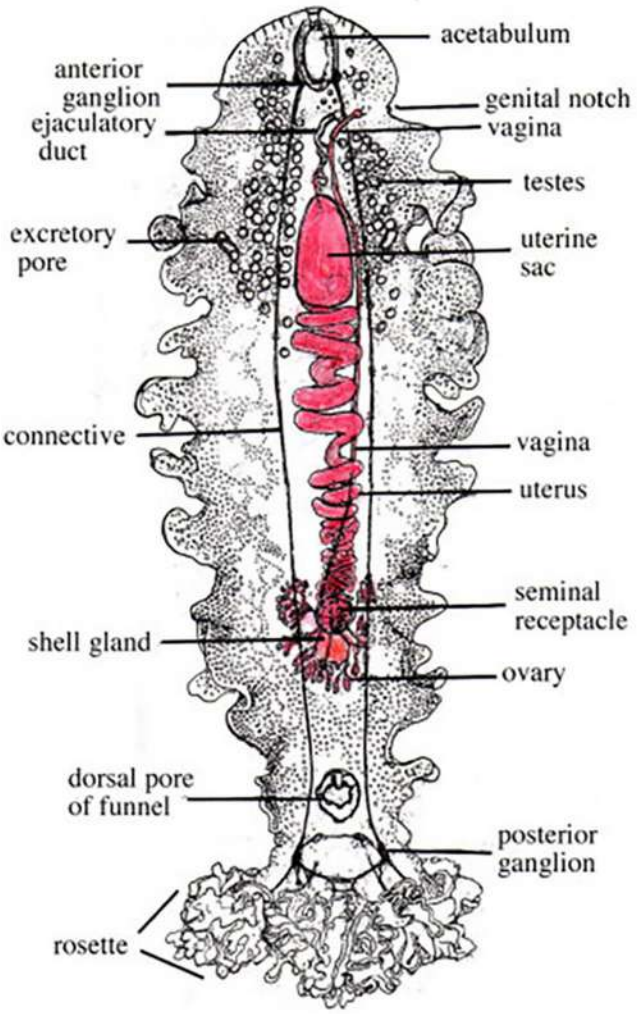

Figure 2. Adult *Gyrocotyle fimbriata*, dorsal view. Female reproductive system except vitellaria drawn red. Source: Adapted from Lynch (1945), Cheng (1986), and other sources. License: CC BY-NC-SA 4.0.

Gyrocotylids are hermaphroditic. Follicular **testes** are located in the anterior part of the body and connect to **sperm ducts** (**vasa efferentia**) which unite to form 1 large sperm duct (**vas deferens**) whose terminal part is muscular forming an **ejaculatory duct**. It opens near the anterior end. The female reproductive system consists of a **germarium** (**ovary**) and a **vitellarium**. The ovary is located in the posterior part of the body and is composed of many **follicles**. The **oviduct**, into which the egg cells are discharged, leads to the **ootype** surrounded by the **Mehlis' gland**, into which or near which the **yolk ducts** and **vagina** open, as well. A very high number of **vitelline** (**yolk**) **follicles** are scattered throughout the body from the anterior to the posterior end (most are located laterally). The compound **eggs** (consisting of a single fertilized egg cell and many yolk cells surrounded by a shell originating from glands in the ootype, the Mehlis' gland and material

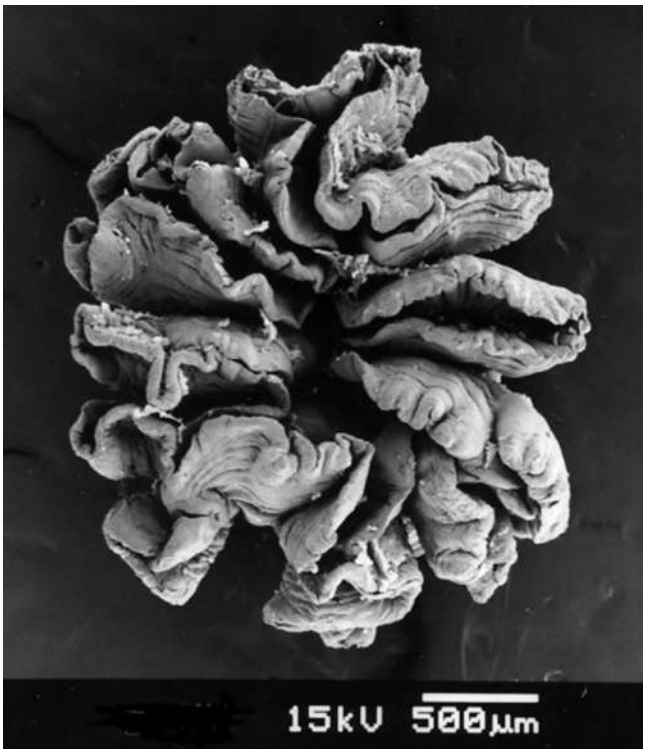

Figure 3. Scanning electron micrograph of the rosette of *Gyrocotyle* sp., probably *G. rugosa*, from the holocephalan *Callorhinchus milii* in Tasmania, Australia. Source: K. Rohde. License: CC BY-NC-SA 4.0.

discharged from the **vitellocytes**) are formed in the ootype. Fertilized eggs are stored for weeks in a large **uterine sac** and then are set free via a **uterine pore** near the anterior end. The vagina terminates at that point.

The main parts of the **nervous system** consist of an anteriorly located **brain** (or **cephalic ganglia**), large lateral **nerve cords** (and many **smaller nerves**) and large posterior **nerve ring** in the vicinity of the rosette. More than 10 different **sensory cells** have been found in mature *Gyrocotyle* specimens (Xylander, 1992b; Xylander and Poddubnaya, unpublished data).

Gyrocotylids, like all tapeworms, lack an intestine. Food must be absorbed by the neodermis. The neodermis is completely covered by regularly shaped typical tapeworm **microtriches** (Figure 4); these microtriches may be responsible for nutrient uptake, or may instead be involved in protection against the digestive enzymes of the host (Xylander, 2001).

For some recent ultrastructural studies see Poddubnaya and colleagues (2006; 2009; 2015), and Levron and colleagues (2016).

Structure of the Larva

The lycophora larva (decacanth) is about 0.2 mm-long and is completely surrounded by a syncytial ciliated **epidermis**

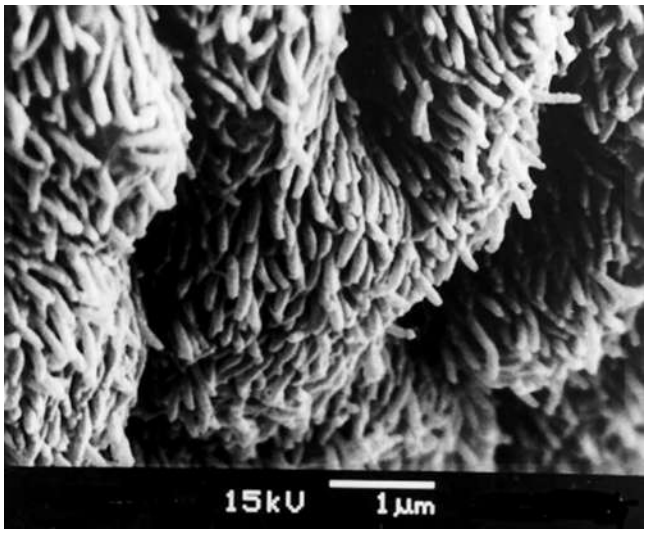

Figure 4. Scanning electron micrograph of the neodermis of *Gyrocotyle* sp., probably *G. rugosa,* from the holocephalan *Callorhinchus milii* in Tasmania, Australia. Note the numerous microvilli. Source: K. Rohde. License: CC BY-NC-SA 4.0.

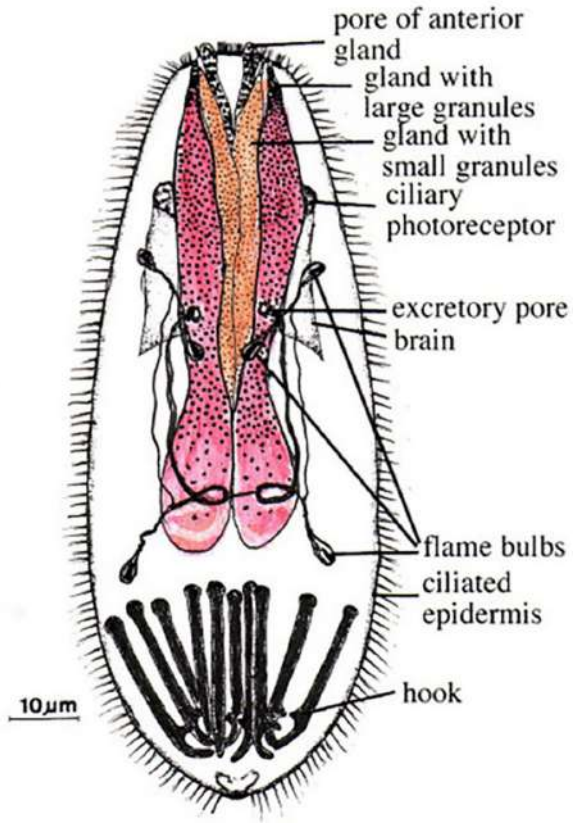

Figure 5. Lycophora larva of *Gyrocotyle urna*. Adapted from Xylander (1997c; 1990; 1991; 2001; 2006b) and Rohde (1994). License: CC BY-NC-SA 4.0.

(Xylander, 1987a). There are 4 pairs of **gland cells**, each pair with a different secretion extending from the posterior half of the body to the anterior body tip where they open (Xylander, 1990). Lycophores have a well-developed **brain**, at least 7 different ciliary **sensory receptors** (the majority at the anterior end) as well as a paired **photoreceptor** located at the anteriolateral margins of the brain (Figure 5, Xylander 1984; 1987b). Such a well elaborated nervous system is lacking in the larvae of more derived tapeworms (such as an oncosphere or coracidium). At the posterior end they bear 10 **hooks** resembling the hooks of oncomiracidia (Xylander, 1991). The **protonephridial** (excretory/osmoregulatory) system consists of 3 pairs of **terminal cells** connected to **capillaries**, which unite in 2 **ducts** terminating in **excretory pores** at the transition between the anteriormost to the middle-third of the body (Xylander, 1987c).

Life Cycle

The complete life cycle of all species of the Gyrocotylidea is still unknown. However, Xylander (1989; 2006a) has argued for a 2-host life cycle in Gyrocotylidea based on: 1) Even the earliest stages of *Gyrocotyle* found in the spiral valve show an anterior pit which develops in other tapeworms within the first (crustacean) intermediate host; and 2) infection of hosts is correlated with feeding. Young holocephali restricted to yolks are not infected, whereas young host specimens which have already preyed on invertebrates (mainly smaller crustaceans) very often are infected; so, it is highly probable that gyrocotylids do not infect a fish directly but that a (crustacean) intermediate host is involved in the life cycle.

The lycophora larva hatches from the egg after a maturing period of more than 30 days. In vitro, lycophores swim for about 24 hours before dying.

Host individuals are usually (but not always) infected by only one gyrocotylid species, but each holocephalan host species can harbor 2 species (though in *Chimaera monstrosa*, each can harbor 3 species), usually attached to different sites along their spiral valve. One of each species pair belongs to the *urna group*, and the other to the *confuse group*. The former has many marginal body undulations and very elaborate folds of the rosette, whereas the latter has a smaller rosette with fewer folds, a more elongate body, and less elaborate body undulations.

Unique to this group are the post-larvae which may be present in the parenchyma of larger gyrocotylids of the same species (see, for example, Halvorsen and Williams, 1968). They seem to disintegrate after a while; the biological function of this phenomenon is unclear. It may be an intraspecific regulation procedure to reduce the number of gyrocotylids per host; so, young hosts may harbor many, larger ones, but seldom more than 2 parasites.

Ecological and Economic Importance

As for pathogenic effects on hosts, inflammation of the epithelium of the spiral valve has been observed, but this observation is mostly restricted to heavily infected individuals. Due to the small number of species occurring in a host group (chimaeras) restricted to specific habitats and of low economic relevance, the group is unlikely to have any economic importance, and further, probably negligible ecological importance.

Acknowledgement

The authors wish to thank Tim Littlewood for information on latter developments in the phylogeny of parasitic platyhelminths including the gyrocotylids.

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ENDOPARASITIC TREMATODES

33

Aspidogastrea

Aspidogastrea (Subclass)

Klaus Rohde

Phylum Platyhelminthes

Class Trematoda

Subclass Aspidogastrea

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 33

Aspidogastrea (Subclass)

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Introduction

Trematodes (also sometimes called flukes) are one of the largest groups of platyhelminths (parasitic worms) with thousands of species. They comprise the Digenea and **Aspidogastrea**. Many species of digeneans have great economic or medical importance. The Aspidogastrea (= Aspidobothria, Aspidobothrea), in contrast, are a very small group of trematodes with around 60 species, none of them of economic importance. But they are of great interest because of their unique structure, their simple life cycles (which may well be the most primitive or ancestral one among the trematodes; Rohde, 1971a), and the extraordinarily complex sensory/nervous systems found in some species. Extensive lists of references of the group have been compiled by Rohde (1999; available at http://tolweb.org/Aspidogastrea/20399 and Alves et al., 2015).

Main Characteristics

The larvae always have a posterior **sucker**, and an anterior **pseudosucker**, or **false sucker**, may also be present, which is not separated from the surrounding tissue by a genuine connective tissue sheath (Figure 1).

Adults do not have a posterior or ventral sucker, but a ventral **adhesive disc** consisting of transverse grooves (**rugae**) and a single row of well-separated small **suckers** (**suckerlets**) or 3 to 4 rows of **alveoli** (**suckerlets**) on a ventral disc (see Figures 2–4).

Hosts include vertebrates and molluscs. In the molluscan host, there is no multiplication of larval stages, that is, a single egg produces a single adult.

All species of Aspidogastrea are hermaphroditic, that is, the adults possess male as well as female reproductive systems. This is demonstrated in the example below, in the

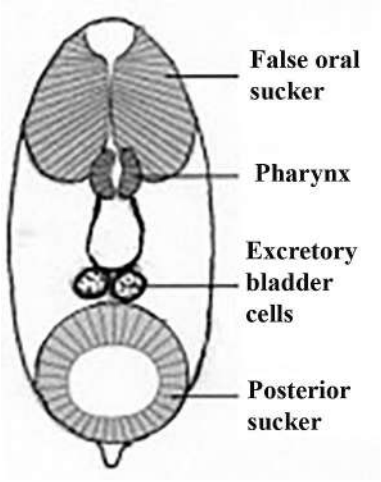

Figure 1. Larva of *Lobatostoma manteri*. Note the **false anterior sucker**, the **pharynx**, the **blind ending cecum**, and the 2 **excretory bladder cells**. The posterior end is drawn out into a short appendage of unknown function. Source: K. Rohde. License: CC BY-NC-SA 4.0.

Figure 2. Ventral view of *Rugogaster hydrolagi*, an aspidogastrean from the rectal glands of the elephant shark *Hydrolagus* in Tasmania, Australia. Note the row of **transverse grooves** (**rugae**). Source: K. Rohde. License: CC BY-NC-SA 4.0.

Figure 3. Part of the aspidogastrean *Multicalyx* sp, from the intestine of a shark. Note the single row of **alveoli** (**suckerlets**) separated by **transverse septa**. Source: K. Rohde. License: CC BY-NC-SA 4.0.

Figure 4. Scanning electron micrograph of the aspidogastrean *Lobatostoma manteri* from the intestine of the teleost fish *Trachinotus blochi*, ventral view. Note the **anterior head** and the **adhesive disc** consisting of 4 rows of **alveoli**. Source: K. Rohde. License: CC BY-NC-SA 4.0.

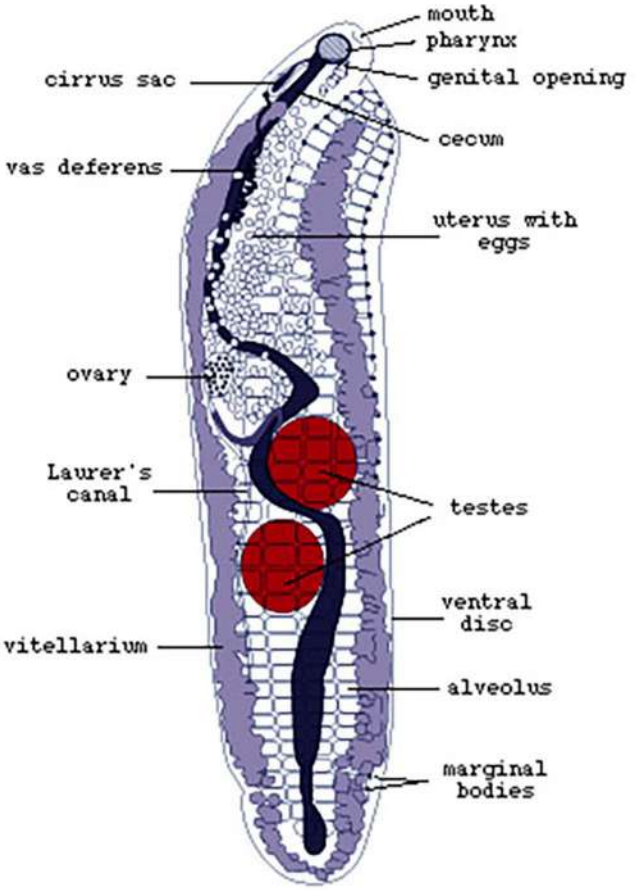

Figure 5. Adult *Multicotyle purvisi*. Note the single and blind ending **cecum** (**intestine**), the **ventral** (**adhesive**) **disc** consisting of 4 rows of **alveoli**, the **male genital system** consisting of 2 large **testes** opening into the **vas deferens** (**sperm duct**) and the **terminal cirrus pouch** (**copulatory organ**), as well as the **female genital system** with a single **ovary** and **vitellarium** (**yolk gland**). The common (to both the male and female systems) **genital opening** is located at the anterior end. The **marginal bodies** are located between the outer rows of **alveoli** and have a glandular function. **Laurer's canal** extends from the female reproductive system to the dorsal surface where it opens to the outside. Source: K. Rohde. License: CC BY-NC-SA 4.0.

image of *Multicotyle purvisi*, an aspidogastrean found in the stomach and intestine of freshwater turtles in Southeast Asia. It reaches a length of about 10 mm and contains both a fully mature male reproductive system as well as a fully mature and gravid female reproductive system (Figure 5).

Unique features of the Aspidogastrea include a septate **oviduct** (that is, the oviduct carrying the egg cells from the ovary has a number of concentric constrictions), and **marginal bodies**, which were long considered to be sensory in nature but are in fact secretory organs (Rohde, 1971d; Rohde and Watson, 1989b) (Figures 6 and 7).

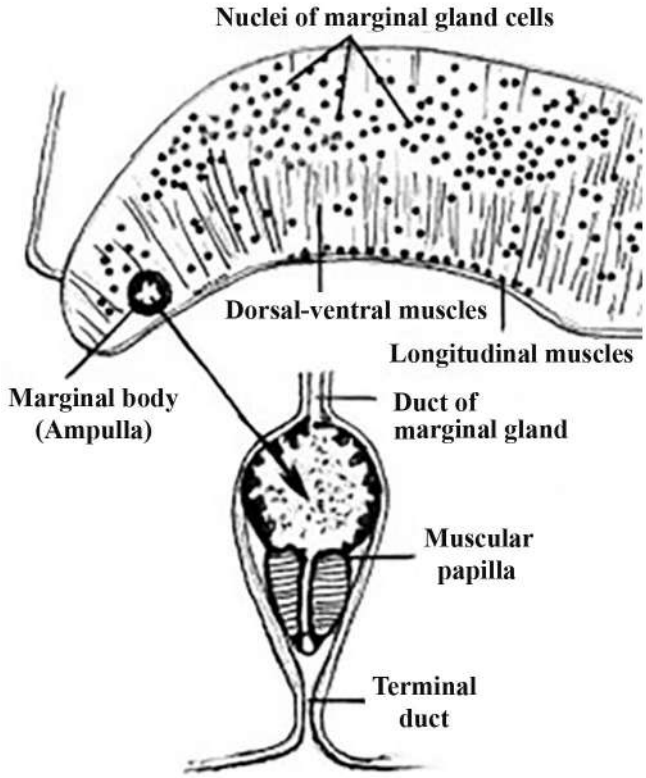

Figure 6. Cross section through the lateral part of the **adhesive disc** of *Lobatostoma manteri* showing the **nuclei** and the **terminal ampulla**, **papilla**, and **terminal duct** of a **marginal gland**. Source: K. Rohde. License: CC BY-NC-SA 4.0.

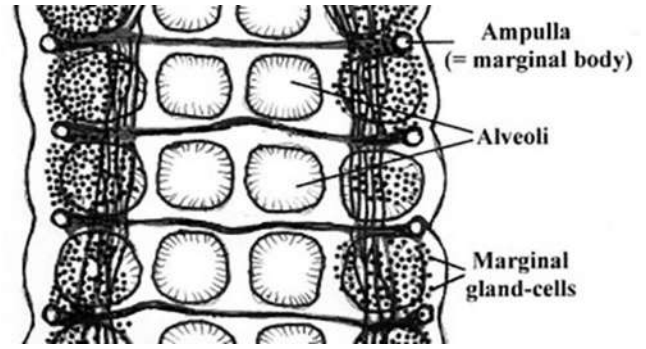

Figure 7. Ventral view of part of the ventral disc of *Lobatostoma manteri* showing the marginal glands and bodies connected by longitudinal and transverse ducts. Source: K. Rohde. License: CC BY-NC-SA 4.0.

Juvenile and Adult Sensory Receptors

Two species of Aspidogastrea in particular, *Multicotyle purvisi* from Malaysian turtles and *Lobatostoma manteri* from Australian marine fish, were examined using light microscopy, as well as scanning electron microscopy. It is important to understand that juveniles of Aspidogastrea from the intermediate host that are infective to the final host differ little from adults; either stage will therefore give identical results. Rohde (1966; 1968a) drew attention to the great variety of **sensory receptors** and their great numbers in *M. purvisi* based on examination of serial sections impregnated with silver under a light microscope. Subsequently, numerous studies, also using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), confirmed this not only for *Multicotyle*, but for *Lobatostoma*, as well.

Location and Number of Surface Receptors

Scanning electron microscopy only shows **surface receptors** (Figure 8). Rohde (1973) counted the surface receptors on scanning electron micrographs of 1 specimen of *Lobatostoma manteri* supplemented by counts of another specimen impregnated with silver, and reported the numbers as follows (see Table 1).

Receptors close to the surface form only a small proportion of receptors, therefore, the total number is far greater. Considering this, Rohde (1989) estimated that a fully grown worm of this species (4 mm-long, unpressed, or unflattened) has a total of 20,000–40,000 receptors, which appears to be extraordinary for a worm of such a small size.

Interior Structures

Transmission electron microscopy is not restricted to the surface but can be used to examine interior structures, as well. Comparison of serial ultrathin sections has shown that juvenile and adult *Lobatostoma manteri* have at least 8 and possibly up to 14 types of **receptors** (Rohde, 1989; Rohde and Watson, 1989a). The receptors are distinguished by the presence or absence of a **cilium**. The length of the receptors is

Table 1. Surface receptors on *Lobatostoma manteri*.

Location	Number
In each anterior marginal alveolus	35
In each marginal alveolus in the middle of the body (among all 60 marginal alveoli, 2,700; and in all 29 median alveoli, 870) (total of 3,570)	50
In a marginal row of papillae just dorsal to the alveoli	780
On the dorsal part of the body	1,200
Along the ventral margins of the ventral head lobes	1,600
On the anterior side of the median dorsal head lobe	140
On the anterior side of the ventral head lobes	300
On the anterior sides of the lateral dorsal head lobes	300
On the posterior side of the dorsal head lobes	200
On the posterior side of the ventral head lobe	150
On the neck	200
Overall total	**8,475**

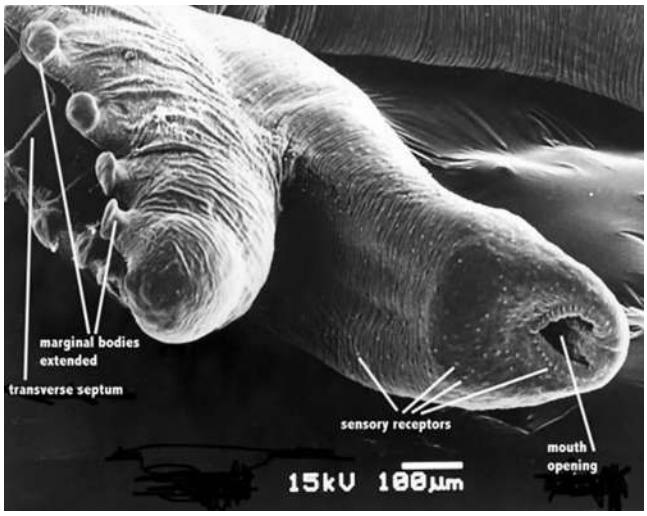

Figure 8. Scanning electron micrograph of anterior end of *Multicalyx elegans*. Source: K. Rohde. License: CC BY-NC-SA 4.0.

determined by the absence or presence of ciliary **rootlets** and their shape, by the number of axonemal **microtubules** in the **axoneme** of the cilium, and whether they are part of a complex organ or not. Juvenile and adult *Multicotyle* have 7 and possibly up to 9 types of receptors. A few major types of receptors that are found in *L. manteri* are illustrated in the following images by single sections (although for distinguishing different receptor types, in all cases serial sections were used). All receptors represent differentiated endings of **dendrites** (that is, **nerve fibers**), usually with ciliary structures within them. For example, the receptor illustrated in Figure 9 has a short cilium at the end of the dendrite, which is embedded in the worm's surface layer (also called its **tegument**). Typically, cilia have a 9 + 2 structure of the **axoneme**, that is, each contains 9 pairs (doublets) of microtubules in the periphery and 2 single microtubules in the center, but there may be deviations from this pattern. Figure 10 shows a receptor without a free cilium, in which the rootlet is widened to form a large disc. The receptor in Figure 11 has a branched ciliary rootlet.

Electron microscopic studies of *Multicotyle purvisi* (Rohde, 1990) have shown the following receptor types, in many respects similar to those of *Lobatostoma*, but differing in some aspects:

1) Disc-like receptor with many dense collars and a modified ciliary rootlet forming a disc;
2) Non-ciliate receptor with a long rootlet;
3) Non-ciliate receptor with a branching rootlet and a dense mass of irregularly arranged microtubules;
4) Non-ciliate receptor with a rootlet fanning out from a basal body, cross-striated in the upper region and with electron-dense structures in the lower part;

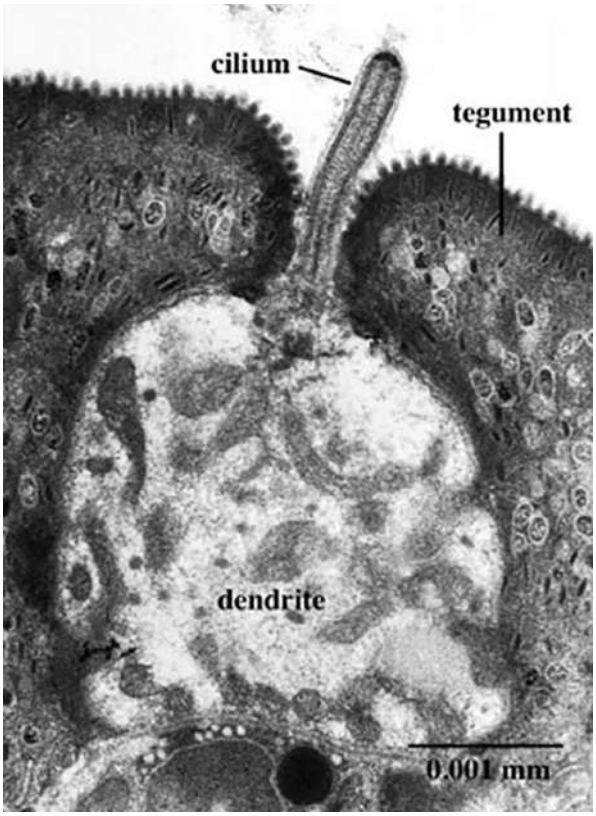

Figure 9. Ciliated surface receptor of *Lobatostoma manteri*. A single cilium arises from the terminal dendritic swelling. Source: K. Rohde. License: CC BY-NC-SA 4.0.

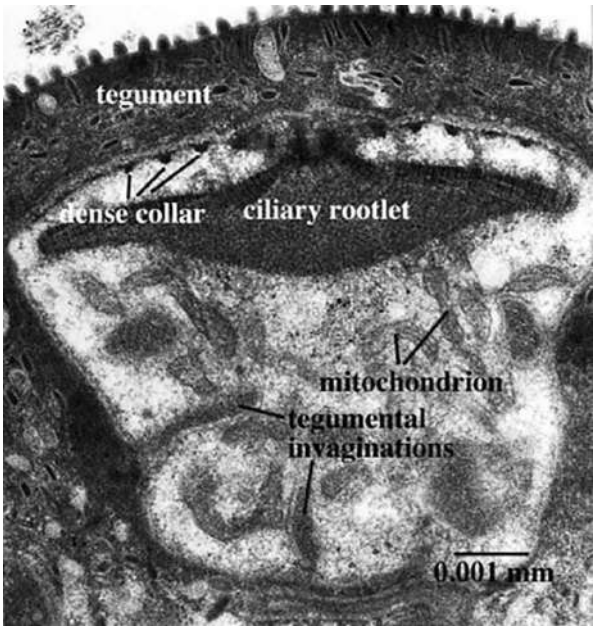

Figure 10. The receptor of *Lobatostoma manteri* without a free cilium, but with an expanded ciliary rootlet. Also note the sections through the dense collars around the upper part of the dendrite. Source: K. Rohde. License: CC BY-NC-SA 4.0.

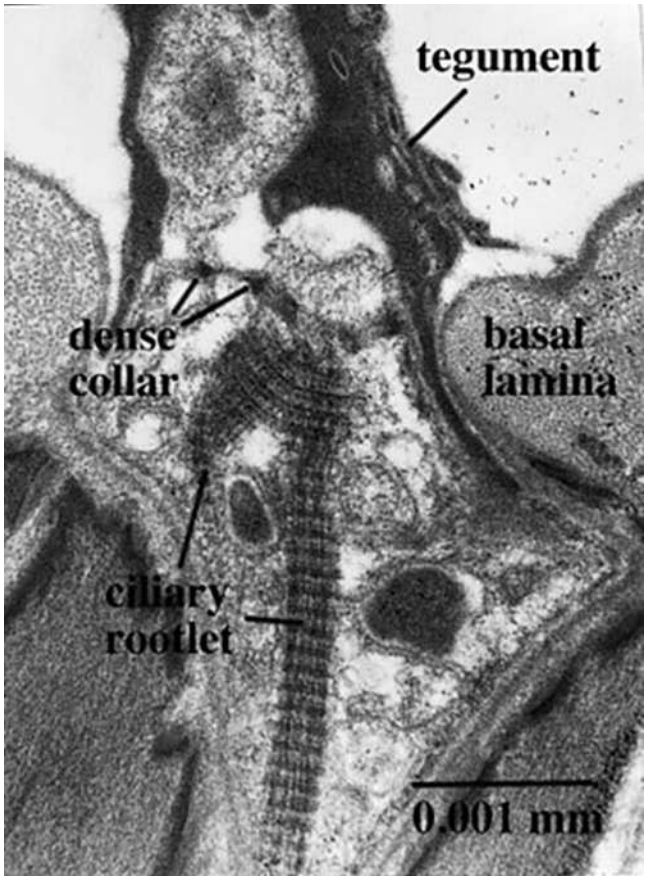

Figure 11. Non-ciliate receptor of *Lobatostoma manteri* located in a deep pit, with branching ciliary rootlet. Source: K. Rohde. License: CC BY-NC-SA 4.0.

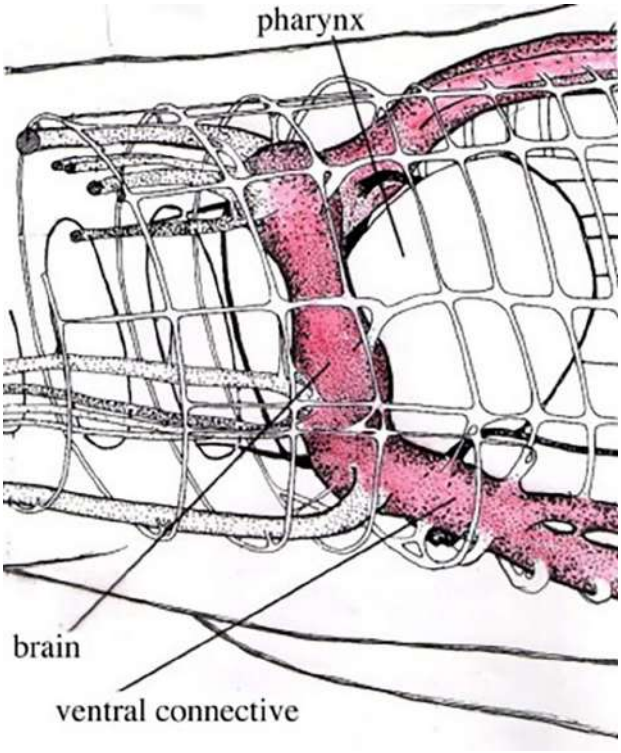

Figure 12. **Nervous system** of *Multicotyle purvisi* in the anterior part of the body. Some nerves on the right-hand side of the worm have been cut in order to show the arrangement of the nerves in cross section. Note the 2 rings of commissures, and the very large brain (**cerebral commissure**) and **main ventral connective** (pink). Source: K. Rohde. License: CC BY-NC-SA 4.0.

5) Uniciliate receptor with a thick layer of cytoplasm around the axoneme;
6) Receptor with a short cilium, at the base of a deeply invaginated tegument;
7) Receptor with a short cilium terminating in an electron-denser apical cap;
8) Uniciliate receptor with a long cilium.

In addition, there may be a small non-ciliate receptor with a long ciliary rootlet at the base of the thick dorsal tegument, and uniciliate receptors differing from the uniciliate receptor with a long cilium in the number of dense collars and the length of the cilium and ciliary rootlet.

Juvenile and Adult Nervous System

The **nervous system** of larval and adult *Multicotyle purvisi* has been reconstructed in detail using serial sections impregnated with silver and supplemented by sections stained with various other stains, among them some specific for neurosecretion (Rohde, 1968b; 1971c; review in Rohde, 1972). In most Platyhelminthes, the nervous system consists of **longitudinal nerves** (**connectives**) connected by **transverse nerves** (**commissures**). The dorsal part of one of the most anterior commissures is often particularly well-developed, forming the **cerebral commissure**, or **brain**. In *Multicotyle*, the number of anterior connectives is much greater than in any species of the many turbellarians that have been examined, and there are 2 rings of commissures, 1 close to the tegument, the other deeper in the tissue. The dorsal part of an interior commissure just anterior to the **pharynx** is very large, forming the brain (Figure 12). More posteriorly, the nerves form a typical system of connectives and commissures (with 1 pair of dorsal, 1 pair of lateral and 1 pair of ventral connectives), as well as a complex pattern innervating the **ventral** (**adhesive**) **disc** (Figure 13).

Interestingly, a dense network of nerve fibers (**nerve plexus**) innervates the **intestine** (Figure 14), and the **connective tissue septum** separates the dorsal part of the body from the ventral disc. Transmission electron-microscopy of the nerves of *Multicotyle purvisi* revealed the presence of a nerve sheath around parts of a posterior connective (Rohde, 1970), a structure not known from other flatworms.

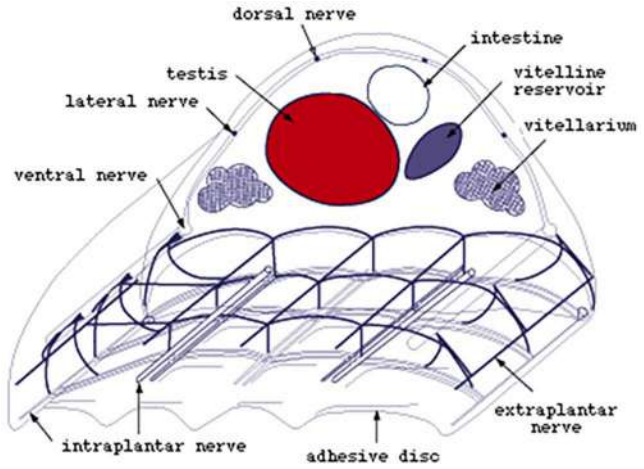

Figure 13. **Nervous system** of *Multicotyle purvisi* in the middle part of the body, showing a typical arrangement of connectives and commissures in the dorsal part of the body, and an intricate pattern of nerves innervating the **ventral** (**adhesive**) **disc**. Source: K. Rohde. License: CC BY-NC-SA 4.0.

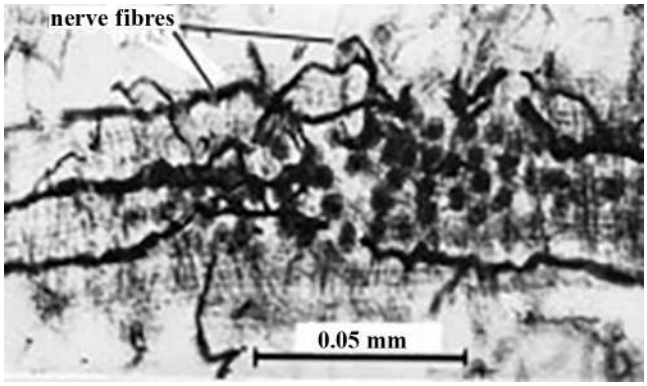

Figure 14. **Nerve plexus** around the intestine of *Multicotyle purvisi*. Section impregnated with silver as seen under the light microscope. Source: K. Rohde. License: CC BY-NC-SA 4.0.

Larval Sensory Receptors and Nervous System

Information about the sensory receptors of the larvae of *Multicotyle purvisi* and *Lobatostoma manteri* has been published in several papers (Rohde and Watson, 1990a; 1990b; 1990c; 1991; 1992a; reviews in Rohde, 1994; 1999). In *M. purvisi*, 13 receptor types were found altogether. *Multicotyle purvisi* has a paired eye and a paired receptor complex dorsal to the mouth cavity, each complex consisting of 2 dendrites. One of the dendrites forms a large liquid filled cavity with at least 10 short cilia lacking ciliary rootlets but possessing basal bodies and lamellate extensions of the ciliary membrane. The other of the dendrites penetrates the anterior wall of the cavity formed by the first dendrite and possesses a single cilium, star-shaped in cross section. Each **eye** (**ocellus**) consists of 1 **pigment cell** and 2 **receptor cells** with **rhabdomeres** (the light-sensitive dendritic endings) (Figure 15).

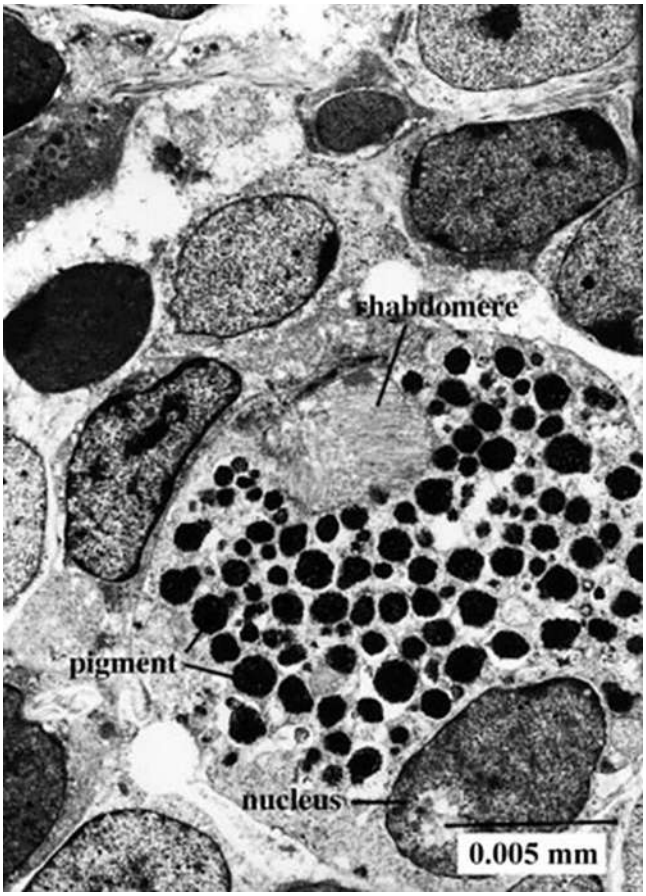

Figure 15. Transmission electron micrograph of ocellus (eye) of larval *Multicotyle purvisi*. Note the pigment cell with pigment granules, nucleus of pigment cell, and rhabdomere (light-sensitive dendritic endings). Source: K. Rohde. License: CC BY-NC-SA 4.0.

The larva of *Lobatostoma manteri* has only about 9 types of receptors. In *L. manteri*, eyes are lacking and anterior receptor complexes are not found, either. The difference between the 2 larvae can be explained by the way infect the intermediate host. *Lobastoma manteri* does not hatch, it is ingested by a snail. *Multicotyle purvisi* hatches, swims in water, is attracted to the surface layer by light stimuli, and is then inhaled by a snail host.

The nervous system of larval *Multicotyle* was reconstructed using serial sections impregnated with silver. It shows the basic pattern also found in the adult, with nerves innervating the pharynx, intestine, and posterior sucker, and a large number of anterior connectives (Rohde, 1971c).

Life Cycles

The life cycles of several species have been worked out. Based on the knowledge available to date, 2 kinds of life

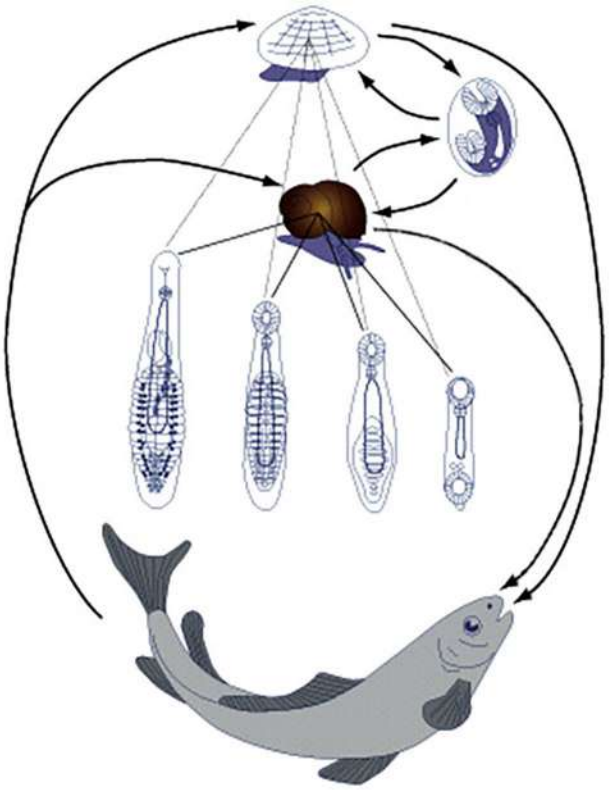

Figure 16. Life cycle of *Aspidogaster conchicola*. Only molluscs (freshwater bivalves or snails) are necessary for the completion of the life cycle. Adult worms produce eggs in which a non-ciliated larva with an anterior and posterior sucker develops. There are conflicting reports on how molluscs become infected: Either by eggs containing larvae or by hatched larvae. The life cycle can be completed without involvement of a vertebrate host, but if a fish eats an infected mollusc, adults can produce eggs in it. Source: K. Rohde. License: CC BY-NC-SA 4.0.

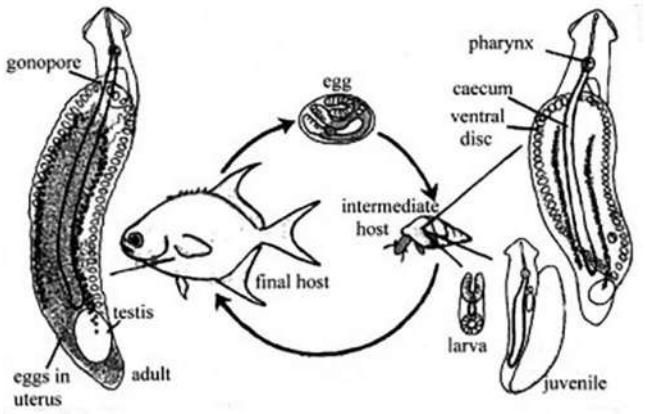

Figure 17. The life cycle of *Lobatostoma manteri*. Adults live in the small intestine of the marine teleost fish *Trachinotus blochi* (family Carangidae). They possess fully mature male and female reproductive systems with 1 large posterior testis, and a uterus filled with eggs which are shed through the gonopore at the anterior end. Eggs are deposited with the feces of the fish on the sea floor, where they are eaten by snails such as *Cerithium* (*Clypeomorus*) *moniliferum*. In the snail, the posterior sucker of the larva develops to the adhesive disk, and reproductive organs develop to (almost) the final state, without, however, maturing and producing sperm and eggs. Adapted from Rohde, 2001. License: CC BY-NC-SA 4.0.

cycles can be distinguished. In one type, the entire life cycle can be completed in molluscs, although vertebrates may act as facultative hosts (not obligate hosts). In the other, both a mollusc and a vertebrate are required for completion of the life cycle. An example of the first kind is *Aspidogaster conchicola*, whose life cycle has been studied by many authors beginning in the 19th century (references in Rohde, 1972; 1999) (Figure 16). Another example is *Cotylaspis insignis,* sexually mature specimens of which were found in molluscs and turtles (references in Rohde, 1972). The species has found considerable attention in the last decade (for example, see Rosen et al. 2016a; 2016b; 2016c; 2017).

An example of the second kind of life cycle is *Lobatostoma manteri* from the small intestine of the marine teleost fish *Trachinotus blochi* (Rohde, 1973) (Figure 17).

At Heron Island on the Great Barrier Reef north of Australia, only juvenile *Trachinotus* (a few cm-long) were found to be infected. They crush very thick-shelled snails with their well-developed pharyngeal plates (Figure 18). When first infected, larvae hatch in the stomach of the snails but move into the digestive gland where they develop (Figure 18).

Like *Lobatostoma manteri*, *Multicotyle purvisi* (Figure 5) also needs a mollusc and vertebrate host for the completion of its life cycle. However, infection of the mollusc is not by an egg that is ingested, but by a larva that hatches in freshwater and then swims for hours by means of its 10 ciliary tufts with support by a flotation mechanism, a thick sheath of microfila (Figure 19). Larvae are inhaled by snails and migrate into the kidneys where they grow to the stage infective to turtles (Rohde, 1971b).

It is possible that other species of aspidogastreans have more complex life cycles. Thus, larvae of *Stichocotyle nephropis*, were found encapsulated in the intestinal wall of lobsters, while adult *S. nephropis* infect elasmobranchs. Immature *Multicalyx* have been recorded from the intestines of teleost fish, while adult *Multicalyx* infect holocephalans (class Chrondrichthyes: subclass Holocephali—that is, ratfish and ghost sharks) and elasmobranchs (class Chondrichthyes: subclass Elasmobranchii—that is, sharks, skates, and rays). This suggests that, in addition to the intermediate and final hosts, a further host acting as a transport host (that is, a host containing immature stages which do not develop in it), may be involved.

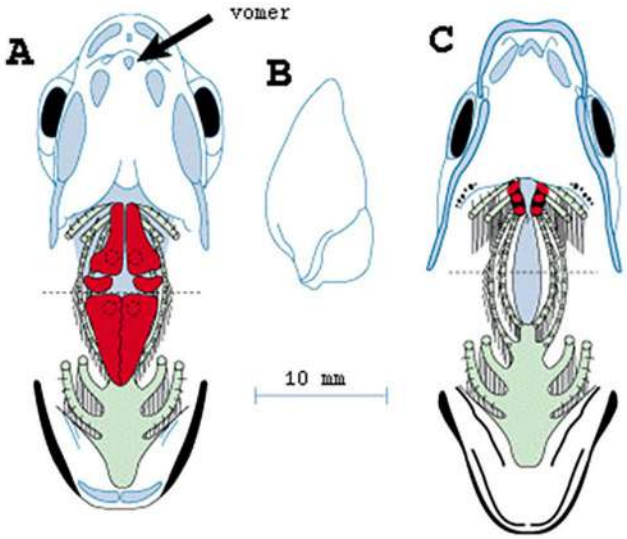

Figure 18. The head of juvenile *Trachinotus blochi* (A) opened along the dotted line. Snails (B) are crushed between the pharyngeal plates (red) which are moved by strongly developed muscles responsible for the peculiarly shaped head of the fish species (snub-nosed dart). The pointed vomer (arrow) prevents the snail from slipping out of the mouth. On the right a close relative of *Trachinotus blochi* of about the same size (C): Note the ostensibly normal pharyngeal plates. This fish species cannot become infected because it cannot crush the snails. Source: K. Rohde. License: CC BY-NC-SA 4.0.

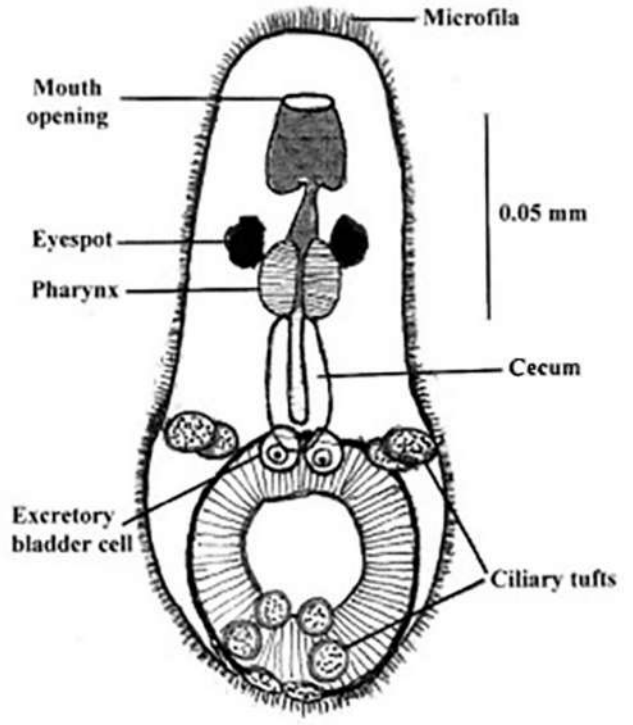

Figure 19. Larva of *Multicotyle purvisi*. Note the anterior mouth not surrounded by an anterior sucker, followed by the pharynx and cecum. As in the larva of *Lobatostoma manteri* (see Figure 1), there are a posterior sucker and 2 excretory bladder cells. In addition, the surface is covered by a thick sheath of microfila which appear to help in flotation. Altogether 10 ciliary tufts enable the larva to actively swim, and a pair of eyespots (ocelli) facilitate reaction to light. Source: K. Rohde. License: CC BY-NC-SA 4.0.

In all species of Aspidogastrea that have been studied, the posterior sucker of the larva is transformed into an adhesive disc. In *Rugogaster*, for example, the rugae are formed by the posterior wall of the sucker (Rohde and Watson, 1992b). In species of *Lobatostoma* and *Multicotyle*, among others, alveoli are formed within the sucker. Detailed studies of *Stichocotyle* have not been made.

Taxonomy and Phylogeny

About 60 species of aspidogastreans in 13 genera have been described. There has been some controversy about the relationships of the various genera of aspidogastreans, but according to the prevailing view, 4 families are distinguished, as follows (Rohde, 2002; see Figure 20):

1) **Rugogastridae** (2 ceca, single row of transverse rugae) comprising a single genus *Rugogaster* with 2 species from the rectal glands of holocephalan fishes;
2) **Stichocotylidae** (1 cecum, single row of well separated suckerlets) with a single species, *Stichocotyle nephropis*, from the intestine of elasmobranchs;
3) **Multicalycidae** (1 cecum, single ventral row of alveoli separated by transverse septa) with a single genus *Multicalyx* from the intestine of holocephalan fishes and elasmobranchs;
4) **Aspidogastridae** (1 cecum, ventral disc with 3 or 4 rows of alveoli) with 9 genera in 3 subfamilies from molluscs, turtles, and teleost fishes (however, note that new work shows that this family to be polyphyletic; see Sokolov et al., 2019).
    4a) Subfamily **Rohdellinae** (terminal part of male and female reproductive ducts united to form a hermaphroditic duct) with a single species *Rohdella siamensis* from freshwater teleosts;
    4b) Subfamily **Cotylaspidinae** (3 rows of alveoli) with 3 genera, *Cotylogaster*, *Cotylaspis*, and *Lissemysia* which differ in the number of testes (1 or 2) and the absence or presence of a cirrus pouch;
    4c) Subfamily **Aspidogastrinae** (4 rows of alveoli) with 6 genera, *Multicotyle*,

Lobatostoma, *Aspidogaster*, *Lophotaspis*, *Sychnocotyle*, and *Neosychnocotyle*, which differ in the number of testes (1 or 2), the absence or presence of a cirrus pouch, and the absence or presence of head lobes and/or papillae on the ventral disc.

Whereas the Aspidogastridae have an adhesive disc bearing 3 or 4 rows of alveoli and use teleosts or turtles as hosts, all the other families share the characters (synapomorphies) rugae or a single row of deep suckers/alveoli, as well as the use of elasmobranchs or holocephalans as hosts. Gibson and Chinabut (1984) distinguished 2 orders: 1) **Aspidogastrida** with the single family Aspidogastridae, and 2) **Stichocotylida** with the other families. Since DNA studies on the relationship of the families with each other have not been made, the following diagram illustrates the likely relationship of the aspidogastrean families with each other based on morphology and hosts.

The sister group of the Aspidogastrea is the very large group Digenea, with thousands of species and many families (for example, see Park et al., 2007). The ancestor of the Digenea split from the ancestor of the Aspidogastrea early in evolutionary history of the flatworms, probably more than 400 Ma (= million years ago; Littlewood et al., 1999a). Comparative studies using 18S rDNA (Littlewood et al., 1999b), 28S rDNA (Litvaitis and Rohde, 1999b), as well as extensive electron microscopic studies (for example, Ehlers, 1985; Littlewood et al., 1999b) have demonstrated that all the major groups of parasitic Platyhelminthes, the Trematoda, Eucestoda, Gyrocotylidea, and Amphilinidea, as well as the Polyopisthocotylea and Monopisthocotylea—have 1 common ancestor, that is, from 1 monophylum, the Neodermata (for example, see Ehlers, 1985; Rohde, 1997). This hypothesis was also confirmed by later DNA studies (for example, see Egger et al., 2015). Note, however, that the Polyopisthocotylea and Monopisthocotylea are commonly put in the Monogenea which is not a monophyletic group (for example, see Littlewood, 2006).

Infection Process and Localization in the Host

As discussed above, the 2 species of Aspidogastrea examined in detail possess some intriguing differences both in morphology and life cycles. The larva of *Multicotyle purvisi* has a larger variety of sensory receptors than that of *Lobatostoma manteri*, including a pair of eyes and an anterior paired receptor complex which are absent in the latter species. It also has 10 ciliary tufts and a coat of microfila, that is, very thin processes of the tegument. The larva of *L. manteri,* on the other hand, has a well-developed pseudosucker absent in the former species.

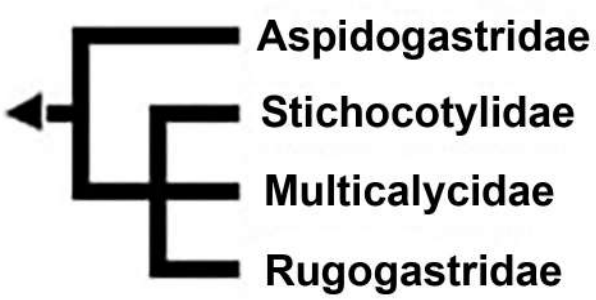

Figure 20. The likely relationship of the aspidogastrean families with each other based on morphology and hosts. Source: K. Rohde. License: CC BY.

Adults of *Multicotyle purvisi* reach a length of at least 10 mm (unpressed, or unflattened). Adults of *Lobatostoma manteri* reach a length of about 7 mm (pressed) and 4 mm (unpressed, or unflattened). The former species has a uterus coiled up in the anterior part of the body, with relatively few eggs. The latter species has a uterus filling most of the body, with a large number of eggs. The juvenile and adult of both species have a large number of marginal organs (terminal parts of glands) between the marginal alveoli of the adhesive disc. In the following sections, these differences will be explained by distinctions in the life cycles of the species.

***Multicotyle purvisi*: Infection Process and Localization in the Intermediate Host**

Rohde (1971b) described the infection process of *Multicotyle purvisi* from the stomach and occasionally the anterior part of the duodenum of several species of Malayan turtles, with freshwater snails as intermediate hosts, as follows:

Eggs containing embryos at the 1–3 cell stage are laid. Larvae develop in the egg after it has escaped in the feces of turtles into freshwater. In experiments at temperatures of 27–29 ºC, first hatching occurred 25 days after egg laying, at 21–28 ºC first hatching occurred after 35–40 days, at 19–22 ºC after 103 days. Environmental temperatures in Malaysia are 21–32 ºC (in the shade in the lowlands). At higher temperatures the hatching process takes only a few minutes. Hatching in cultures under normal diurnal fluctuations of light and temperatures occurs, with few exceptions, in the early hours of the morning. In cultures kept in the dark beyond the normal time of hatching, hatching occurred only after illumination. However, when cultures were kept in the dark over days, hatching occurred also without a light stimulus.

Immediately after hatching, larvae swim usually with an extended anterior end, rotating around their longitudinal axis, either along the bottom or straight upwards to the surface, but also irregularly in all directions in the water. They often

remain attached to the surface, slowly rotating, or sink slowly to the bottom with the posterior end directed downwards, or faster with the anterior end directed downwards. Larvae can also float in the middle of the water column rotating slowly around their longitudinal axis, carried sidewards by water currents. They sometimes remain at the bottom, appearing to touch and feel the substratum.

Larvae are positively phototrophic and survive at 26–30 ºC for 5 to over 33 hours. They reach the host less by their own movements, but rather by water currents produced by snails, which carry them into the inhalant opening.

Localization of larvae in the snails was determined in 3 specimens of the snail *Pila scutata*. At 50 and 69 days, respectively, after infection, a larva was found in the anterior kidney chamber; 108 days after infection, a larva was found in the posterior kidney chamber of the snail. Experiments showed that turtles become infected by ingesting infected snails.

The smallest specimens of *Multicoyle* found in a large number of naturally infected turtles had 17 and 18 transverse rows of alveoli, respectively. It therefore seems that specimens must be of a certain minimum size before they become infective. Fully grown up and mature specimens have 50 transverse rows of alveoli.

Functional Morphology

These features of the life cycle suggest that the larval eyes are responsible for phototaxis which keeps them in the water column where they can encounter snails, but they may also contribute to hatching in the morning.

The paired anterior sensory complex may have the function of a balancing organ, as suggested by the ciliary structure extending into interior liquid filled cavities.

The coat of microfila increases the surface area without increasing the weight, suggesting that it makes floating in the water column more effective.

Ciliary tufts are necessary for swimming, which leads the larvae not directly to the snails, but into the water column where snails may inhale them. This kind of infection behavior is possible only because the habitat of these freshwater snails is relatively undisturbed, that is, eggs and larvae after hatching do not run a great risk of being swept away into a less favorable site by adverse currents.

The numerous sensory receptors may enable the parasite to keep damage to the very delicate tissues of the host, in particular their kidneys, on which it depends for survival, at a minimum. But they may also contribute to finding the snails' habitat and to mate-finding.

The uterus of the adult can be kept relatively short, because larvae in the eggs develop only after leaving the worm.

***Lobatostoma manteri*: Infection Process and Localization in the Intermediate Host**

Rohde (1973; 1975) described the infection process of *Lobatostoma manteri* as follows: Eggs are laid which contain already fully developed larvae. Snails become infected by eating eggs containing larvae. In experiments, larvae hatched in the stomach of snails (*Planaxis sulcatus* and *Cerithium moniliferum*) and migrated immediately along the ducts of the digestive gland into the digestive follicles.

Host Tissue Reactions

Larvae of *Lobatostoma* feed on secretions and probably epithelial cells of the follicles of the digestive gland of snails. The posterior sucker and developing ventral disc are used for adhesion to the epithelium, and they contribute to its erosion. In heavy experimental infections, 47–49 and 65–66 days after infection, only small parts of the epithelium are still secretory, and the larvae live in large, fused cavities. Juveniles are usually found in a cavity formed by an enlargement of the main duct and 1 (or maybe more?) side duct of the digestive gland near the stomach in *Cerithium moniliferum*, or in the stomach and main ducts of the digestive gland of *Peristernia australiensis*. They may creep from the ducts into the stomach and back into the ducts. Fish become infected by eating snails.

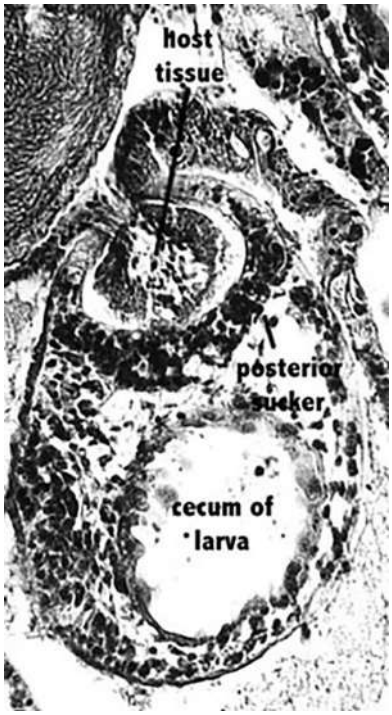

Figure 21. Young larva of *Lobatostoma manteri* (47–49 days old) feeding on the digestive gland of an experimentally infected *Cerithium moniliferum*. Note the posterior sucker around some host tissue. Source: K. Rohde. License: CC BY-NC-SA 4.0.

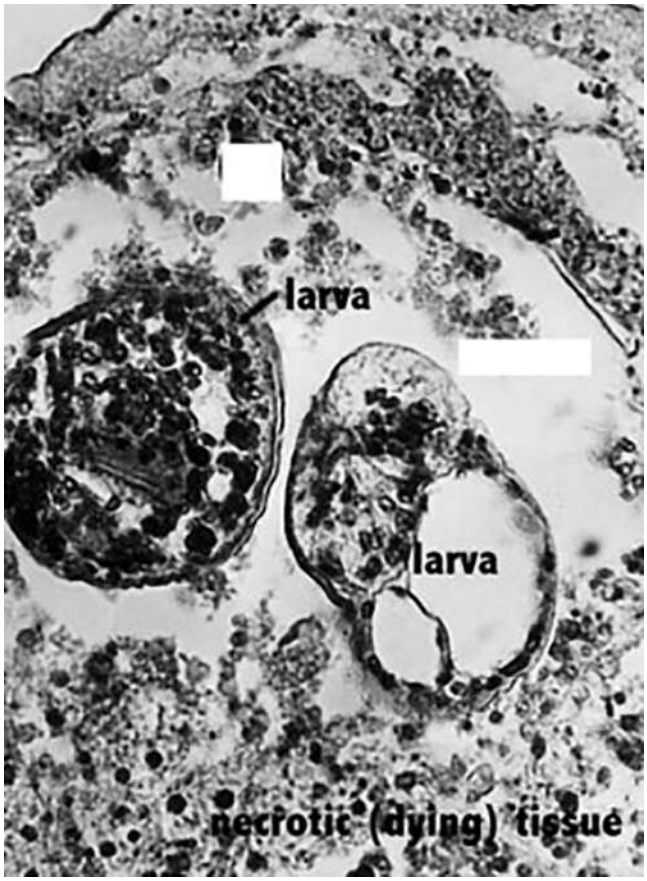

Figure 22. Two larvae (65–67 days old) of *Lobatostoma manteri* in the digestive gland of an experimentally-infected *Cerithium moniliferum*. Note the functional glandular tissue replaced by necrotic (dead and dying) cells. Source: K. Rohde. License: CC BY-NC-SA 4.0.

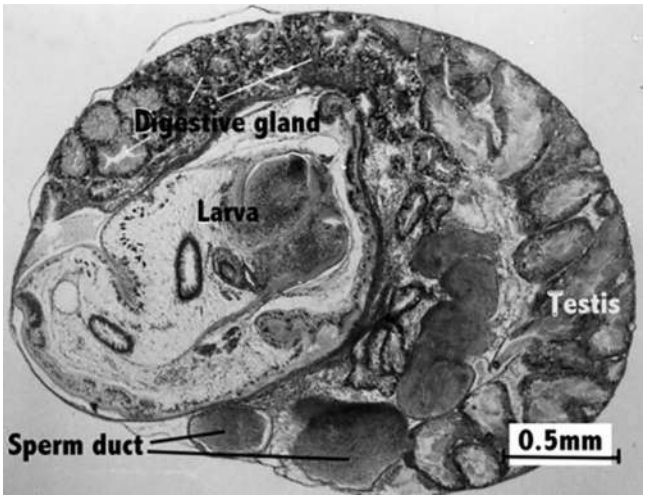

Figure 23. *Cerithium moniliferum* with one large juvenile *Lobatostoma manteri* ("larva") in the digestive gland. Source: K. Rohde. License: CC BY-NC-SA 4.0.

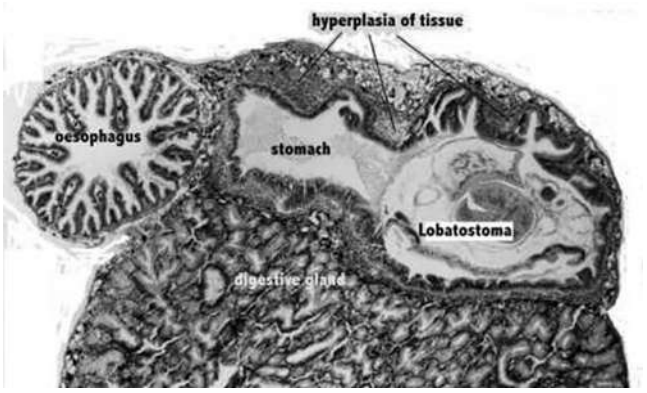

Figure 24. Large juvenile of *Lobatostoma manteri* in the stomach of naturally infected *Peristernia australiensis*. Note the enlarged subepithelial tissue (hyperplasia), that is, fibrosis (abnormally thick connective tissue) around parts of the stomach. Source: K. Rohde. License: CC BY-NC-SA 4.0.

Rohde and Sandland (1973) examined histological sections of *Cerithium moniliferum* and *Peristernia australiensis* infected with *Lobatostoma manteri*. In the former species (much smaller than the latter), a single parasite is usually present, coiled up in a cavity formed by the main digestive gland, and perhaps 1 or more side ducts of the digestive gland, causing metaplasia of the duct epithelium, hyperplasia of the inter-follicular connective tissue, an increase in the number of amoebocytes, and necrosis of some glandular follicles. The latter species may harbor up to 6 parasites in the stomach and in the large ducts of the digestive gland, with a thickening of the subepithelial connective tissue layer.

Some stages of pathogenesis caused by larval and growing *Lobatostoma* are illustrated in Figures 21–23. Note in particular that in the small snail species infected, *Cerithium moniliferum,* a single large juvenile worm is usually located in the digestive gland, in which only a few digestive follicles remain functional, whereas in the much larger *Peristernia australiensis* several large juveniles are often found in the stomach, with tissue reactions around it but most glandular follicles functional (Figures 24–26).

The illustrations show the considerable damage done to the hosts by the infection, although it should be pointed out that, during investigations, naturally infected snails never had as many parasites as experimentally infected ones. Reasons may be that snails in their natural habitat never encounter so many eggs, that heavily infected snails die, or that in natural infections later infections are suppressed by larvae or juveniles already present, either by predation of large on smaller larvae, or by tissue reactions induced by older parasites.

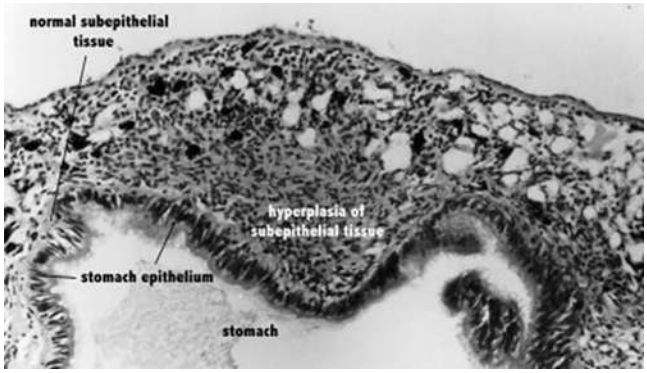

Figure 25. Another view of the *Lobatostoma manteri* specimen in the stomach of naturally infected *Peristernia australiensis* shown in Figure 24. Source: K. Rohde. License: CC BY-NC-SA 4.0.

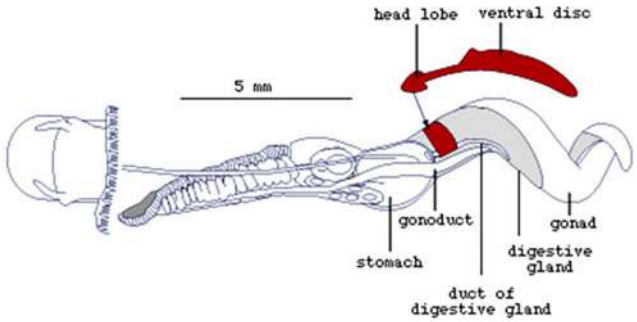

Figure 26. *Peristernia australiensis* infected with *Lobatostoma manteri.* This is an enlarged portion of Figure 25. Source: K. Rohde. License: CC BY-NC-SA 4.0.

Functional Morphology

In view of the pathological findings, it seems reasonable to assume that one function of the variety and number of sensory receptors may be to limit damage done to the delicate host tissue by the parasites. However, they may also play a role in mate finding. Rohde (1973) discussed the adaptive value of the ventral disc: It could be an adaptation for locomotion in or on the soft tissues of the host (snails), perhaps facilitating adhesion of only small portions to a small area of the host's tissue and preventing damage to it. Observations of digeneans and *Multicotyle* and *Lobatostoma* showed that the ventral disc is not more effective for attachment to the vertebrate intestine than the suckers of digeneans, suggesting that it is indeed an adaptation to life in molluscs. It is also very rarely used for tight attachment to the surface of containers or snails. The secretion produced by the marginal glands on the discs has not been examined, but it may be digestive, contributing to the erosion of the digestive gland follicles of the snails, as seen in histological sections. The long uterus of *Lobatostoma* is necessary, because eggs leave the worm only after larvae infective to snails have developed in them. This is necessary because the habitat is rather violent, exposed to strong tidal currents, and may dry out at low tide, making rapid ingestion of eggs by snails essential.

Ecology: Infection Dynamics

At Heron Island, Australia, prevalence of infection of several snail species with various Digenea and *Lobatostoma* was monitored around the island over an extended period (Rohde, 1981). Only snails of the species *Cerithium moniliferum*, *Peristernia australiensis*, and *Planaxis sulcatus* were found to be infected with *Lobatostoma*. The aspidogastrean and 11 species of larval digeneans were found in *Cerithium moniliferum* (Rohde and Sandland, 1973). *Peristernia australiensis* did not harbor any digeneans. Except for a few exceptions, *Lobatostoma* was found only in a small part of Heron Island's Shark Bay (which has a flat bottom formed by beach rock), and on beach rock close to it, possibly carried there by snails that had acquired their infection in Shark Bay (Figure 27) (Rohde, 2013). Examination of beach rock in Shark Bay showed a large number of shell fragments on it, mainly of *Cerithium*.

Netting in Shark Bay yielded small snubnosed dart, *Trachinotus blochi*. Its name is derived from the strongly developed muscles in the forehead which move the large pharyngeal plates, an adaptation to crushing the very thick shell of snails. Dissection of these fish revealed *Lobatostoma* in the small intestine and shell fragments in the stomach. Other fish caught in Shark Bay without this structure were never found to be infected.

From January 1971 to April 1972, there was a strong decrease in the relative number of infected snails. During the period of high frequency of infection, *Cerithium* infected with Digenea contained *Lobatostoma* relatively more frequently than snails without Digenea. Snails with double infections disappeared first. Infection with *Lobatostoma* did not affect the relative number of egg-producing *Cerithium* during the period of high frequency of infection. *Lobatostoma* from fish with single infections produced eggs with the haploid number of 7 chromosomes and development did not proceed beyond the blastula stage (Rohde, 1973).

Populations and Communities in Equilibrium or Non-Equilibrium?

There has been much debate about how common equilibrium conditions, largely determined by competition, are in ecological populations and communities. An attempt has been made to interpret the findings on *Lobatostoma* using 2 ecological paradigms, referred to as the demographic and autecological paradigms by Walter and Hengeveld (2000; see also, Hengeveld and Walter, 1999; Walter, 2013).

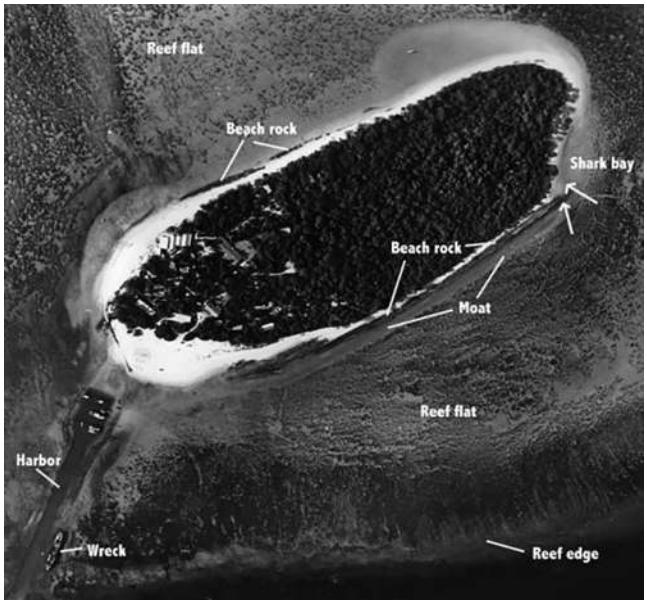

Figure 27. The distribution and infection of snails with *Lobatostoma manteri* at Heron Island at the southern end of the Great Barrier Reef in January–April 1971. Note: Beach rock around much of the island, the harbor and the moat (shallow channel) extending from the harbor area towards Shark Bay. At incoming tide, the moat fills first and fish swim from the reef edge into Shark Bay. The snails *Cerithium moniliferum*, *Planaxis sulcatus*, and *Peristernia australiensis* were infected with *Lobatostoma*, but infection was practically restricted to a small area of Shark Bay with a bottom of flat beach rock (arrows), although snails occurred all around the island on beach rock and rubble. A few *Lobatostoma* were also found a short distance from Shark Bay, possibly acquired in Shark Bay by snails which subsequently migrated along the beach rock. Source: K. Rohde, Vertikalphoto Royal Australian Air Force Number 2 Squadron. License: CC BY-NC-SA 4.0.

In the **demographic paradigm**, species are thought to be demographically similar but have different functions in communities. Intra- and interspecific competition have great importance, leading to co-evolution by optimization processes (that is, processes that bring about optimal adaptation to environmental conditions), to saturation of communities with species, and to equilibrium. Optimization is thought to be possible over short time spans because the abiotic environmental component is, on average, constant.

According to the **autecological paradigm**, species are dissimilar entities affected by abiotic and demographic factors. Optimization is impossible because of the greatly variable environment.

The demographic paradigm gives rise to the question: Why do so many species share the same resources? The autecological paradigm leads to the question: How did species arise and how do they survive in a variable and heterogeneous environment? It focuses on the unique nature of adaptations and on species with their spatial responses to environmental conditions. Walter and Hengeveld (2000) claim that the 2 paradigms are mutually exclusive.

Which of the 2 paradigms is better suited to explain the unique adaptations of the 2 aspidogastreans discussed, and the distribution of *Lobatostoma*? As pointed out above, the Aspidogastrea are a very ancient group, having diverged from the digenean trematodes more than 400 Ma. Its unique features (such as the adhesive disc, marginal glands, great variety and number of sensory receptors, and no multiplication of larvae in the intermediate host) also are likely to be very ancient. It is unlikely that they have evolved due to short-term adaptations to particular environments.

Possible competitors with the 2 aspidogastreans are larval Digenea in the snails. However, the distribution of *Lobatostoma* and Digenea at Heron Island clearly shows that prevalence of infection with Digenea is greatest in a small habitat which also has the heaviest infections with *Lobatostoma*, making it unlikely that interspecific competition plays any role in determining the distribution of *Lobatostoma* at Heron Island. Intraspecific competition, that is competition between individuals of *Lobatostoma* in the snails, may well occur, as suggested by the observation that the smallest of the 3 snail species infected, *Cerithium moniliferum*, very rarely contains more than 1 juvenile of *Lobatostoma*. More individuals simply cannot be accommodated. But it is difficult to see how this could have led to any of the adaptations and to the distribution of the species. Clearly, each species has features that are long-term adaptations to a particular kind of life cycle and habitat. In other words, only the autecological paradigm can explain them.

However, caution is necessary in accepting the statement that the 2 paradigms are mutually exclusive. Rohde (2005), in discussing the relative frequency of equilibrium (caused by competition) and non-equilibrium conditions in biological systems, stressed that groups with certain characteristics will tend to exist in equilibrium, others will tend to exist in non-equilibrium. Both conditions are possible and depend on the size of populations and individuals, and on the vagility of the species. If all these are small (as in the aspidogastreans), a tendency towards non-equilibrium results (Rohde, 2005).

Acknowledgment

The foregoing text is based on the author's online articles (further details therein):

https://krohde.wordpress.com/2011/12/31/the-aspidogastrea-a-parasitological-xk923bc3gp4-13/

https://krohde.wordpress.com/2011/12/31/the-aspidogastrea-a-parasitological-xk923bc3gp4-15/

https://krohde.wordpress.com/2011/12/31/the-aspidogastrea-a-parasitological-xk923bc3gp4-16/
https://krohde.wordpress.com/2011/12/31/die-aspidogastrea-ein-parasitologisches-xk923bc3gp4-20/
https://krohde.wordpress.com/2011/12/31/sacculinisierung-bei-parasiten-die-xk923bc3gp4-37/
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34

Digenea, Diplostomida

Introduction to Diplostomida Olson et al., 2003 (Order)

Lucrecia Acosta Soto, Bernard Fried, and Rafael Toledo

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Diplostomida

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 34

Introduction to Diplostomida Olson et al., 2003 (Order)

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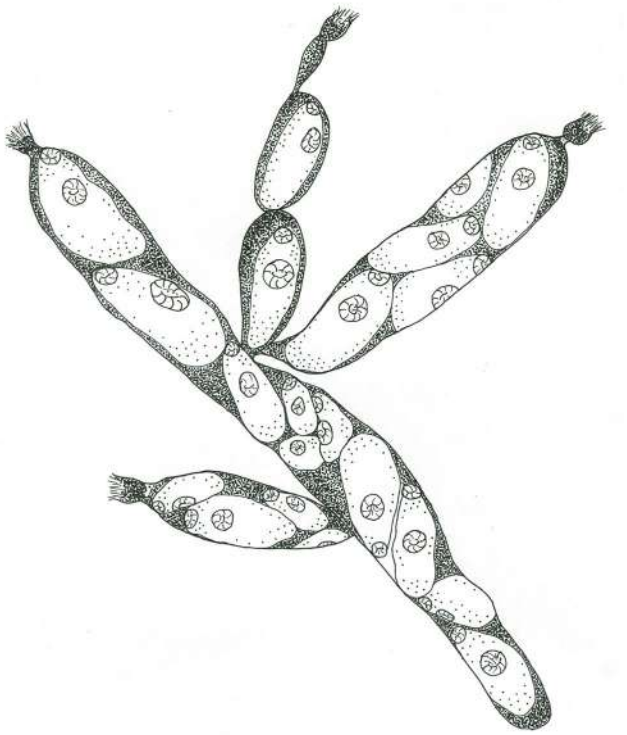

Figure 1. Branched sporocyst of Brachylaimoidea. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

Introduction

The order **Diplostomida** constitutes 1 of the 2 main lineages from which digeneans (subclass **Digenea**) have diversified (Olson et al., 2003). Using a Bayesian analysis, the order Diplostomida was established by Olson and others in 2003 and includes only 1 suborder, **Diplostomata** (Olson et al., 2003). Although some discrepancies have been found between the mt (meaning, mitochondrial) genomic phylogeny and the rDNA genomic phylogeny, a study by Locke et al. (2018) used a much larger genomic dataset which supported the validity of Diplostomida, arriving at a similar result as that of Olson and colleagues (2003).

Probably, the most relevant feature of the morphology of these digeneans is the position of the genital pore posterior to the ventral sucker in the adult stage.

Diplostomida Systematics

This order comprises 3 main lineages or superfamilies: **Brachylaimoidea**, including **Leucochloridiidae**; **Diplostomoidea**, in which members of **Diplostomidae** and **Strigeidae** are intermingled; and the blood flukes, or superfamily **Schistosomatoidea**, in which **Schistosomatidae**, **Sanguinicolidae**, **Spirorchidae**, and **Clinostomidae** are included (Olson et al., 2003). Following are descriptions of some Diplostomida groups.

Superfamily Brachylaimoidea

The Brachylaimoidea comprises a group of digeneans that are difficult to identify and that have poorly understood phylogeny. Larvae in the mollusc intermediate host have branched sporocysts (Figure 1) and cercariae with a poorly developed or absent tail are probably the most relevant morphological features. The main diagnostic features are the position of the genital pore, the extent of the vitellaria through the body, the arrangement of the gonads, the presence of a cirrus sac, and the pattern of the life cycle. Adult worms are characterized by a very short or absent esophagus, which may be more patent in the cercariae. The body may be of variable shape and armed or unarmed with fine spines. The pharynx is stout and muscular and, commonly, preceded by a prepharynx. Usually, the 2 blind ceca reach close to the posterior extremity. Gonads in tandem or a triangle are posterior to the ventral sucker. For more detailed data on the morphology of Brachylaimoidea, see Pojmańska (2002h).

Members of Brachylaimoidea are mainly parasites of birds and mammals, affecting domestic animals, poultry, wild game birds and, rarely, amphibians or reptiles (Joyeux et al., 1934; Harkema, 1939; Heneberg et al., 2016). Some members of Brachylaimoidea have been found to parasitize humans (Butcher et al., 1996; 1998; Butcher and Grove, 2001).

The history of the superfamily Brachylaimoidea shows a rather controversial classification. The superfamily was first recognized by Allison (1943), with Brachylaimidae as the type family (Joyeux and Foley, 1930), and retained in the classifications published in the 1960s and 1970s. A phylogenetic analysis by Olson and colleagues (2003) included this superfamily within the order Diplostomida and the suborder Diplostomata.

Although several phylogenetic and molecular studies have been performed to help differentiate the members of Brachylaimoidea (Machalska, 1978; Casey et al., 2003; Olson et al., 2003; Iwaki et al, 2009; Locke et al., 2012; Zhukova et al., 2012; Heneberg et al., 2016), the phylogenetic relationships are still mainly based on morphology and life cycles. However, conflicting opinions exist and there are several genera, such as *Michajlovia, Urorygma*, or *Zeylamurotrema*, that are currently considered as incertae sedis (Pojmańska, 2002h).

The life cycles of Brachylaimoidea differ markedly from one another. Together with Dicrocoeliidae, they are the only trematodes that are able to complete their life cycle outside of wetlands, sometimes even in more xeric, arid habitats. Only species of the Leucochloriodiomorphidae complete their life cycle in an aquatic environment (Sirgel et al., 2012). According to Pojmańska (2002h), 3 main patterns of life cycle can be distinguished within Brachylaimoidea:

- Life cycle with 2 intermediate hosts completed in an aquatic environment (as that of the Leucochloriodiomorphidae). This pattern includes a mother sporocyst, a daughter sporocyst, furcocercous cercariae with a poorly developed tail, unencysted metacercariae in the second intermediate host, and water birds as the definitive host.
- Life cycle with 2 intermediate hosts completed in a terrestrial environment (for example, Brachylaimidae and Panopistidae). These life cycles include a single generation of sporocysts, cercariae without a tail leaving the first intermediate host, encysted or unencysted metacercariae within the second intermediate host, and adults in birds and mammals, though sometimes (rarely) in amphibians or reptiles.
- Life cycle with 1 intermediate host completed in a terrestrial environment (for example, Hasstilesiidae and Leucochloridiiade). This example includes a single generation of sporocysts, cercariae with an absent or rudimentary tail not leaving the sporocyst, encysted or unencysted metacercariae within the sporocyst, and adults in birds and mammals.

Following is the taxonomy of Brachylaimoidea proposed by Pojmańska and colleagues (2002h), including a total of 8 families and 4 genera incertae sedis.

Family Brachylaimidae

The family Brachylaimidae contains numerous species of terrestrial trematodes that infect mammals, birds, reptiles, and, rarely, amphibians (Gibson and Bray, 1994). *Brachylaima* is the most representative genus within this family and the type genus. Adult worms of Brachylaimidae are characterized by an elongated, or occasionally oval or subglobular, body, sometimes with fine spines. The suckers are well-developed and usually at the anterior end. There is usually a prepharynx, the pharynx is muscular, the esophagus, if present, is short and the ceca long, terminating near the posterior end. The gonads are posterior to the ventral sucker and the genital pore opens on the ventral surface.

Regarding the larval stages, the main characteristic features are branched daughter sporocyts, and cercariae with rudimentary, stumpy tails. Metacercariae, encysted or not, inhabit the kidney or pericardium of the second intermediate host.

Pojmańska (2002a) recognizes subfamilies within Brachylaimidae. The subfamily Brachylaiminae, including 5 genera (*Brachylaima, Ectosiphomus, Glaphyrostomum, Parabrachylaima*, and *Postharmostomum*) and the subfamily Ityogoniminae with 2 genera (*Ityogonimus* and *Scaphiostomum*). Both subfamilies are differentiated based on the adult morphology. Brachylaiminae is characterized by adults with a plump or elongate body, well developed suckers, and an esophagus that is practically absent. In contrast, adult worms of Ityogoniminae are filiform, with small suckers and a short esophagus.

Adult flukes in this family are found in mammals and birds, and occasionally amphibians, and have a complex 3-stage life cycle. There are 2 intermediate hosts, both terrestrial molluscs. The cercariae leave the first intermediate host with easily shed, rudimentary tails, and the metacercariae in the second intermediate host may or may not be encysted.

As mentioned above, *Brachylaima* is the type genus of this family. This genus has had many synonyms with no fewer than 4 spellings having been used in the literature (Yamaguti, 1971; Kamiya and Machida, 1977). Many of the species have been poorly described, with incomplete life cycles and a lack of detailed information for accurate identification. This problem is compounded by the morphological similarity of many of the adult worms. The first and second intermediate hosts of brachylaimids are either the same or 2 different species of terrestrial snail species. The definitive host can be either a mammal or a bird. Humans have also been reported as an incidental definitive host for 1 species of the

genus *Brachylaima*, namely, *B. cribbi* (Butcher et al., 1996; 1998). These infections were reported from South Australia (Butcher et al., 1996; 1998; Butcher and Grove, 2001) where the life cycle is maintained between mice, *Mus musculus*, and helicid and hygromiid snails (Butcher et al., 1996).

Humans often accidentally ingest these snails with vegetables from house gardens or local markets (Butcher et al., 1996; 1998). Infections in humans usually become chronic and can persist as long as 18 months (Butcher et al., 1996). Clinical symptoms depend on the parasite load and heavy infections are associated with diarrhea, abdominal pain, low-grade fever, and fatigue (Butcher et al., 1996; Toledo et al., 2006).

Family Hasstilesiidae

The family Hasstilesiidae has been often recognized as a subfamily of Brachylaimidae. However, its status at the family level has been recognized with *Hasstilesia* as the type genus (Pojmańska et al., 2002b). Hasstilesiidae are similar to Brachylaimidae in the position of the genital pore between or anterior to the testes on the ventral side, but differ in the pattern of the life cycle. They are intestinal parasites of mammals such as rabbits, pikas, bats, goats, and sheep (Rowan, 1955; Nogueira et al., 2004), and the life cycle includes 1 intermediate host, a cercarium with a rudimentary tail, and metacercariae that remain unencysted within the ramified sporocysts.

Adult worms are characterized by their wide body and small, ovoid suckers, testes larger than the ovary in a triangle or in tandem, and an elongated cirrus sac in the median line of the body. There is an ovary located between the testes and short vitelline fields. This family includes 2 genera (*Hasstilesia* and *Strzeleckia*) that can be differentiated by body shape, extent of the ceca, position of the genital pore, and the arrangement of the gonads (Pojmańska et al., 2002b).

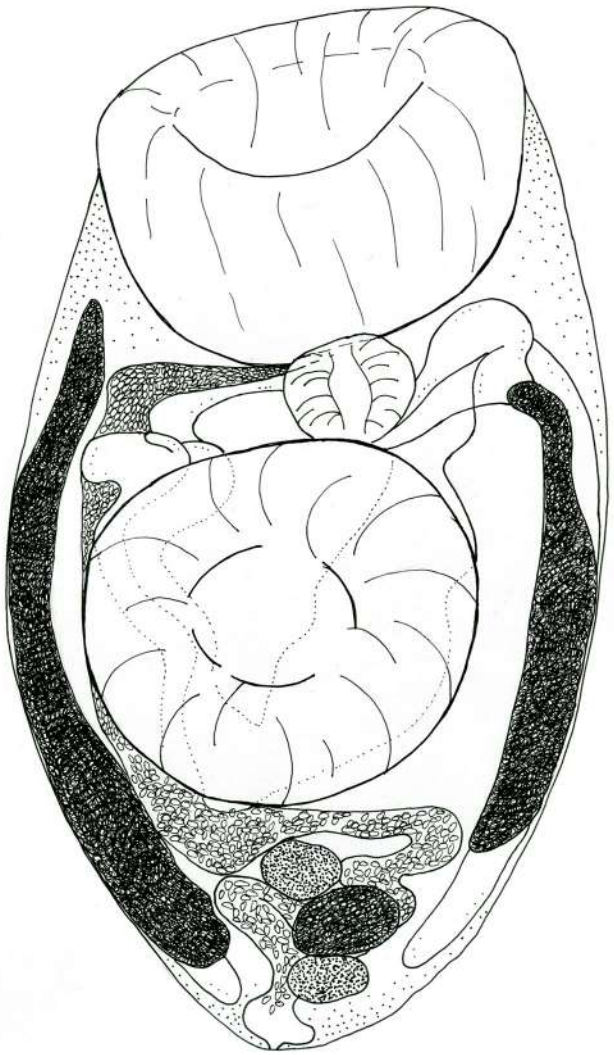

Figure 2. General scheme of an adult specimen of *Urogonimus* sp. (Leucochloridiidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

Family Leucochloridiidae

The family Leucochloridiidae has a very long and confused taxonomic history and, still, the systematics of this group remain uncertain (Heneberg et al., 2016). Traditionally, it was considered to be a monotypic taxon, but currently, a total of 3 genera (*Leucochloridium, Urogonimus*, and *Urotocus*) are included within the family (Pojmańska, 2002c), *Leucochloridium* being the type genus. In fact, several genera that were included within Leucochloridiidae, such as *Urorygma* or *Michajlovia*, are now considered as incertae sedis (Pojmańska, 2002c; Heneberg et al., 2016). Moreover, the existence of subfamilies and the composition distributed among them is still being discussed (Heneberg et al., 2016).

Adult worms are oval or lanceolated, often covered with fine spines, with well-developed suckers and a ventral sucker situated about the middle of the body. The pharynx is well-developed, the esophagus is absent, and the ceca terminate at the posterior extremity of the body. The testes are in tandem or a triangle, with an ovary between them. Genera of this family are distinguished on the basis of the proportions of the suckers, the position of the ventral sucker, the arrangement of the gonads, the position of the genital pore, and the course of the uterus (Figure 2) (Pojmańska, 2002c).

Leucochloridiidae include adult worms that parasitize the alimentary tract (cloaca and bursa Fabricii) of birds, especially passerine birds (order Passeriformes) (Heneberg et al., 2016). The life cycle is terrestrial, with only 1 intermediate host, a single generation of strongly branched sporocysts, cercariae lacking tails, and encysted or unencysted metacercariae within the sporocyst (the sporocyst with encysted metacerariae also being called the brood sac).

Family Leucochloridiomorphidae Yamaguti, 1958

The members of the family Leucochloridiomorphidae differ from other brachylaimids since they develop in an aquatic environment and have free-living cercariae (Allison, 1943). Although these digeneans were traditionally considered as a subfamily, Travassos and Kohn (1966) raised this subfamily to full family status and, currently, 3 genera (*Amblosoma, Leucochloridiomorpha*, and *Ptyalincola*) are admitted within the family, *Leucochloridiomorpha* being the type genus.

The main family characters are the length of the cecae, the position of the genital pore and ovary, the extent of the vitellarium, the course of the uterus, the presence of a prominent pars prostatica, and of spines on the surface of the cirrus (Pojmańska, 2002d). Members of Leucochloridiomorphidae are common parasites of aquatic birds and, more rarely, of mammals. The life cycle is aquatic and includes 2 intermediate hosts (both molluscs belonging to the family Viviparidae), with 2 generations of sporocysts, branched daughter sporocysts, furcocercous cercariae that leave the first intermediate host, and unencysted metacercariae developing in the gonads or surface of the hepatopancreas of the second intermediate host.

Family Moreauiidae Johnston, 1915

Moreauiinae was erected as a monotypic subfamily by Johnston (1915) to include a single species (*Moreauia mirabilis*) and originally included within the family Harmostomidae. After some controversy, the group was raised to full family status and placed within Brachylaimodea (Yamaguti, 1958; Travassos and Kohn, 1966).

Moreauia mirabilis is a parasite of mammals characterized by the asymmetrical position of the gonads in relation to the cirrus sac. In view of this character, Pojmańska (2002e) suggested that the inclusion of this family within Brachylaimodea is uncertain.

Family Ovariopteridae Spaskii & Kulikov, 1963

The family Ovariopteridae was created by Leonov and colleagues (1963) and includes only 1 species, *Ovarioptera sobolevi*, a parasite of the aquatic bird *Tringa nebularia*. This species differs from other brachylaimids by the morphology of the ovary and the ventral sucker, and the absence of a cirrus sac and cirrus.

The life cycle of this species remains unknown.

Family Panopistidae Yamaguti, 1958

Members of the family Panopistidae differ from other brachylaimids in the general morphology of the adults. This genus can be distinguished by the extent of the vitellarium, the course of the uterus, and the position of the genital pore (Pojmańska, 2002f). Adult worms are characterized by their oval body, armed or unarmed with spines, and well-developed suckers in the anterior part of the body. Moreover, they lack an esophagus and the ceca reach the posterior end of the body. The genital pore opening is ventral and posterior to the gonads.

A total of 4 genera (*Dasyurotrema, Dollfusinus, Panopistus*, and *Pseudoleucochloridium*) are included within Panopistidae, *Panopistus* being the type genus.

They are parasites of mammals and the life cycle is terrestrial with 2 intermediate hosts.

Family Thapariellidae Srivastava, 1953

Although the status of Thapariellidae at family level has been strongly discussed, Pojmańska (2002g) retained this status based on several characters such as the lack of a true cirrus sac, the post-testicular position of the ovary, and vitelline fields posterior to testes that approximate those of the brachylaimids. The family comprises only 1 genus, *Thapariella*.

They are parasites of birds and the sporocysts are not known. Little is known regarding its life cycle. The metacercariae develop in snails of the family Viviparidae (river snails).

Superfamily Diplostomoidea

Members of the superfamily Diplostomoidea are characterized by possessing a single holdfast, or trobocytic organ, found posterior to the ventral sucker. This sucker-like, or bi-lobed, structure plays both adhesive and digestive roles (Niewiadomska, 2002g; Blasco-Costa and Locke, 2017). Moreover, all the Diplostomoidea, except for species of the family Cyathocotylidae, have the cirrus sac and cirrus replaced by a copulatory apparatus and terminal genitalia. A copulatory bursa, with an opening and with or without a genital cone or bulb, occupies the posterior end of the body. The seminal vesicle leads into the ejaculatory duct. The uterus and ejaculatory duct may have separate pores. Other structures such as a circular muscle ring in the copulatory bursal wall, sucker-like structures, a preputial fold around the genital cone, para prostate, or an ejaculatory pouch may be present and characteristic in different taxonomic groups (Niewiadomska, 2002g).

The morphology of the metacercariae of diplostomoids is similar in species of related genera and commonly metacercarial forms were distinguished by generic names. Niewiadomska (2002g) reduced the metacercarial forms of Diplostomoidea to 4 main types:

- Diplostomulum (in the genera *Diplostomum, Neodiplostomum,* and *Alaria*). These are round or elongate, free or without a cyst of parasite origin, or encapsulated

with or without a cyst of parasite origin. Pseudosuckers are either present or absent. There is a reserve bladder of 3 longitudinal canals. This type of metacercariae develops in fishes, amphibians, reptiles, and mammals.

- Neascus (in the genera *Uvulifer, Posthodiplostomum,* and *Bolbophorus*). These are encapsulated commonly with a cyst wall of parasite origin. Clusters of cysts may be formed (also called sincysts). The forebody is foliaceus or oval. Pseudosuckers are either present or absent. There is a reserve bladder composed of a ramified median and 2 lateral canals forming a net in the forebody. This type can be found in fishes and oligochaetes.
- Prohemistomulum (in the genera *Cyathicotyle, Holostephanus,* and *Paracoenogonimus*). These are encapsulated commonly with thick-walled cysts of parasite origin. Pseudosuckers are absent. There is a reserve bladder composed of 4 main canals forming 2 loops. This type may be found in fishes, amphibians, and leeches.
- Tetracotyle (in the genera *Strigea, Cotylurus*, and *Apatemon*). These are encapsulated with a well-defined cyst wall of parasite origin. There is a cup-shaped forebody, or it may be flattened and concave ventrally. Pseudosuckers are present. There is a reserve bladder which forms a network filling the entire body. This type of metacercariae develops in snails, leeches, oligochaetes, fishes, amphibians, reptiles, and rarely in birds and mammals.

The Diplostomoidea contains 6 families. Two of them, Brauninidae and Bolbocephalodidae, are monotypic but there is enough morphological evidence to support their validity. The other 4 families are rich in species (Niewiadomska, 2002g).

The arrangement of the systematics of the Diplostomoidea has varied from the late 1930s onward (Niewiadomska, 2002g; Blasco-Costa and Locke, 2017). Currently, the higher systematics are based on: Morphological features like the structure and shape of the holdfast organ and forebody, the distribution of the vitellarium, the presence or absence of bisegmentation of the body, a cirrus sac and paraprostate, and the structure and shape of the reproductive organs. Moreover, the specificity toward the definitive host has been used as a criterion for classification at the subfamily level, though several authors have questioned the validity of this parameter (Blasco-Costa and Locke, 2017). Molecular studies on this group are somewhat conflicting (Olson et al., 2003; Brabec et al., 2015), however, some discrepancies exist between both studies since Brabec and colleagues (2015) suggested that Diplostomoidea form a lineage basal to the Plagiorchiida, clustering as sisters to the schistosomes. The discrepancy appears to be due to the low number of species included in the studies and the lack of overlap and sequences analyzed between studies (Blasco-Costa and Locke, 2017).

Most diplostomoids have an aquatic 3-host life cycle. Adult worms inhabit the intestine of amniote vertebrates (birds, mammals including cetaceans, and reptiles including crocodilians, snakes, and turtles), shedding eggs that pass in the feces of the host. After hatching from eggs, the free-living miracidium swims to infect a gastropod first intermediate host. In the snails, diplostomoids undergo 3 generations (mother sporocyst, daughter sporocysts, and furcocercariae). Furcocercous cercariae emerge from the snail in the aquatic environment and swim to actively locate and penetrate the second intermediate host to form metacercariae. The definitive host becomes infected after it preys on the second intermediate host harboring the metacercariae.

The genus *Strigea* (family Strigeidae) represents an exception in this general pattern since species of this genus have an obligate 4-host life cycle including a non-encyted premetacercaria (mesocercaria) in the second intermediate host (amphibians). The mesocercaria is ingested by the third intermediate host (an amphibian, reptile, bird, or mammal) which it develops into an encysted tetracotyle-type metacercaria. The definitive host (a bird) becomes infected after ingestion of the metacercaria within the third intermediate host (Blasco-Costa and Locke, 2017).

Family Diplostomidae Poirier, 1886

In general, species of the Diplostomidae have a 3-host life cycle, though some variations on this pattern can be found. Fork-tailed (furcocercous) cercariae are produced in sporocysts in the gastropod first intermediate host. The cercariae emerge from the snails and penetrate and form metacercariae in fishes, amphibians, molluscs, and annelids (Hong et al., 1982). In some Diplostomidae, the life cycle is expanded to incorporate 4 hosts by inclusion of a mesocercaria stage. The definitive hosts become infected by the ingestion of the second intermediate host or the paratenic host harboring metacercariae. Eggs typically hatch and penetrate the first intermediate host (Cribb et al., 2003).

In summary, the family Diplostomidae contains digeneans from numerous orders of birds and mammals. Although there have been some problems with regard to the subdivision of Diplostomidae into subfamilies (Dubois, 1970; Yamaguti, 1971), Niewiadomska (2002d) recognized a total of 4 subfamilies according to host range, morphological features, and type of metacercariae. These are the Diplostominae, Crassiphialinae, Alariinae, and Codonocephalinae. However,

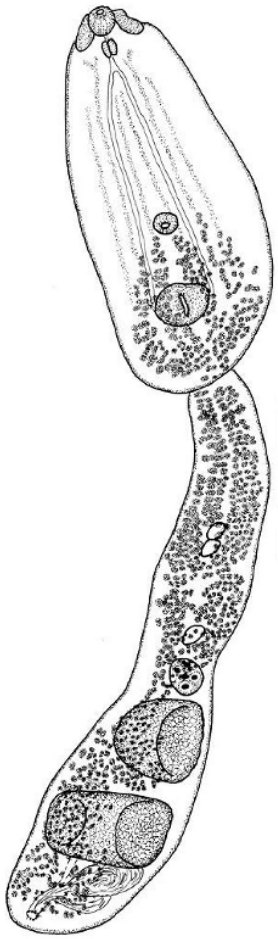

Figure 3. Adult specimen of *Diplostomum pseudospathaceum* (family Diplostomidae). Source: Pérez del Olmo et al., 2014. License: CC BY 4.0.

a recent study suggested that Crassiphialinae should be raised to the family level (Locke et al., 2018). Members of Diplostomidae are characterized by their 2-part body: 1) The forebody is foliate, spatulate, caliciform, or bulbiform; and 2) the hindbody is cylindrical or coniform and apart from the oral and ventral sucker holdfast organ, which is ventrally located and, in some representatives, pseudosuckers (lappets) can be found (Figure 3) (Horák et al., 2014).

Human pathogenesis

Members of at least 3 genera of Diplostomidae (*Neodiplostomum*, *Fibricola*, and *Alaria*) are known to parasitize humans. In the intestines, only *N. seoulense* and *F. cratera* parasitize humans. In the case of *Alaria* spp., humans serve as paratenic hosts harboring metacercariae in different tissues (Fernandes et al., 1976; Freeman et al., 1976; McDonald et al., 1994; Kramer et al., 1996).

Species *Neodiplostomum seoulense*.

Twenty-eight cases of human infections with *Neodiplostomum seoulense* have been reported in South Korea, but none in other countries (Chai and Lee, 2002). This species was first implicated when an infected human was found suffering severe enteritis with abdominal pain, fever, diarrhea, bloating, and anorexia (Seo et al., 1982). The patient had a history of eating raw snakes, which appears to be the most important food source for human infections (Hong et al., 1984a; 1984b). Chai and Lee (2002) extrapolated that the total number of human cases may be 1,000 in South Korea. There are no available studies on the pathology of *N. seoulense* infections in humans.

Species *Fibricola cratera*.

Fibricola cratera is a trematode species indigenous to North America. Human infections with *F. cratera* are more anecdotal than those of *Neodiplostomum seoulense* as Shoop (1989) reported an experimental inoculation of a human volunteer producing a patent infection that lasted 40 months. Symptoms exhibited by the volunteer were similar to those described with the *N. seoulense* infections.

Black-spot disease.

Black-spot disease is caused by the encystment of the metacercariae stage of diplostomoids on the skin, fins, and flesh of freshwater fishes (Williams and Chaytor, 1966; Williams, 1967). Currently, more than 30 fluke parasite species, mainly of the genus *Neascus*, are known to cause black-spot disease or similar symptoms. A fibrinous capsule with melanocytes around the metacercariae cyst gives name to the disease in relation to the small black-spotted appearance (1–2 mm in diameter) (Kurochkin and Biserova, 1996; Williams, 1967). Most metacercarial infections are non-pathogenic for the fish although its unsightly appearance can reduce value of the fish in a market situation.

Family Bolbocephalodidae Strand, 1935

The family was created as Bolbocephalidae by Dubois (1934) for *Bolbocephalus intestiniforax*, though Strand (1935) renamed it as *Bolbocephalodes* and the family Bolbocephalodidae, since the generic name was pre-occupied. This monotypic family parasitizes Ciconiiformes birds in Italy and Syria. The aberrant morphology of adults of *Bolbocephalodes*, the only genus within the family, shows unclear relationships with other Diplostomidae (Niewiadomska, 2002a).

Family Brauninidae Wolf, 1903

This taxon was created by Wolf (1903) as a subfamily for the genus *Braunina*. Thereafter it was raised to family level by Dubois (1938; 1953). *Braunina* is the only genus included in this family. Although members of Brauninidae have characteristics of both the Diplostomoidea and those of other digeneans, the main features of this family are the structure of

the holdfast organ, the presence of gonads, part of the uterus, and ceca within, and the definitive hosts which are marine mammals (cetaceans). They are distributed in Europe and subtropical North America (Niewiadomska, 2002b).

Family Cyathocotylidae Mühling, 1898

Members of this family also have characteristics of diplostomids and other digeneans. Cyathocotylidae are characterized by possessing a generally undivided body and a cirrus sac, but they also have a holdfast organ and a terminal genital pore (Niewiadomska, 2002c). The testes and ovary are round or oval and variable in position. In general, cyathocotylids exhibit great morphological variability at the adult and metacercarial stages. By contrast, all cyathocotylid cercariae have a homogeneous morphology. Cercariae from different genera, or even subfamilies, can be differentiated on the basis of the number and arrangement of the flame cells, the length of the furca, and the presence or absence of fin folds (Niewiadomska, 2002c). As adults, cyathocotylids are parasites of reptiles, birds, and mammals. Mother- and daughter sporocysts develop in gastropods (Prosobranchia), while the metacercariae are found in fishes, amphibians, and aquatic invertebrates (Niewiadomska, 2002b; Hernández-Mena et al., 2017).

Although the number subfamilies within Cyathocotylidae and the genera assigned to each subfamily differ according to various authors, Niewiadomska (2002c) recognizes 5 subfamilies—**Cyathocotylinae**, **Muhlinginae**, **Prohemistominae**, **Prosotephaninae**, and **Szidatiinae**—that can be differentiated based on body shape, the structure and position of the holdfast organ, the presence or absence of a ventral sucker, and the extent of the vitellarium. The number of genera within each family ranges from 1 to 5.

Family Proterodiplostomidae Dubois, 1936

The Proterodiplostomidae are a relatively small group of diplostomids found exclusively in reptiles. The proterodiplostomids are morphologically very similar to, and share several morphological synapomorphies with, strigeids and diplostomids (Hernández-Mena et al., 2017). Morphologically, they are characterized by a bipartite body, a flattened forebody, and a cylindrical, oval, or claviform hindbody. Pseudosuckers may be present or absent, and they include a holdfast organ that may be variable in size (Niewiadomska, 2002e). Moreover, proterodiplostomids have an independent paraprostatic gland—or paraprostate—that was considered a morphological automorphy of the family (Shoop, 1989). This organ has the shape of a thin- or thick-walled tubule or pouch, surrounded by gland cells (Niewiadomska, 2002e). A total of 4 subfamilies and 17 genera are included by Niewiadomska (2002e), *Proterodiplostomum* being the type genus.

Family Strigeidae Railliet, 1919

Members of the family Strigeidae are distinguished morphologically by having bodies divided into 2 segments (a forebody and hindbody) and a cup-shaped forebody containing a holdfast organ with 2 lobes (ventral and dorsal). These trematodes parasitize the intestine, bursa Fabricii, and, rarely, liver as endoparasites of birds worldwide and they are especially common in raptors (Niewiadomska, 2002f; Heneberg et al., 2018).

This family has been the subject of a large number of taxonomic studies. Strigeids are considered a phylogenetically unsettled group. They are likely paraphyletic, with Diplostomidae nested within it (Sitko et al., 2017; Heneberg et al., 2018). There are some discrepancies with regard to the number of subfamilies recognized. The genus *Pseudapatemon* has been ranked as subfamily (Pseudapatemoninae) by several authors (Sudarikov, 1984; Zarzanova and Sysoev, 1993). However, Niewiadomska (2002f) only considers 2 subfamilies, Strigeinae and Duboisiellinae, within Strigeidae. Currently, the family contains 13 genera with approximately 110 nominal species. All these species are specific to birds, with the exception of the members of the genus *Duboisiella* that infect mammals. For this reason, together with several morphological characters, this genus was reassigned to the

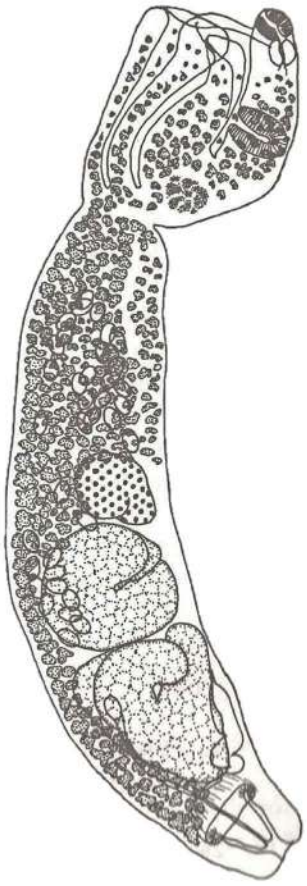

Figure 4. Adult specimen of *Strigea falconis* (family Strigeidae). Source: Dubois, 1968. License: Public domain.

monotypic subfamily Duboisiellinae (Niewiadomska, 2002f). According to Niewiadomska (2002f), the remainder species of Strigeidae are included within the subfamily Strigeinae.

Strigeidae have 3- or 4-host life cycles in which vertebrates often serve not only as definitive, but also as intermediate or paratenic hosts. Pathology is usually associated with migration of metacercariae and mesocercariae within the host tissues. The impact of these trematode infections on both farm and wild animals may be significant. The metacercariae is of the tetracotyle type and mesocercariae occurs in the genus *Strigea* (Figure 4).

Superfamily Schistosomatoidea: The Blood Flukes

Members of the superfamily Schistosomatoidea are exceptional trematodes that inhabit the circulatory system of their hosts. For this reason, they are collectively called blood flukes. This superfamily constitutes a monophyletic group that includes 3 families: 1) The fish blood flukes or **Aporocotylidae** (or **Sanguinicolidae**; see discussion below); 2) **Spirorchiidae** including blood flukes of reptiles, mainly turtles; and 3) **Schistosomatidae** that comprises parasites of birds, reptiles, and mammals including humans. Human schistosomiasis affects over 230 million people in tropical and subtropical regions, causing about 300,000 human deaths annually (van der Werf et al., 2003).

Schistosomatoids have a common ancestor and share several characteristics such as the structure of the tegumental outer membrane, which is different from other trematodes, and various other biological features. Two hosts are involved in the life cycle of the blood flukes. Cercariae are produced in the sporocytst of the first intermediate host and directly and actively penetrate the definitive host, without a metacercarial stage (Smith, 2002b). However, this subfamily is one of the most diverse trematode groups. For example, both aporocotylids and spirorchiids are monoecious, meaning having both male and female organs in the same individual. By contrast, schistosomatids are dioecious, meaning having distinct male and female individuals.

Morphologically, members of Schistosomatoidea are very variable. They are elongate worms that may include spines with variable shape and distribution. Oral and ventral suckers may be present or absent. The number of testes ranges from 1 to numerous and vary in size and shape, and the cirrus sac and cirrus can be present or absent. There is a single ovary and it may vary in shape (Smith, 2002b).

Family Aporocotylidae Odhner, 1912

Family Aporocotylidae includes the fish blood flukes. The family-group name has been historically unstable, with both Aporocotylidae Odhner, 1912 and Sanguinicolidae Graff, 1907 being used for the single family (Smith, 2002b). Bullard and colleagues (2009), after a critical review of the relevant literature, concluded that Aporocotylidae Odhner, 1912 is the earliest available family name and it has been generally accepted rather than Sanguinicolidae Graff, 1907.

As occurs with other schistosomatoids, a high degree of variability occurs within Aporocotylidae and there are many gaps in the knowledge of this group. Therefore, further research is required. While the absence of suckers has been regarded as diagnostic of the family, an oral sucker can be present in a number of aporocotylids, though it is poorly developed (Kirk and Lewis, 1993; Smith, 2002a).

Smith (2002a) included 20 genera within this family, though about 6 new genera have been described since then, *Aporocotyle* being the type genus. These genera are mainly characterized by the possession of variously shaped and variously disposed tegumental spines and oral structures (Smith, 2002a). Although most of these 20-or-so genera are monospecific, more than 105 nominal species have been included in the Aporocotylidae. About 5 of them infect cartilaginous fishes (Chondrichthyes) and more than 100 are parasites of bony fishes (Osteichthyes, Actinopterygii: Teleostei) (Cribb et al., 2011).

Relatively few complete life cycles of aporocotylids have been described, especially in marine species (Cribb et al., 2011); all of them are dixenous. There are still many associations between intermediate hosts and aporocotylid larval stages with unresolved species determination. Molecular approaches would help to solve these problems.

The family Aporocotylidae is discussed in greater detail in the chapter following this one (see Yong, 2024).

Family Spirorchiidae Stunkard, 1921

The family Spirorchiidae includes trematodes that inhabit the circulatory and lymphatic system of turtles worldwide. This family has about 100 species grouped into 20 genera. Ten genera include parasites of green turtles, loggerhead turtles, and hawksbill turtles (Roberts et al., 2016).

Spirorchiids are small to medium trematodes with oral and ventral suckers present or absent, and without a pharynx. There are a variable number, shape, and distribution of testes, and a uterus containing commonly a single, voluminous egg. Genera within Spirorchiidae are mainly differentiated on the basis of the structure of the intestinal ceca, testes, cirrus sac, and genital pore. *Spirorchis* is the type genus. Spirorchiids have a 2-host life cycle, commonly using freshwater snails as intermediate hosts (Platt, 2002).

The history of spirorchiids is plagued by disagreements over nomenclature and synonymy in relation to poorly described species and divergent morphology, aggravated by the

fact that many specimens are unavailable for examination or are in poor condition (Platt, 2002; Snyder, 2004). This has made it difficult to characterize the family. Although the evident differences between Spirorchiidae and Schistosomatidae (that is, dioecious versus monoecious and different definitive hosts), both are closely related and considered sister taxa within the Schistosomatoidea. Representatives of both families have similar furcocercous cercariae and life cycles. In fact, studies based on molecular data suggest that members of Spirorchiidae should be included within Schistosomatidae as 1 or various subfamilies (Snyder, 2004).

Family Schistosomatidae Stiles & Hassall, 1898

The Schistosomatidae constitutes an important family of trematodes since species of the genus *Schistosoma* cause a neglected tropical disease that affects at least 230 million people worldwide resulting in extensive social and economic burdens. Besides the massive public health burden caused by schistosomes in the tropical areas of the world, additional species are the causative agents of human cercarial dermatitis, for example, members of the genera *Austrobilharzia, Bilharziella, Gigantobilharzia,* and *Trichobilharzia* (Horák et al., 2014).

Members of the family Schistosomatidae are exceptional organisms among digenean trematodes: For one, they are dioecious and gonochoristic, with males and females mating in the blood vessels of definitive hosts. Furthermore, the lateral edges of the adult male worm fold to form a groove (gynecophoral canal) where the female worm resides (Figure 5). Although the systematic position of Schistosomatidae is widely accepted, Azimov (1975) separated the schistosomes of mammals and birds into 2 different families. The Schistosomatidae, with subfamilies including parasites of mammals, and the Ornithobilharziidae with 4 subfamilies comprising the parasites of birds. However, most parasitologists have not accepted this division and consider all schistosomes as members of the single family, Schistosomatidae (Khalil, 2002).

There are 4 subfamilies: **Schistosomatinae**, **Griphobilharziinae**, **Bilharziellinae**, and **Gigantobilharziellinae**. They are differentiated mainly on the basis of the development of the gynecophorous canal, bifurcation of the ceca, and the position of the female genital pore. A total of 14 genera parasitizing mammalian and avian hosts are included within the family Schistosomatidae. Besides the genus *Schistosoma* having medical and veterinary importance (human and mammalian parasites), 3 genera (*Bivitellobilharzia, Heterobilharzia,* and *Schistosomatium*) infect mammals and 10 genera (*Allobilharzia, Anserobilharzia, Austrobilharzia, Bilharziella, Dendritobilharzia, Gigantobilharzia, Jilinobilharzia, Macrobilharzia, Ornithobilharzia,* and *Trichobilharzia*) infect birds (Horák et al., 2014).

As with most other trematodes, the schistosomes have a

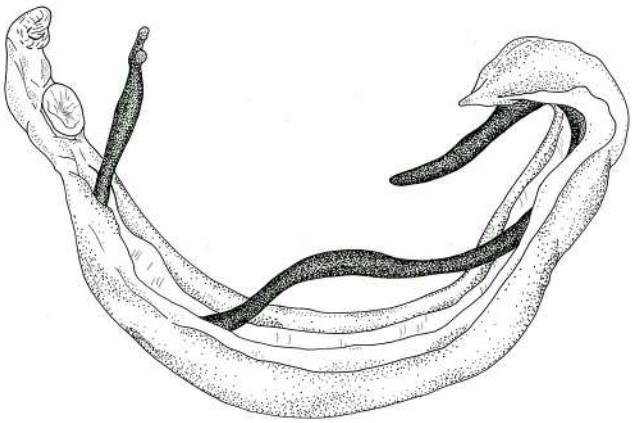

Figure 5. Adults of *Schistosoma* spp. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

2-host life cycle. Generally, the eggs containing mature miracidia (motile ciliated larvae) are released into the environment via feces or urine, where they have arrived from the circulatory system of the mammalian host. In an aquatic environment, eggs hatch and the miracidia that are released from the egg then seek out the intermediate host, which are freshwater, brackish, or saltwater snails. Within the snail, the miracidium transforms into a sporocyst and asexual multiplication occurs finally producing cercariae. Several weeks after exposure to the miracidia, cercariae begin to leave the snails. Free-living cercariae in fresh water can penetrate the skin of the vertebrate definitive host. The anterior end of the body of the cercariae enters the skin whereupon the tail is lost. Once in the host, the cercarial body transforms into a schistosomulum. The schistosomulae then travel through the circulatory system, where they mature into adult worms and mate. Depending on the species, the schistosomes migrate to the destination of their final infection where the females begin egg production. These eggs are attached to the wall of the lumen, where the eggs then penetrate the wall. They are then expelled in the feces or urine of the host.

Schistosomatoids are of great importance since some species cause avian disease and, as noted, can occasionally cause cercarial dermatitis in humans. Cercariae of some genera of bird schistosomes (for example, *Austrobilharzia, Bilharziella, Gigantobilharzia*, and *Trichobilharzia*) have been confirmed as the causative agent of human cercarial dermatitis. Human skin possesses components that may be recognized by cercariae as signals for attachment and penetration of the accidental host resulting in the cercaria to attack to the incompatible, human host. As far as human infections are concerned, an allergic skin reaction involving cellular and humoral reactions (cercarial dermatitis) develops in sensitized persons after repeated contacts with the agent. These reactions are

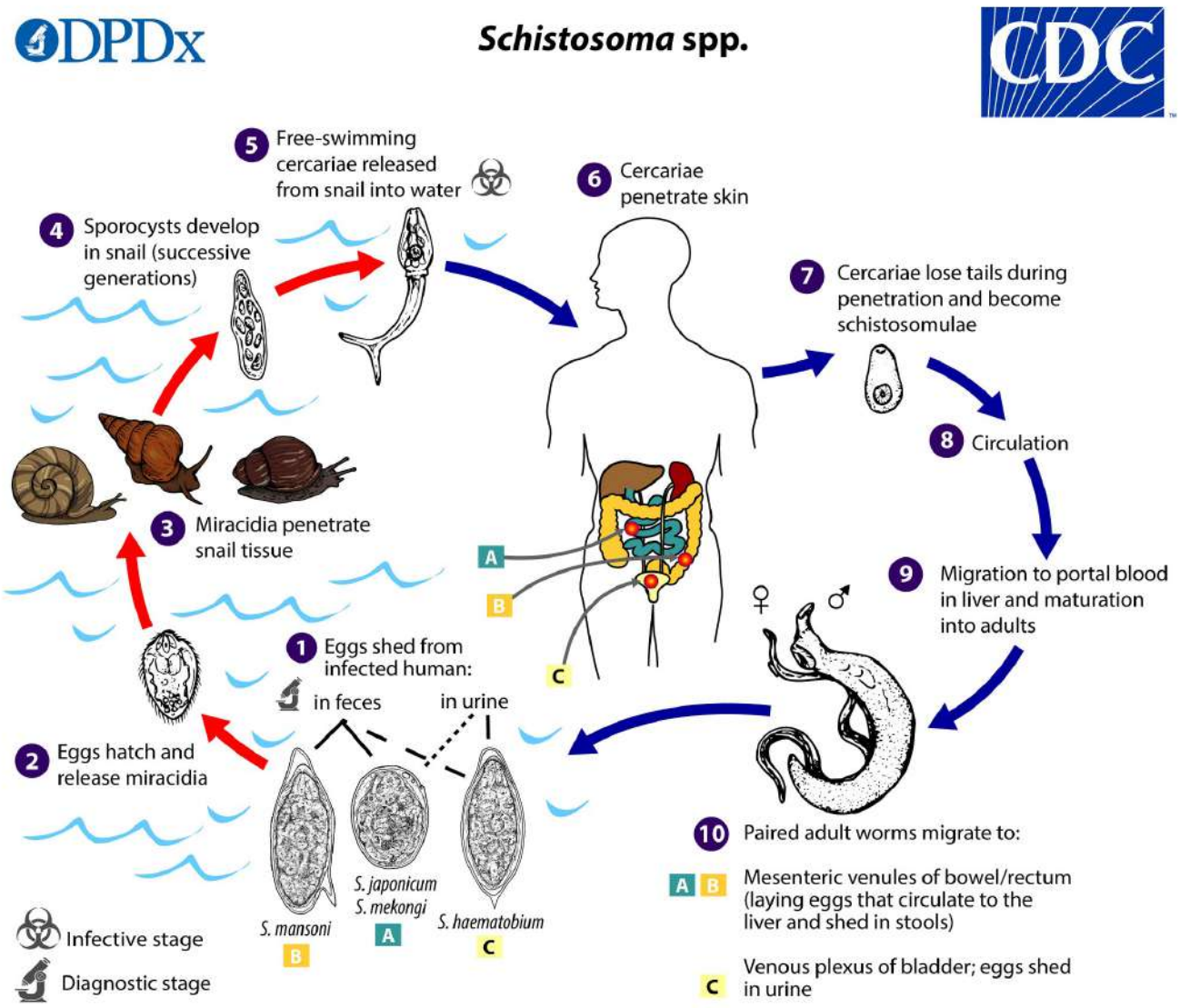

Figure 6. *Schistosoma* eggs are eliminated with feces or urine, depending on species (1). Under appropriate conditions the eggs hatch and release miracidia (2), which swim and penetrate specific snail intermediate hosts (3). The stages in the snail include 2 generations of sporocysts (4) and the production of cercariae (5). Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host (6), and shed their forked tails, becoming schistosomulae (7). The schistosomulae migrate via venous circulation to the lungs, then to the heart, and then develop in the liver, exiting the liver via the portal vein system when mature (8, 9). Male and female adult worms copulate and reside in the mesenteric venules, the location of which varies by species (with some exceptions) (10). For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine (A), and *S. mansoni* occurs more often in the inferior mesenteric veins draining the large intestine (B). However, both species can occupy either location and are capable of moving between sites. *Schistosoma intercalatum* and *S. guineensis* also inhabit the inferior mesenteric plexus but lower in the bowel than *S. mansoni*. *Schistosoma haematobium* most often inhabits in the vesicular and pelvic venous plexus of the bladder (C), but it can also be found in the rectal venules. The females (size ranges from 7–28 mm, depending on species) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*/*guineensis*) and of the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, respectively (1). Source: Division of Parasitic Diseases and Malaria, United States Centers for Disease Control and Prevention, 2019. https://www.cdc.gov/dpdx/schistosomiasis/index.html. Public domain.

unpleasant, but they provide immediate protection against further infection by the invading worms because the parasites are killed in the skin (Horák et al., 2014).

Human schistosomiasis

Several members of the genus *Schistosoma* are the causative agents of human schistosomiasis. Schistosomiasis, also known as bilharziasis in the Middle East and also called snail fever, is a neglected tropical disease caused by several species of the genus *Schistosoma*. It constitutes one of the most important parasitic diseases globally in terms of public health impact, just behind malaria (Steinmann et al., 2006). Blood flukes infect almost 230 million people worldwide and more than 779 million people are at risk of infection at any one time (Steinmann et al., 2006; Utzinger et al., 2009). The main species of *Schistosoma* infecting humans are: *S. mansoni*, which is transmitted by *Biomphalaria* snails and causes intestinal and hepatic schistosomiasis in Africa, the Arabian Peninsula, and South America; *S. haematobium*, transmitted by *Bulinus* snails and causes urinary schistosomiasis in Africa and the Arabian Peninsula; and *S. japonicum*, transmitted by the amphibian snail *Oncomelania* and causing intestinal and hepatosplenic schistosomiasis in China, the Philippines, and Indonesia (Gryseels et al., 2006) having been declared to be eliminated from Japan in 1996 (Tanaka and Tsuji, 1997). Other less epidemiologically important species are *S. intercalatum, S. guineensis*, and *S. mekongi*. Overall, around 80–90% of the schistosomiasis cases worldwide occur in sub-Saharan Africa (Lewis and Tucker, 2014).

All human schistosomes have a generally similar life cycle. The particular intermediate snail that is implicated in its transmission and the location of the adult within the human definitive host, are the main differences among species. The human host becomes infected by active penetration through the skin of infective cercariae (free-swimming larval stages). As the cercariae enter the skin, the tails drop off on the outside epidermis and the cercariae transform into schistosomulae. The schistosomulae migrate to the liver via the hepatic portal into the blood circulation and form pairs of adults. Adult couples migrate to mesenteric venules in the species *Schistosoma mansoni*, *S. japonicum*, and *S. mekongi*) or the venous system of the bladder, in the case of *S. haematobium* (see the life cycle in Figure 6). The females produce eggs about 5 weeks after infection. The adult average life span in a human host is about 5 years but they may survive for up to 30 years. The inflammatory immune responses (including granulomas) caused by the eggs trapped in the organs and surrounding tissues can result in intestinal, hepatosplenic, or urogenital disease. The eggs are released in the bloodstream and pass through the intestinal wall or urogenital system and are excreted in either the feces or in urine, respectively, reaching a freshwater environment. The miracidium stage (free-swimming ciliated larvae) hatches from an egg and swims to actively search for and penetrate the intermediate snail host. Within the snail, the miracidia develop into various sporocyst generations, finally transforming to cercariae which emerge from the snail in the freshwater environment, and the life cycle continues (Gryseels et al., 2006).

After the cercariae penetrate the human and end up in their final site within the host, there are 3 distinct phases of clinical disease progression: Acute infection (characterized by cercarial dermatitis and Katayama fever or Katayama syndrome); established active infection (characterized by an inflammatory immune response and formation of granuloma around eggs trapped in tissues); and late chronic infection (which affects people continuously exposed to infection in endemic areas) (MacManus et al., 2018).

The definitive diagnosis for a schistosome infection is the detection via microscopy of eggs in stool (for *Schistosoma mansoni*, *S. japonicum*, and *S. mekongi*) or urine (for *S. haematobium*) samples. Antigen or antibodies point-of-care tests detection in serum can be useful for people living in endemic areas. Praziquantel is currently the most widely used drug and is safe and effective against adult worms of all *Schistosoma* species known to infect humans (MacManus et al., 2018).

Schistosomiasis elimination requires a multifactorial or integrated approach, including: Snail control; improved water sanitation and hygiene; information, education and communication; accurate diagnostics; and mass treatment of infected people in endemic areas (Gryseels et al., 2006; MacManus et al., 2018).

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35

Digenea, Diplostomida

Aporocotylidae (Family): Fish Blood Flukes

Russell Q.-Y. Yong

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Diplostomida

Family Aporocotylidae

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 35

Aporocotylidae (Family): Fish Blood Flukes

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Introduction

The family Aporocotylidae Odhner, 1912, formerly known as the family Sanguinicolidae, refers to blood flukes which infect fishes. This relatively small family contains just over 160 described species, the vast majority of the species described occurring in bony fishes (class Osteichthyes) and only a handful being reported from cartilaginous fishes or members of the class Chondrichthyes, including sharks, skates, rays, and chimaeras. Aporocotylids have, throughout their history, been regarded as some of the more enigmatic and mysterious trematodes. Their unusual body form and infection sites led to misidentifications by early workers in this area and, to this day, they are regarded as difficult to study. As a consequence, aporocotylids are often neglected in parasitological assessments or biotic surveys and are overall regarded as under-surveyed. Nevertheless, they are an important family of digeneans, with many species being of high commercial significance due to their deleterious impacts on fish stocks grown in aquaculture (Bullard and Overstreet, 2002; Ogawa, 2014). Their unique evolutionary history and diversity of life cycles, meanwhile, being intimately connected with that of their hosts, provides an important system for the study of evolution of the Trematoda (Orélis-Ribeiro et al., 2014).

Identifying Aporocotylids

The infection site for most aporocotylids—the circulatory system—makes them unique among fish-infecting trematodes. Species of most genera can be found in the vascular organs (gills and heart), as well as blood vessels throughout the body including the mesenteric, neuro-cephalic, and renal vessels, where they feed on blood. Several species, such as some in the genera *Sanguinicola* Plehn, 1905 and *Skoulekia* Alama-Bermejo et al., 2011 show remarkable affinity for the vessels of specific organs, such as the eyes and brain (Schell, 1974; Alama-Bermejo et al., 2011).

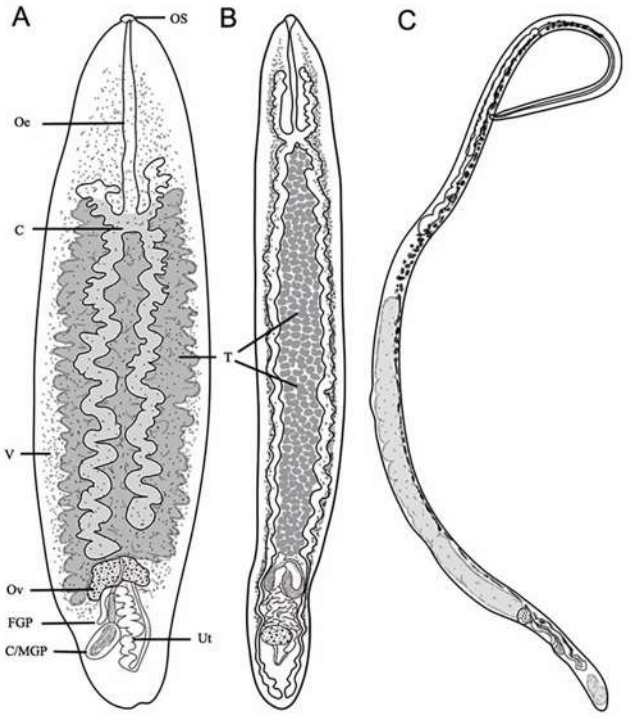

Figure 1: Selected aporocotylid species demonstrating their general body plans. A) *Psettarium pandora* Yong et al., 2018 (after Yong et al., 2018), a good example of a typical aporocotylid; B) *Aporocotyle simplex* Odhner, 1900 (after Thulin, 1980), showing a similar body plan but possessing multiple testes; C) *Phthinomita hallae* Nolan & Cribb, 2006 (after Nolan & Cribb, 2006), showing a threadlike body form. Legend: C: Ceca; C/MGP: Cirrus/male genital pore; FGP: Female genital pore; Oe: Esophagus (at times spelled oesophagus); OS: Oral sucker; Ov: Ovary; T: Testis/testes; Ut: Uterus; V: Vitelline follicles. Source: R. Q.-Y. Yong. License: CC BY-NC-SA 4.0.

The body form of most aporocotylids is one of a broadly-flattened, blade- or oval-shaped worm, with little in the way of modifications or protuberances (Figure 1A, B). Species of 2 genera, *Ankistromeces* Nolan & Cribb, 2004 and *Phthinomita* Nolan & Cribb, 2006 have delicate, thin, threadlike bodies (Figure 1C). These species live intertwined in the spaces and chambers of the walls of the cardiac muscle or heart (Nolan and Cribb, 2005; 2006). Still others, of the genera *Deontacylix* Linton, 1910, and *Plethorchis* Martin, 1975, are free-living in the body cavity of their hosts; they apparently still feed on blood, but it is not clear how they obtain it (Yamaguti, 1970; Martin, 1975).

Aporocotylids range between 350 and 12,000 µm in length, with the larger species occurring in chondrichthyans. Rarely among digeneans, all aporocotylids lack ventral suckers, and many also lack or have highly-reduced oral suckers.

This unusual body form was a source of confusion for early authors who characterized them, variously, as gill ectoparasites, endoparasitic turbellarians, and even so-called monozoic tapeworms (Odhner, 1900; Plehn, 1905; 1908). It was not until 1911 that their true affinities to digeneans were recognized (Odhner, 1911). Most aporocotylids have tegumental spines to some degree; in most species, these spines are arranged in serial rows along the lateral margins, in a manner that recalls the treads of a tractor tire (Figure 2). Other, more extensively armed forms, like species of *Hyperandrotrema* Maillard & Ktari, 1978, have spines over most of the body. These spines, in lieu of a ventral sucker, presumably aid in attachment to surfaces and provide traction when the worm moves. The neural systems of many aporocotylid species are well-developed and readily observed in mounted specimens; the nerve cords and neural networks are often well-defined. This may relate to the fact that many species are active movers and have been observed crawling vigorously, thrashing, and even swimming, albeit poorly (Bullard and Overstreet, 2003). Yong (in unpublished observations) has even observed 1 species exhibit tactile sensory responses, crawling in 1 direction and then changing course when sensing a disturbance ahead. The exception to the rule seems to be those species that live in the heart of their host and have a threadlike body, which exhibit only feeble movement even when extricated from cardiac tissue.

Like the overwhelming majority of digenetic trematodes, all aporocotylids are hermaphrodites. The male terminal genitalia are usually simple, consisting of unarmed cirri, lacking the modifications seen in many other trematode groups, such as spines or stylets. The exception is *Rhaphidotrema kiatkiongi* Yong & Cribb, 2011, which has a sclerotized stylet that protrudes via the male genital pore (Yong and Cribb, 2011). The testes and ovary of most species are prominent and occupy large proportions of the body. The number of testes varies from 1 to over 100 (see the examples in Figure 1). Aporocotylids shed their eggs passively into the host bloodstream and range in size from 10 to 40 µm-long and vary in form between species, with some being spherical, others ovoid or oblong, and some with spines (McMichael-Phillips et al., 1992; Kirk and Lewis, 1993; Yong et al., 2013). The fecundity of most aporocotylids is such that even a moderate infection can result in the production of a large number of eggs. For instance, it was estimated that a single tuna infected by about 50 worms had over 4.5 million eggs in the gills of just 1 side of its body (Shirakashi et al., 2012).

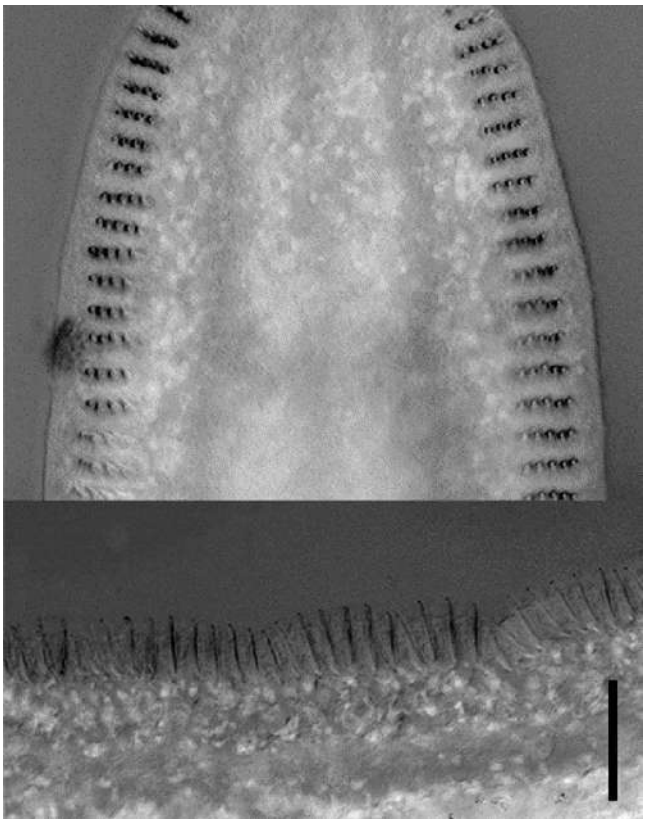

Figure 2: Ventral (top) and lateral (bottom) views of the tegumental spines of aporocotylids, as exemplified by *Cardicola suni* Yong et al., 2016. Note the hooked ends of the spines (seen most clearly in lateral view), and their arrangement in regular rows along the ventrolateral body margins. Scale bar: 20 µm. Source: R. Q.-Y. Yong. License: CC BY-NC-SA 4.0.

Aporocotylids in Relation to Other Schistosomatoidea

The **Aporocotylidae** form 1 of 3 families within the Schistosomatoidea, which includes the 2 other families of blood flukes: The **Spirorchiidae**, which infect reptiles, and the **Schistosomatidae**, which infect endothermic tetrapods (that is, mammals and birds) (Smith, 2002). The overwhelming majority of species occur in actinopterygian fishes, with only 9 known from chondrichthyans (Cutmore et al., 2018).

The phylogeny of the Aporocotylidae is of great interest, because there is evidence of broad cospeciation with their host fishes, that is, the more phylogenetically basal parasite species infect phylogenetically basal fish taxa (Figure 3). Only 1 group of 3 species which infect the milkfish *Chanos chanos*, a basal teleost, bucks this trend, with all 3 species grouping in a monophyletic clade formed otherwise by species infecting more derived Teleostei. This is interpreted as an example of secondary host-switching into ancient or less derived fish taxa (Yong et al., 2016). Interestingly, the fact that the chondrichthyan-infecting blood flukes form a monophyletic clade with those of actinopterygian fishes puts the understanding of aporocotylid evolutionary history at odds with the current understanding of craniate evolution, which

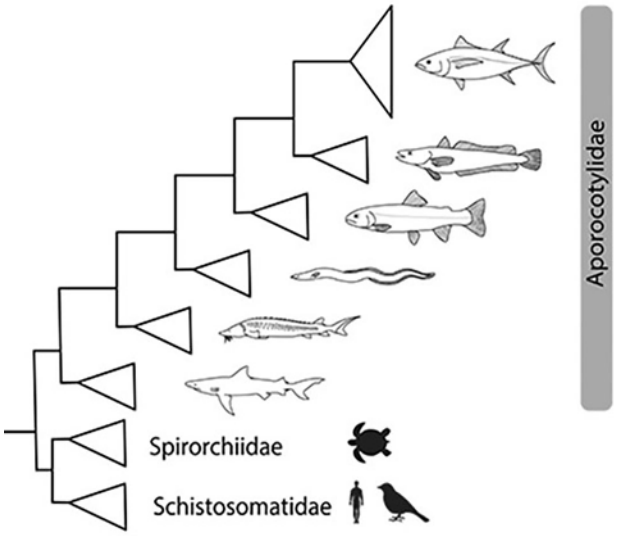

Figure 3: A simplified phylogenetic tree of the Aporocotylidae showing its relationships to the 2 other schistosomatoid families, the Schistosomatidae (blood flukes of mammals and birds) and the Spirorchiidae (blood flukes of reptiles), and cophyly with progressively evolutionarily-advanced groups of fishes from chondrichthyans, to chondrosteans (for example, sturgeon), to basal teleosts (such as, elopomorphs, like eels, and basal euteleosts, like salmoniforms and gadiforms), to advanced euteleosts (perciform fishes). Source: R. Q.-Y. Yong. License: CC BY-NC-SA 4.0.

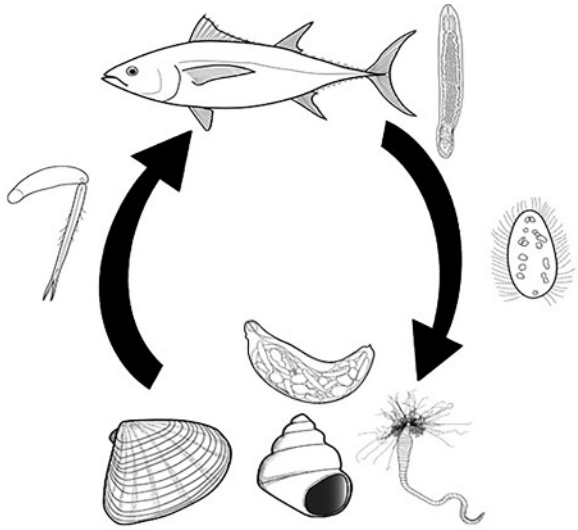

Figure 4: A generalized life cycle for aporocotylids. Adult worms live in the fish host and produce eggs (top); miracidial larvae (right) hatch, leave the fish and infect either a bivalve (bottom left; chondrichthyan-infecting species), gastropod (bottom middle; freshwater teleost-infecting species), or polychaete worm (bottom right; marine teleost-infecting species) intermediate host. The miracidium develops into a sporocyst, which in turn clonally produces daughter sporocysts or rediae (bottom center), both of which asexually produce cercariae (left) that leave the intermediate host, infect the definitive fish host, and develop into sexual adults. Source: R. Q.-Y. Yong. License: CC BY-NC-SA 4.0.

holds that tetrapods (that is, terrestrial craniates) and sarcopterygian fishes (such as lungfishes and coelacanths) diverged from the common ancestor of actinopterygian fishes (Orélis-Ribeiro et al., 2014).

Aporocotylids show varying patterns of radiation among different groups. Species of some genera, such as those of *Psettarium* Goto & Ozaki, 1930, and *Paradeontacylix* McIntosh, 1934, have radiated only among particular fish taxa. In the case of the former, in tetraodontiform fishes (Yong et al., 2018b) and, in the latter, perciform fishes of the family Carangidae (Repullés-Albelda et al., 2008). Others, like those of *Aporocotyle* and *Cardicola* Short, 1953, infect much broader ranges of hosts; 5 orders and 10 families of fishes in the case of the former, and 5 orders and 17 families in the latter. Some radiations, such as those of *Ankistromeces*, *Phthinomita*, and *Cardicola* Short, 1953 that infect rabbitfishes (Perciformes: Siganidae), show highly-conserved morphologies and relatively little molecular divergence, indicating cryptic or incipient speciation (Nolan and Cribb, 2006; Brooks et al., 2017). Other lineages of aporocotylids have shown limited capacity for radiation in other fish groups; for instance, only 1 species is known to infect damselfishes (Pomacentridae) and only 2 are known from wrasses (Labridae), 2 of the most speciose fish families in the world, with over 400 and 500 species, respectively, despite both these families being surveyed extensively for aporocotylids (Nolan and Cribb, 2005; 2006; Yong et al., 2018a).

Aporocotylids in Relation to Other Organisms

All known aporocotylid life cycles involve 2 hosts, an invertebrate intermediate host and a fish final host (Figure 4). As for all blood flukes, sexual reproduction occurs within the host circulatory system and the eggs are shed into the host bloodstream. The eggs travel either to the gills, where they cause erosive pathology which eventually brings them into contact with seawater, or to the gut via the mesenteric vessels, where they exit with the feces. In both routes, egg escape is probably mediated by significant antigenic reactions on the part of the host and, in cases of heavy infection, can be compounded by environmental stress and cause host mortality.

Once free of the fish, the eggs hatch and a ciliated miracidium infects an intermediate host. Among freshwater species, the intermediate host is a gastropod mollusc or snail, whereas those of marine bony fishes infect polychaete worms

(Cribb et al., 2017). No full life cycles are known for chondrichthyan-infecting aporocotylids, but Cribb and colleagues (2017) found intermediate stages in a bivalve mollusc which formed a molecular phylogenetic clade with sequence data for elasmobranch-infecting species. Having penetrated the host, the miracidia infect the digestive and reproductive organs and develop into mother sporocysts, which in turn asexually produce daughter sporocysts. These asexually produce cercariae, which emerge from the intermediate host and enter the final hosts by direct penetration (Kirk and Lewis, 1993). Aporocotylid cercariae have varying capacities to seek out their final hosts; some species have well-developed tails and actively swim to find their final hosts. Others, such as those of tuna-infecting species of *Cardicola*, have rudimentary tails and are evidently poor swimmers. It is not known how they come into contact with their final hosts (Cribb et al., 2011; Shirakashi et al., 2015). The act of penetration by cercariae can be traumatic for the hosts, particularly if the larvae are densely concentrated (Wales, 1958). Infection by aporocotylids causes pathological reactions which, if sufficiently severe, can permanently impair or kill the host. This is discussed in further detail below.

Most Important Groups

Aporocotylids are that rare aquatic trematode family which receives human attention due to the propensity for high infection rates to cause mass mortalities in the fish aquaculture industry. Several species in both freshwater and marine systems cause diseases that reduce production in aquaculture production facilities (reviewed in Ogawa, 2014). Rainbow trout (family Salmonidae: *Oncorhynchus mykiss*) farms in the western United States have reported losses of up to a million fish in a short amount of time due to infection with these trematodes (Wales, 1958), while significant losses have also been reported by farmers of brook trout (family Salmonidae: *Salvelinus fontinalis*) in the United States (Hoffman et al., 1985), tiger pufferfish (family Tetraodontidae: *Takifugu rubripes*) in China and Japan (Ogawa et al., 2007), amberjack (family Carangidae: *Seriola* spp.) in Japan and Spain (Crespo et al., 1994; Ogawa and Fukudome, 1994), and bluefin tuna (family Scombridae: *Thunnus* spp.) in Japan (Ogawa et al., 2010).

The cumulative pathogenic effects of aporocotylid infection are collectively known as sanguinicoliasis. The symptoms of sanguinicoliasis fall into 2 phases: Those which result from initial infection of fish by cercariae, and those related to mature infections. In the first phase, the physical trauma resulting from penetration by large numbers of cercariae can rapidly and severely compromise, and even kill, a fish. In the second phase, the consumption of host blood and the release of eggs into the organs lead to severe pathological effects such as inflammation, ulceration of the gut wall (Yong et al., 2018a), and erosion of gill tissue (Bullard and Overstreet, 2002). One species, *Cardallagium anthicum* (Bullard & Overstreet, 2006), which infects cobia (family Rachycentridae: *Rachycentron canadum*), even induces dramatic alterations to its attachment site. By lacing itself into spaces in the heart tissue, it induces a fibromatous tissue response, leading to the formation of a sort of fibrotic collar which surrounds the worm (Bullard and Overstreet, 2006; Warren et al., 2017). Although some fish are able to survive these effects, they may still incur significant loss of body condition and may have increased susceptibility to infection by other pathogens (Iqbal and Sommerville, 1986; Kumon et al., 2002). More typically, however, particularly in aquaculture settings, these effects lead to severe trauma and, ultimately, death. Through all these phases, the severity of disease suffered is directly proportionate to the number of worms to which fishes are exposed. Since most aquacultured fish are kept in stationary facilities, often adjacent to large populations of intermediate hosts, they are unable to escape high concentrations of cercariae. Treatment of sanguinicoliasis hence depends not just on treatment of the infection using drugs (anthelminthics such as praziquantel), but on interrupting the fluke life cycle, either by removing intermediate hosts from the immediate area (Malevitskaya, 1950) or moving the fish away from infection sites (Kirchhoff et al., 2011). To cite an example, bluefin tuna ranchers in South Australia combat infection of their stock by *Cardicola* spp. by shifting their sea cages further offshore to minimize encounters between the tuna and the aporocotylid intermediate hosts, which are polychaete worms of the family Terebellidae (Kirchhoff et al., 2011).

Other Relevant Related Topics

The inherent difficulties associated with working on aporocotylids mean that, for most of the family's history, the rate of discovery lagged behind those of many other trematode groups. The fragility of individuals upon collection of most species poses problems with collecting specimens for preservation and study, with specimens readily fragmenting, even during targeted dissections. Once removed from their infection sites, they often rapidly degrade, losing their tegumental spines and quickly dying (Bullard et al., 2008). Compounding the issue is the fact that their chief infection sites—blood vessels, gills, and cardiac tissue—are difficult to dissect, requiring great care and precision. As a result, aporocotylids have often been discoveries of chance, appearing in washings of guts and other dissections, their real sites of infection unknown until systematic searching was performed. This is a pervasive and characteristic problem of collecting small helminth parasites from larger hosts with little time, and usually with inadequate training of the persons doing the necropsies.

However, since the turn of the 21st century, the number of described aporocotylid species has increased rapidly because of concerted efforts to characterize the fauna of this family, to try for a better understanding of both the nature of parasites affecting aquaculture and how to optimize targeted searching. Seventeen of the 36 known genera and 86 of the 164 known species were described or proposed post-2000, including many of the species which infect fish of aquacultural significance. The pace of discovery for this family continues unabated, with new taxa continuing to be proposed and described year after year. Nevertheless, many fish groups are still under-surveyed or unstudied for the presence of blood flukes, many regions of the world have been little-studied for parasites in general, and a high proportion of the genera within the Aporocotylidae are monotypic. The fish blood flukes, therefore, can still be regarded as a highly exciting potential trove of discovery.

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Digenea, Plagiorchiida

Introduction to Plagiorchiida La Rue, 1957 (Order)

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Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

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Chapter 36

Introduction to Plagiorchiida La Rue, 1957 (Order)

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Order Plagiorchiida La Rue, 1957

The order **Plagiorchiida** constitutes the second fundamental branch of the **Digenea**, together with Diplostomida, according to Olson and colleagues (2003). This is a large order of trematodes comprising a vast diversity of forms. Included within the Plagiorchiida are digeneans with marked morphological characteristics (that is, absence versus presence of suckers, or simple tail versus forked tail in the cercariae) and biological characteristics (aquatic versus terrestrial life cycles, or infective free-living miracidia versus eggs eaten by the first intermediate host, among others) (Cribb et al., 2003). This makes it difficult to generally characterize the order Plagiorchiida. In fact, Olson and colleagues (2003), in their revision on the classification of the Digenea, divided the class into the order Diplostomida and the remaining digeneans were included within the Plagiorchiida. According to this division, the Plagiorchiida includes a large number of independent lineages that were classified as suborders. The traditional division of the Echinostomida, Plagiorchiida, and Strigeida was considered non-natural. Based on DNA analysis, Olson and colleagues (2003) defined the order Plagiorchiida, including a total of 13 suborders and 19 superfamilies.

Following is a classification of the Plagiorchiida after Olson and colleagues (2003). The bolded suborders are each discussed in greater detail following this introductory section, with special attention to the Xiphidiata.

Order **Plagiorchiida** La Rue, 1957
- Suborder Apocreadiata Olson et al., 2003
  - Superfamily Apocreadioidea Skrjabin, 1942
- Suborder **Bivesiculata** Olson et al., 2003
  - Superfamily Bivesiculoidea Yamaguti, 1934
- Suborder Bucephalata La Rue, 1926
  - Superfamily Bucephaloidea Poche, 1907
  - Superfamily Gymnophalloidea Odhner, 1905
- Suborder **Echinostomata** La Rue, 1926
  - Superfamily Echinostomoidea Looss, 1902
- Suborder **Haplosplanchnata** Olson et al., 2003
  - Superfamily Haplosplanchnoidea Poche, 1925
- Suborder **Hemiurata** Skrjabin and Guschanskaja, 1954
  - Superfamily Azygioidea Lühe, 1909
  - Superfamily Hemiuroidea Looss, 1899
- Suborder Heronimata Skrjabin and Schulz, 1937
  - Superfamily Heronimoidea Ward, 1918
- Suborder Lepocreadiata Olson et al., 2003
  - Superfamily Lepocreadioidea Odhner, 1905
- Suborder **Monorchiata** Olson et al., 2003
  - Superfamily Monorchioidea Odhner, 1911
- Suborder Opisthorchiata La Rue, 1957
  - Superfamily **Opisthorchioidea** Braun, 1901
- Suborder Pronocephalata Olson et al., 2003
  - Superfamily Pronocephaloidea Looss, 1899
  - Superfamily Paramphistomoidea Fischoeder, 1901
- Suborder **Transversotremata** Olson et al., 2003
  - Superfamily Transversotrematoidea Witenberg, 1944
- Suborder **Xiphidiata** Olson et al., 2003
  - Superfamily **Allocreadioidea** Looss, 1902
  - Superfamily Gorgoderoidea Looss, 1901
  - Superfamily Microphalloidea Ward, 1901
  - Superfamily Plagiorchioidea Lühe, 1901

Furthermore, Olson and colleagues (2003) considered that Bivesiculata, Transversotremata, and Hemiurata constituted the most basal forms of Plagiorchiida. The remaining suborders were considered to be among the higher Plagiorchiida. Note that Gorgoderoidea Looss, 1901 replaces Allocreadioidea Looss, 1902, according to some taxonomists (Bray et al., 2020; Gibson and Cribb, 2014).

Each selected suborder of Plagiorchiida will be addressed in a separate section with the exception of the monotypic suborder **Heronimata** Skrjabin & Schulz, 1932 since this

suborder is only represented by a single species *Heronimus chelydrae* within the superfamily Heronimoidea Ward, 1917. This superfamily is the only plagiorchiid restricted to tetrapods, specifically, freshwater turtles in North America (mainly Chelyddridae, Emydidae, and Kinosternidae). Moreover, other characteristic features of Heronimata are: 1) An anterior and dorsal excretory pore; 2) a single asexual generation within the snail first intermediate host; 3) absence of a ventral sucker in the adult stage; 4) a cercaria with a functional ventral sucker that disappears as the adult worm develops; and 5) mother-sporocysts with enormous lateral branches.

Simple-tailed cercariae are produced in sporocysts within the gastropod intermediate host (mainly physid snails). The cercariae do not emerge from the snail intermediate host but are eaten with the snail by the definitive host (which is turtles). The egg hatches and the miracidia actively penetrates the snail intermediate host (Cribb et al., 2003).

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Digenea, Plagiorchiida

Bivesiculata Olson et al., 2003 (Suborder): Small, Rare, but Important

Thomas H. Cribb and Scott C. Cutmore

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Suborder Bivesiculata

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Chapter 37

Bivesiculata Olson et al., 2003 (Suborder): Small, Rare, but Important

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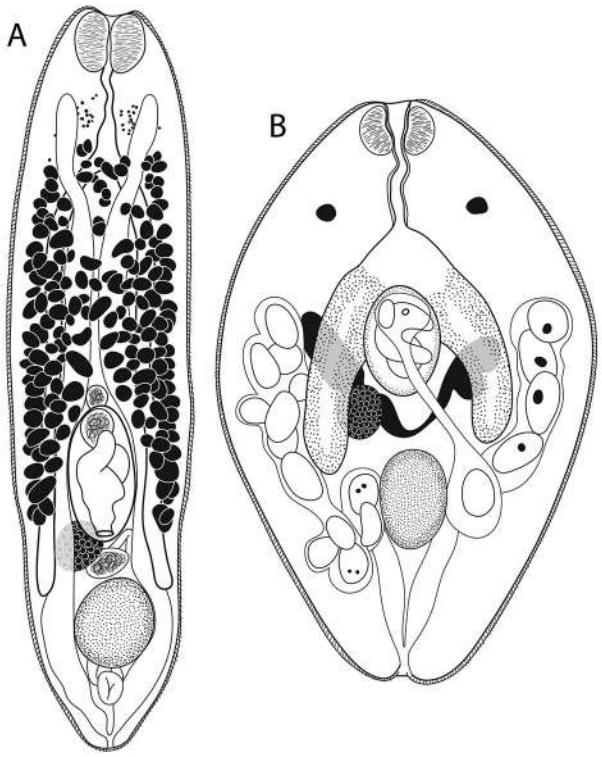

Figure 1. Sexual adult worms of family Bivesiculidae. All species lack oral and ventral suckers but have a well-developed pharynx. Species of the genus *Paucivitellosus* are unique within the family in having a highly reduced vitellarium and eggs that embryonate and grow in utero. A) *Bivesicula claviformis*. B) *Paucivitellosus fragilis*. Source: T. H. Cribb. License: CC BY-NC-SA 4.0.

Introduction

The Bivesiculata Olson et al., 2003 is a suborder of digenean trematodes, the species of which are found in the intestine of marine bony fishes, species of Osteichthyes. The single superfamily and family contain just 5 genera and 29 species (Decock et al., 2013). As none of the species have been reported to have any economic significance, why should we be interested in them? Their greatest significance lies in their evolutionary position and what their morphology and life cycle may therefore imply for the evolution of the Digenea as a whole.

Identifying Bivesiculids

Bivesiculids are small trematodes, the largest reported species (*Bivesicula congeri* Yamaguti, 1970) reaching just barely over 5 mm in length (Yamaguti, 1970) and the smallest (*Paucivitellosus fragilis* Coil, Reid & Kuntz, 1965) maturing at under 0.5 mm in length (Pearson, 1968) (Figure 1). They occur in a wide range of fishes; currently 36 families are known hosts. They are principally recognized by what they lack, namely, oral and ventral suckers. The absence of the ventral sucker is clear and unambiguous; However, the supposed absence of an oral sucker has been contentious because there is one anterior **muscular structure** surrounding the **gut** which sometimes been interpreted as an oral sucker and sometimes as a pharynx. Cribb and Cutmore take the view that it is a **pharynx** as it is well inside the body instead of around the **mouth**. The expression of the absence of oral and ventral suckers is most clear in living worms. They have a distinctive movement marked by pronounced peristaltic contraction of the body; comparable movement in any other trematodes has never been observed.

Beyond the absence of suckers, bivesiculids have few strongly distinguishing characters. They have **cecae**, a single **testis**, a well-developed **cirrus sac**, an **ovary**, extensive **vitelline follicles** (except in species of *Paucivitellosus*), and a deeply V-shaped **excretory bladder** (from which the type-genus *Bivesicula* takes its name).

Life Cycles

The known life cycles of trematodes in the Bivesiculidae are highly distinctive. All known bivesiculid cercariae develop in gastropods of the family Cerithiidae, a massively speciose family of marine snails known generally as sand-creepers. Le Zotte (1954) described 6 distinct bivesiculid cercarial morphotypes from cerithiid snail around Puerto Rico. There are perhaps not quite enough recorded and published data on infections known to be certain, but it seems likely that all bivesiculids infect members of the family Cerithiidae.

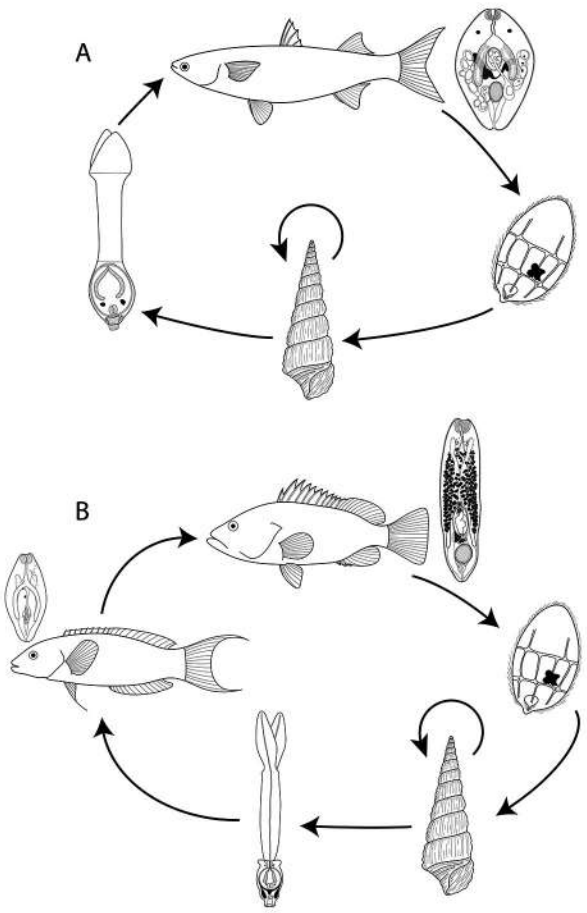

Figure 2. Life cycle of Bivesiculidae. A) *Paucivitellosus fragilis*; cercaria is ingested directly by definitive host. B) *Bivesicula claviformis*; cercaria is ingested by small fish, develops into a juvenile in the gut, and is then ingested with its host by a larger fish-eating grouper. The worms never mature in the small fish. Source: Source: T. H. Cribb. License: CC BY-NC-SA 4.0.

The most complete known life cycle for the family is that of *Paucivitellosus fragilis* as described by Pearson (1968) (Figure 2). A mother-sporocyst generation was not reported for this or any other bivesiculid, but presumably it exists and produces the generation (or generations) of rediae which are known, and which in turn produce cercariae. The cercaria is highly distinctive. It is relatively large (just visible to the naked eye), fork-tailed, and the cercarial body is withdrawn into a chamber at the base of the tail. In this respect it resembles cercariae of the Azygiidae, but the cercarial bodies of azygiids have well-developed oral and ventral suckers. The body withdraws into the base of the tail shortly after the cercaria emerges from the gastropod.

The cercaria is free-swimming and is thought typically to be eaten directly by the definitive host. This is certainly the case for *Paucivitellosus fragilis*, which attaches to the substrate and is then ingested (presumably accidentally) by grazing mullet (Mugilidae) and blennies (Blenniidae) (Pearson, 1968). Some bivesiculids infect planktivorous fishes (for example, Apogonidae, Clupeidae) and it is suspected that their cercariae are eaten directly from the plankton (Trieu et al., 2015). Other bivesiculids infect large predatory fishes, which seems incompatible with what is known of the life cycle of *P. fragilis*. However, Cribb and colleagues (1998) used DNA sequence evidence to show that one of these species, *Bivesicula claviformis* Yamaguti, 1934, frequently occurs as juveniles in the intestines of small fish species in which they are never found as sexual adults. They inferred that the small fish ingest the cercariae and that they are in turn ingested by the definitive hosts (Serranidae or groupers), so that the life cycle was facultatively (perhaps obligatorily) 3-host in at least some species. Such a life cycle probably explains the presence of bivesiculids in fishes such as moray eels (Muraenidae) and scorpionfish (Scorpaenidae).

Bivesiculata in Relation to Others in Their Group

In the molecular phylogenetic analysis of the Trematoda by Olson and colleagues (2003), the Digenea was identified as forming 2 major clades recognized as orders—the Diplostomida (including the blood flukes) and the Plagiorchiida (including such groups as *Fasciola,* the sheep blood fluke, and the human liver flukes, *Clonorchis* and *Opisthorchis*). The Bivesiculidae was resolved as basal, or the sister taxon, to all other Plagiorchiida. It is in this respect that the group is most interesting in its key morphological characters (absence of suckers) and the simple nature of the life cycle of some of its members (2-host with direct ingestion of an unencysted cercaria). The argument with respect to these characters is complicated and not entirely settled because it must take into account the fact that the Bivesiculidae is evidently basal only to the remainder of the Plagiorchiida, not to the Digenea as a whole. It thus remains debatable, for example, as to whether the absence of suckers is plesiomorphic (the basal condition) which implies that they evolved separately in both the Diplostomida and other Plagiorchiida or were secondarily lost in the Bivesiculidae. It seems likely, however, that bivesiculids represent a close approximation of the nature of at least one of the earliest forms of the Digenea.

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Digenea, Plagiorchiida

Echinostomata La Rue, 1926 (Suborder)

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Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Suborder Echinostomata

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Chapter 38

Echinostomata La Rue, 1926 (Suborder)

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Introduction

Echinostomata is a suborder, belonging to the order Plagiorchiida, which includes numerous species of trematodes that are parasites of humans and are of great health significance. Moreover, other species of the suborder are of importance in the veterinary sciences. According to Olson and colleagues (2003), this is a monophyletic taxon including only the Superfamily Echinostomatoidea.

Superfamily Echinostomatoidea

Classification

Echinostomatoidea is a large and cosmopolitan group of hermaphroditic digeneans that parasitize, as adult forms, all classes of vertebrates, but exhibit particularly high diversity in birds (Kostadinova and Jones, 2005). Trematodes that are members of this superfamily are characterized by having a morphologically complex structure, high species diversity with substantial species richness (Tkach et al., 2016).

The taxon was first defined by Faust (1929); however, it was first recognized as a natural group (at the subordinal rank, Echinostomata) by Szidat (1939). Subsequently, La Rue (1957) established the order Echinostomida, including the suborders Echinostomata (comprising the superfamily Echinostomatoidea) and Paramphistomata (including Notocotyloidea and Paramphistomoidea). Cribb and colleagues (2001), after a phylogenetic analysis, supported the validity of the Echinostomatoidea as a superfamily, including 4 families, including: Echinostomatidae, Philophtalmidae, Fasciolidae, and Cyclocoelidae. The most recent classifications of Echinostomatoidea have shown that the superfamily is characterized by a broad diversity comprising 80 species representing 8 families and 40 genera (Kostadinova and Jones, 2005; Tkach et al., 2016). Tkach and colleagues (2016), using 28S rDNA gene sequences, performed a detailed analysis of the phylogeny of the superfamily Echinostomatoidea. Herein will follow the systematic summary (classification) of the Echinostomatoidea proposed by Tkach and colleagues (2016) and we will review species in the 8 families that he recognized in addition to a brief review of species of the 2 families that were not represented in their work including species of the families Rhytidodidae and Calycodidae, both comprising parasites of marine turtles. Species representing these 2 families were not represented in the analysis of Tkach and colleagues (2016).

Identification

Members of Echinostomatoidea are elongate, oval, or foliate and usually the tegument is armed (has spines). The oral sucker is commonly subterminal and the ventral sucker is larger and pre-equatorial. A pharynx is commonly present. They possess 2 testes in the hindbody. The ovary is pretesticular and adults of Echinostomatoidea include a Mehlis' gland and a uterine seminal receptacle. The male and female ducts open separately into a genital atrium. The eggs are operculated, except in Philophtalmidae (Kostadinova and Jones, 2005).

Life Cycles

The life cycles of the Echinostomatoidea have a rich and diverse ecological milieu. Miracidia typically hatch from eggs that are passed from the definitive host into an aquatic environment and actively search and penetrate the first intermediate host (which are gastropods). Miracidia respond with positive chemotaxis to glycoproteins emitted by the gastropod for the host finding (Haberl et al., 2000). Cercariae are simple-tailed and produced by rediae in the first intermediate host and may encyst on vegetation to form infective (to the definitive host) metacercariae in the environment (that is, Fasciolidae) or within a second intermediate host (that is, Echinostomatidae), commonly molluscs, frog tadpoles, crabs, or fishes, among others. To find the second intermediate host, the free-swimming cercariae use different cues than the miracidia used in order to locate the second intermediate host.

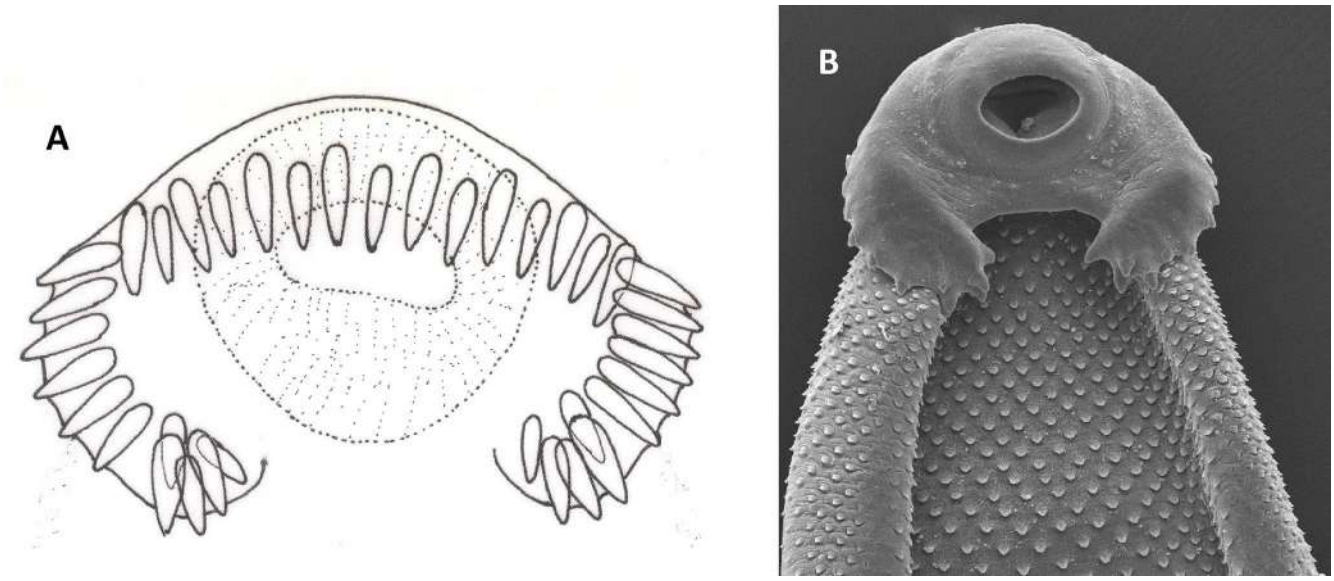

Figure 1. A) Cephalic collar of spines of *Echinostoma* sp. (Echinostomatidae) arranged in a double row (original); B) SEM microphotography of the forebody of *Echinostoma* sp. (Echinostomatidae) showing the cephalic collar of spines. Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

Cercariae swimming in the water commonly respond to low molecular weight molecules such as organic acids (Haberl et al., 2000). A vertebrate definitive host becomes infected after ingestion of the metacercariae.

Family Echinostomatidae Looss, 1899

The family Echinostomatidae is a heterogeneous group that predominately parasitize as adult forms a great spectrum of vertebrate hosts, such as birds, mammals and, occasionally, reptiles and fishes (Toledo et al., 2009). They are also able to parasitize humans, when people eat uncooked vegetables or crabs or crayfish, causing the foodborne infection echinostomiasis.

This family exhibits substantial taxonomic diversity and the morphological criteria adopted by different authors has resulted in a huge number of subfamilies. Kostadinova (2005a) accepted 11 subfamilies and 44 genera within Echinostomatidae as a result of a comparative morphological study, based on the examination of type materials and an evaluation of the previously published data, *Echinostoma* being the type genus.

Identification

Species of the family Echinostomatidae are mainly characterized by the presence of a prominent cephalic collar of spines (Figure 1). The spines of the cephalic collar may be arranged in 1 or 2 circles and the number of spines is usually constant within the individuals of a species. The tegument contains scale-like spines on both dorsal and ventral surfaces, though the number and size of the spines is reduced in the posterior half of the body. The oral and ventral suckers are close to each other. The 2 testes, usually situated in the body in tandem, are located posterior to the ovary. The uterus is intercecal and normally pre-ovarian. The vitellarium is follicular, in 2 lateral fields, usually in the hindbody but may extend into the forebody (Figure 2). Considerable variation exists in the size of echinostomes depending upon species and range from 5 mm to longer than 10 mm.

At the generic level, the main characters for identification are the morphology and the degree of development of the collar, the morphology of the male terminal genitalia, the position of the ovary and testes, the location and structure of the internal seminal vesicle, and the structure of the tegumental armament (Kostadinova, 2005a). Specific diagnosis within this family is difficult due to the morphological similarity of several species and, sometimes, molecular analysis is required.

Life cycles

Echinostomatid adults are hermaphroditic digeneans that live in the intestine and bile ducts of numerous vertebrates. To be viable, eggs released with feces must reach freshwater such as ponds, streams, or lakes. The fertilized eggs are undeveloped when laid and take about 2–3 weeks to reach the fully developed miracidial stage. Miracidia hatch from eggs and actively locate the first intermediate snail host in response to host signals and emitted products. Several species

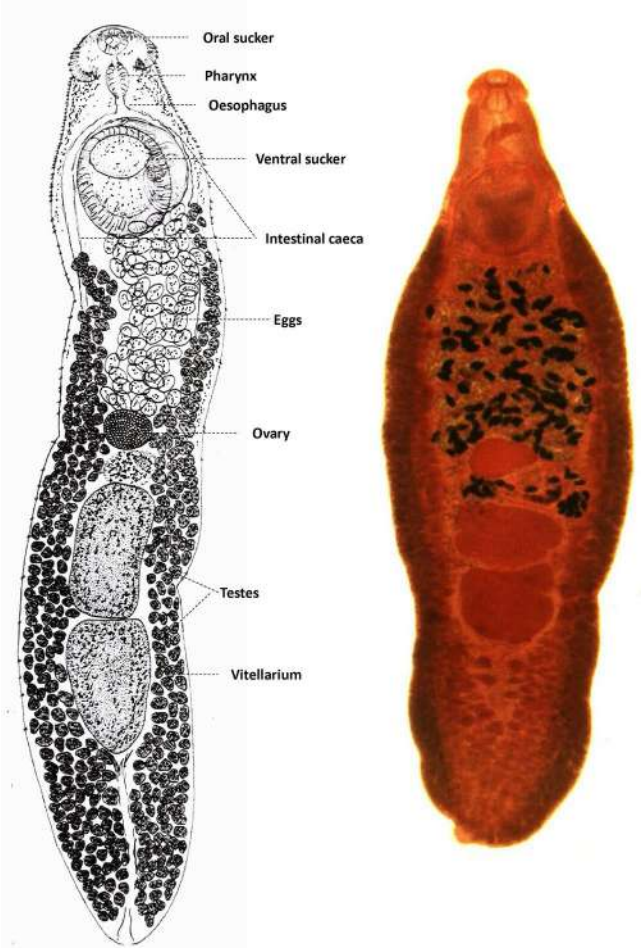

Figure 2. A) Adult *Echinostoma* sp. (Echinostomatidae); B) Adult specimen of *E. caproni* (Echinostomatidae) stained with Grenacher's borax carmin. Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

of planorbids, lymnaeids, and bulinids have been recorded as the first intermediate hosts. After penetration of the snail, miracidia transform into sporocysts in the heart and develop mother rediae. Mother rediae reproduce asexually and produce daughter rediae which develop in the digestive gland-ovotesis complex. Cercariae begin to emerge from infected snails from 4 to 6 weeks post-infection. *Echinostoma* cercariae show a low degree of host range and several species of snails, frogs, tapdoles, and fishes may serve as second intermediate hosts. Cercariae encyst within the second intermediate host. Definitive hosts become infected after ingestion of the second intermediate host harboring encysted metacercariae. Following infection of the definitive host, the metacercariae excyst in the duodenum and the juvenile parasites migrate to the small intestine where they attach to the mucosa by the ventral sucker (Figure 3).

Human echinostomiasis

In general, the specificity of echinostomatids toward the vertebrate is low and humans can become infected when they eat raw or inadequately cooked food, especially fish, snakes, amphibians, clams, and snails containing encysted echinostome metacercariae (Figure 3). Distribution of human echinostomiasis is strongly determined by dietary habits. Infections are most prevalent in areas where traditional cultural practices encourage ingestion of raw or undercooked wild animals. Moreover, it has been shown that drinking untreated water containing echinostome cercariae can be a source of human infection (Toledo et al., 2014; Toledo and Esteban, 2016). Most human infections are reported from foci in East Asia and Southeast Asia. Echinostomiasis is relatively rare, yet the foci of transmission remain endemic owing to local dietary preferences as noted above. Most of these endemic foci are localized in China, India, Indonesia, Korea, Malaysia, Philippines, Russia, Taiwan, and Thailand. Moreover, occasional cases have also been reported in other countries. Current incidence of human echinostomiasis is difficult to determine with any accuracy because of the lack of availability of epidemiological surveys. A total of 24 species of echinostomatids have been recorded infecting humans (Toledo and Esteban, 2016).

Major clinical symptoms due to echinostome infection may include abdominal pain, diarrhea, easy fatigue, and loss of body weight. Although the clinical signs in echinostomiasis in humans are poorly known, morbidity is due to the prolonged latent phase, symptomatic presentations, and similarity of symptoms with other intestinal helminth infections. The severity of the symptoms depends on the parasite load. Heavy infections are associated with local eosinophilia, abdominal pain, watery diarrhea, anemia, edema, and anorexia, and pathological features include catharral inflammation, erosion, and even ulceration (Toledo et al., 2006). Chai and colleagues (1994), in an endoscopic analysis of a human infection with an echinostomatid, showed that adult worms were attached to an ulcerated mucosal layer in the distal part of the stomach. The lesion was accompanied by stage IIc or stage III early gastric cancer and multiple ulcerations and bleeding in the stomach and duodenum. Ulceration and bleeding appeared to be caused by the worms. Other factors observed by endoscopy are mucosal erosions, ulcerative lesions, and signs of chronic gastritis.

Family Caballerotrematidae Tkach et al., 2016

This family was established by Tkach and colleagues in 2016. These authors analyzed the phylogenetic relationships of several Echinostomatoidea, including *Caballerotrema* spp. They concluded that *Caballerotrema* represents a unique group, comprising 3 valid species (*C. brasiliense, C. aruanense*, and *C. piscicola*) parasitic in the intestine of

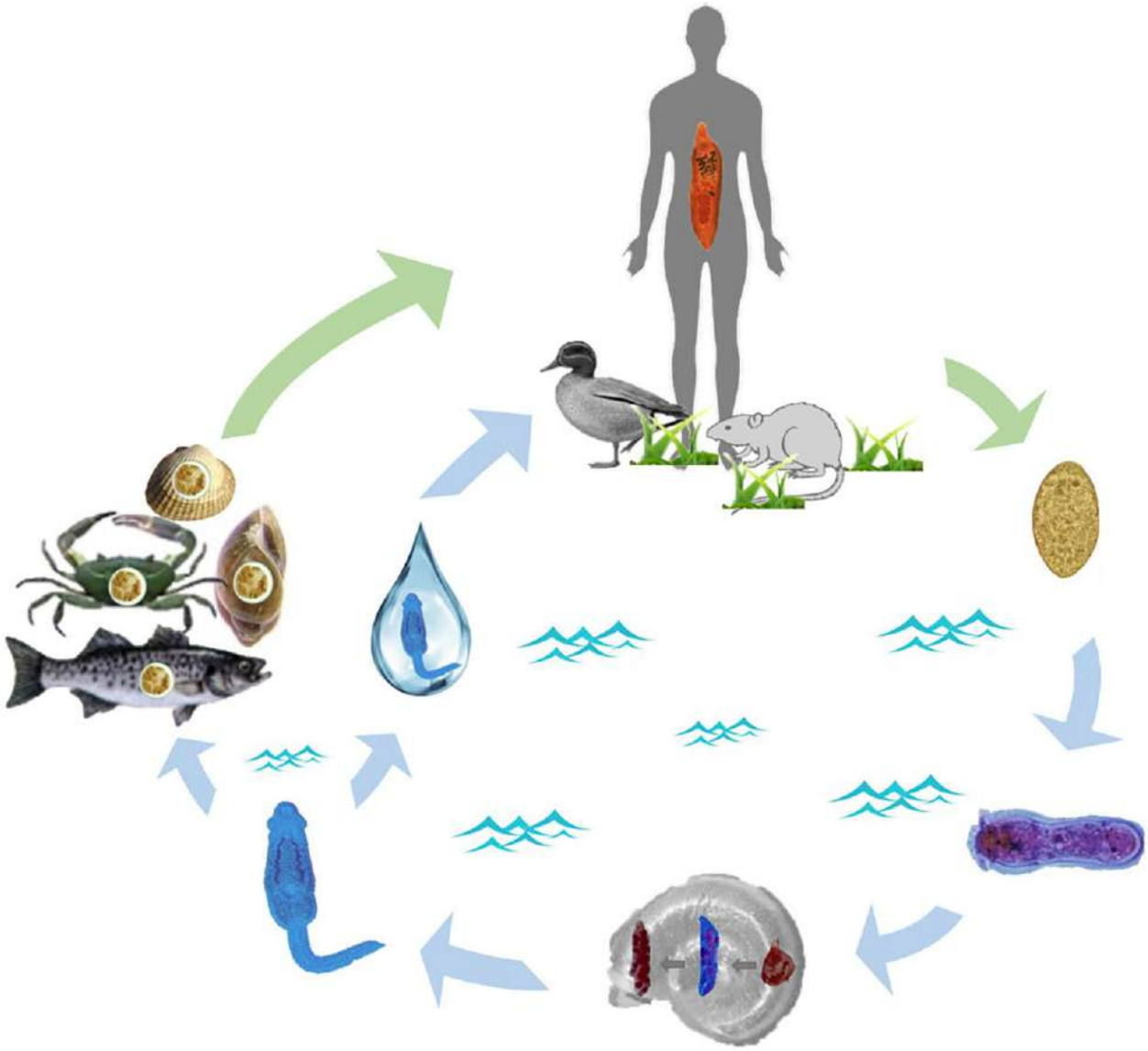

Figure 3. Generalized life cycle of echinostomes: Adult worms inhabiting the small intestine of several vertebrate hosts, including humans; eggs are voided with host feces; miracidia hatch in freshwater and actively infect the snail first intermediate host; intramolluscan stages, that is, sporocysts, mother rediae, and daughter rediae, develop within the snail; cercariae are released by the first intermediate host and swim to locate the second intermediate host (snails, amphibians, bivalves, fishes) which they penetrate; cercariae become metacercariae after encystation within the second intermediate host; and metacercariae are ingested by the definitive host and excyst to become adults. It has also been suggested that drinking water containing cercariae is a source of human infection. Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

freshwater fishes of South America. The genus *Caballerotrema* appeared as a separate branch closest to the Echinostomatidae. This fact together with several morphological characteristics and the use of cold-blooded vertebrates as definitive hosts led to Tkach and colleagues (2016) to distinguish *Caballerotrema* at the family level. Members of Caballerotrematidae are characterized by presenting its maximum width at the level of the collar.

Family Cyclocoelidae Stossich, 1902

Trematodes of the family Cyclocoelidae parasitize, as adult worms, the nasal cavity, hypothalamus, orbit, esophagus, trachea, air sacs, intestine, liver, kidneys, and abdominal cavity of birds feeding on molluscs. Cyclocoelidae has been an unsettled group and its taxonomic status is controversial. Kanev and colleagues (2002) placed Cyclocoelidae within the superfamily Cyclocoeloidea (Plagiorchiida) following La

Rue (1957). However, recent molecular studies have recovered this family within the Echinostomatoidea (Olson et al., 2003; Tkach et al., 2016). Thus, the superfamily Cyclocoeloidea was synonymized with Echinostomatoidea (Tkach et al., 2016).

The number of valid subfamilies, genera, and species within Cyclocoelidae is uncertain due to the continuous revisions of this group. Over 50 genera, tribes, families, and subfamilies have been included in the taxonomic organization of this group (Kanev et al., 2002). Yamaguti (1971) recognized 3 subfamilies (Cyclocoelinae, Promptenovinae, and Typhlocoelinae) and a total of 22 genera. Kanev and colleagues (2002) clarified the taxonomic situation of Cyclocoelidae by recognizing 3 subfamilies (Cyclocoelinae, Ophtalmophaginae, and Haematotrephinae) based on the relative position of the ovary respect the testes (Figure 4). Studies by Dronen (2007) and Dronen and Blend (2015) recognized a total of 6 subfamilies:

- **Cyclocoelinae** in which the ovary is intertesticular forming a triangle with the testes;
- **Haematotrephinae** in which the position of the ovary ranges from being pretesticular to opposite to the anterior testis forming a triangle with the testes;
- **Szidatitreminae** Dronen, 2007 in which the position of the ovary ranges from being posttesticular to opposite to the posterior testis forming a triangle with the testes;
- **Ophthalmophaginae** in which the ovary is posttesticular forming a straight, or nearly straight line with the tandem testes;
- **Hyptiasminae** in which the ovary is intertesticular and the testes are tandem to nearly tandem; and
- **Skrjabinocoelinae** in which the ovary is intertesticular and nearly in a straight line with the side-by-side testes.

Moreover, 22 genera and 128 species were recognized. Genera were assigned to these subfamilies based primarily on the position of the genital pore relative to the pharynx, the distribution of the vitelline fields posteriorly, and the orientation of the testes and ovary (Dronen, 2007; Dronen and Blend 2015). *Cyclocoelum* is the type genus (Figure 4).

The Cyclocoelidae are cosmopolitan and are characterized by an abbreviated life cycle in which the tail-less cercaria encysts within the first intermediate host (which are freshwater or terrestrial snails), which is eaten directly by the definitive host (Cribb et al., 2003).

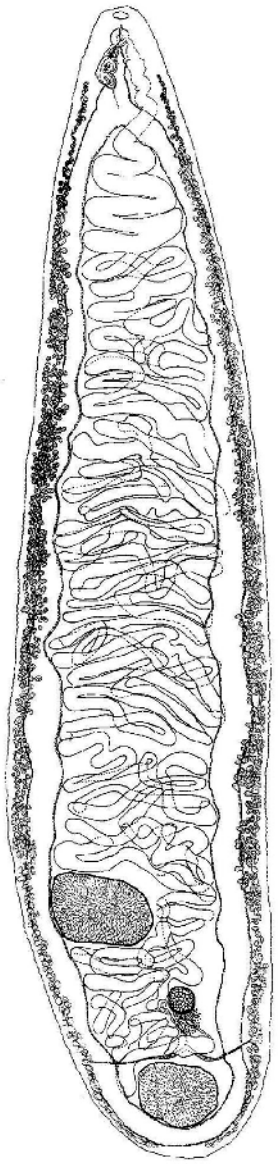

Figure 4. Adult specimen of *Cyclocoelum obscurum* (Cyclocoelidae). Source: Lamothe-Argumedo and Orozco-Flores, 2000. License: CC BY.

Family Echinochasmidae Odhner, 1910

This group was defined by Odhner (1910) as a subfamily (Echinochasminae). Posteriorly, Odening (1963) elevated the subfamily to full family rank. However, this was not followed by several authors (Skrjabin and Bashkirova, 1956; Yamaguti, 1971; Kostadinova, 2005b). Recently, molecular analyses based on concatenated amino acid sequences of 12 protein genes and 28S RNA gene sequences have strongly supported the elevation of the subfamily Echinochasminae to family status, as suggested on the basis of morphological studies by Odening in 1963 (see Le et al., 2016; Tkach et al., 2016).

This family includes cosmopolitan digenean parasites of birds, reptiles, and mammals. Echinochasmidae are characterized by the absence of a ventral connecting ridge on the collar of spines in a dorsally interrupted row (Kostadinova, 2005a). Moreover, echinochasmids have also been differentiated from other Echinostomatoidea by the even number of spines in the collar and the short pre-testicular uterus (Figure 5A) (Odening, 1963).

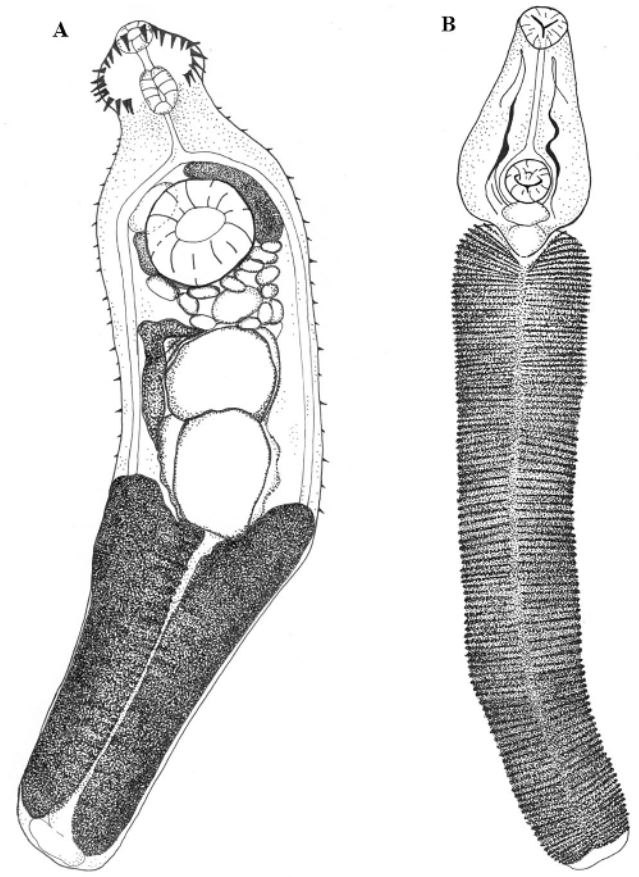

Figure 5. A) Adult specimen of *Stephanoprora* sp. (Echinochasmidae); and (B) cercariae magnacauda of *Stephanoprora* sp. (Echinochasmidae). Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

The life cycle of these species is triheteroxenous and involves brackish and freshwater snails (first intermediate hosts), molluscs, amphibians, and fishes (second intermediate hosts), and reptiles, piscivorous birds, and mammals as definitive hosts. Some members of Echinochasmidae infecting warm-blooded animals can cause diseases in humans, for example ,*Echinochasmus japonicus, E. perfoliatus, E. liliputanus*, and *E. fujianensis*, causing gastrointestinal disorders mainly in Asia (Toledo and Esteban, 2016). Another features of interest of the Echinochasmidae are the first intermediate host used and the morphology of the cercariae. Most other Echinostomatoidea for which life cycles are known use pulmonate snails as first intermediate hosts, whereas echinochasmids and other related families (Philophthalmidae, Psilostomidae) use prosobranch molluscs. Moreover, some species of this group have cercariae of the magnacauda type (Figure 5B).

According to Tkach and colleagues (2016) Echinochasmidae comprises a total of 6 genera (*Dissurus, Stephanoprora, Mehrastomum, Pulchrosomoides, Saakotrema*, and *Echinochasmu*s, the type genus) and more than 120 nominal species. Genera are mainly differentiated by the extension of the vitelline fields, number of spines in the collar, and the position of the ovary and testes.

Family Fasciolidae Railliet, 1895: The Liver Flukes

Fasciolidae is a family of trematodes that includes several parasites of importance in veterinary and medical sciences. In fact, it constitutes one of the most relevant families of digeneans in terms of veterinary and public health. The members of Fasciolidae are collectively referred to as the **liver flukes**.

Fasciolids are really large worms (some species getting as large as 50 mm in length) that parasitize wild and domesticated herbivorous vertebrates but some species can parasitize omnivores, including humans. Most species inhabit the bile ducts and liver, though there some species of the genera, *Fasciolopsis* and *Protofasciola*, that inhabit the intestine of the vertebrate hosts. Geographical distribution varies with the species. Some species are cosmopolitan while others show a more restricted distribution (Jones, 2005). The life cycles are diheteroxenous including a metacercarial stage that encysts on vegetation.

As currently structured, the family comprises 3 subfamilies, differentiated by the morphology of the cecae and the testes, and contains 6 genera: Fasciolinae (*Fascioloides, Fasciola*, and *Tenuifasciola*), Fasciolopsinae (*Fasciolopsis* and *Parafasciolopsis*), and Protofasiolinae (*Parafasciola*) (Jones, 2005). The subfamilies Fasciolinae and Fasciolopsinae includes several species that are of great importance in veterinary and human health.

The subfamily Fasciolinae include the digeneans (*Fasciola hepatica* and *F. gigantica*) that are of great importance in human health. These species infect the liver mammal hosts and are transmitted by snails of the family Lymnaeidae (which serves as the intermediate host). Adults of both species have a leaf-shaped body, with a broadly pointed posterior end. The 2 suckers are relatively small and located close to one another in a cone-like anterior extension of the body. The intestinal ceca are long, reaching the posterior end of the body and presenting lateral branches. The testes are branched and located in tandem, within the second- and third-fourth of the body. The branched ovary is pretesticular and dextral. The vitellaria extend bilaterally up to the hindbody. The short uterus is located between the ovary and the cecal bifurcation (Figure 6). Both species can be differentiated by their respective size. The adult stage of *F. hepatica* has a maximum length of 29.0 mm and a maximum width of 14.1 mm, whereas *F. gigantica* shows a maximum size reaching 52.3 mm and 11.8 mm, respectively. The eggs are operculated, ovoid, yellow, and non-embryonated when laid (Mas-Coma et al., 2014a).

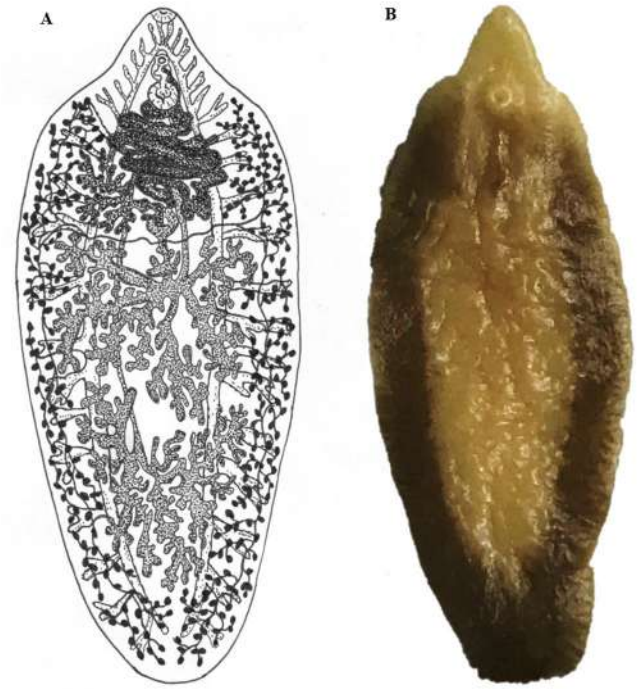

Figure 6. A) Adult *Fasciola hepatica* (Fasciolidae); B) live adult specimen of *Fasciola hepatica* (Fasciolidae). Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

Human fascioliasis

Fascioliasis is a neglected tropical disease caused by infection with the trematodes, *Fasciola hepatica* and *F. gigantica* (Mas-Coma et al., 2009). This is a worldwide water- and foodborne zoonotic infection that occurs on all continents except Antarctica (Hillyer and Apt, 1997; Fuentes et al., 1999; Hotez et al., 2014). *Fasciola hepatica* occurs worldwide, whereas *F. gigantica* is found in tropical areas of Asia and Africa (Cwiklinski et al., 2016; Roberts and Suhardono, 1996). The adult stages of both species inhabit the large biliary ducts and the gallbladder of herbivorous mammals, mainly sheep and cattle. Humans are incidental hosts and become infected by ingesting contaminated watercress or water (Croese et al., 1982; Ashrafi et al., 2006; Berger et al., 2010). It is estimated that 2 to 17 million humans are currently infected in 75 countries, and about 180 million people are at risk of infection (Hotez et al., 2014; Ashafi et al., 2014).

Life cycle

The life cycle of both fasciolids follows a similar pattern and takes around 14–23 weeks (Figure 7). Adult worms produce eggs that are passed with feces that eventually reach freshwater bodies. The miracidium hatches and swims to locate and penetrate the intermediate host, freshwater snails of the family Lymnaeidae (Bargues et al., 2001; Mas-Coma et al., 2009). The development within the intermediate snail host includes sporocyst and redial generations, to finally produce cercariae that are released to reach a solid support (water plants), where they encyst (Rondelaud et al., 2009). The definitive host becomes infected by ingestion of the encysted cercaria (metacercariae) in watercress or by drinking water containing cercariae (Hodasi, 1972). Metacercariae excyst in the small intestine, penetrating the host's intestine wall, and juveniles migrate to the liver across the abdominal cavity. They become sexually mature in the bile ducts. Eventually, infection also can be acquired by eating undercooked sheep or goat livers that contain immature forms of the parasite (Mas-Coma et al., 2014b).

Symptoms and phases of the illness

Several clinical periods may be distinguished in human fascioliasis. The incubation period that lasts from few days to 3 months (from the ingestion of metacercariae to the appearance of the first symptoms) is characterized by fever, abdominal pain, and gastrointestinal and respiratory symptoms. The invasive or acute period involves flukes migrating to the bile ducts. This phase is characterized by mechanical destruction of the hepatic tissue and the peritoneum by migrating juvenile flukes. The major symptoms of this phase are: Fever, abdominal pain, gastrointestinal disturbances, respiratory symptoms, hepato-splenomegaly, ascites, anemia, and jaundice. The latent period is initiated after the establishment of the adult worms in the bile ducts and may last from months to years from the infection. Symptoms during this phase can include eosinophilia, gastrointestinal complaints, inflammation, hyperplasia of the epithelium, and thickening and dilatation of the bile duct and gallbladder walls. The infection may cause obstruction of the bile ducts (Gulsen et al., 2006; Mas-Coma et al., 2014b).

Diagnosis, treatment, and prevention

Fascioliasis can be diagnosed by both direct parasitological techniques or indirect immunological tests. Coprological examination is still the fastest method and the so called "gold standard method" for the diagnosis of fascioliasis, but several serological, intradermal, antigen-detection and PCR methods have been developed (Mas-Coma et al., 2014a).

Triclabendazole is the recommended treatment against fascioliasis and may therefore be employed during the acute and chronic phases (WHO, 2007).

The prevention of human infection may be achieved by strict control of the human infection sources as well as education, especially in endemic zones. Additionally, preventive chemotherapy, mass drug administration, treatment of

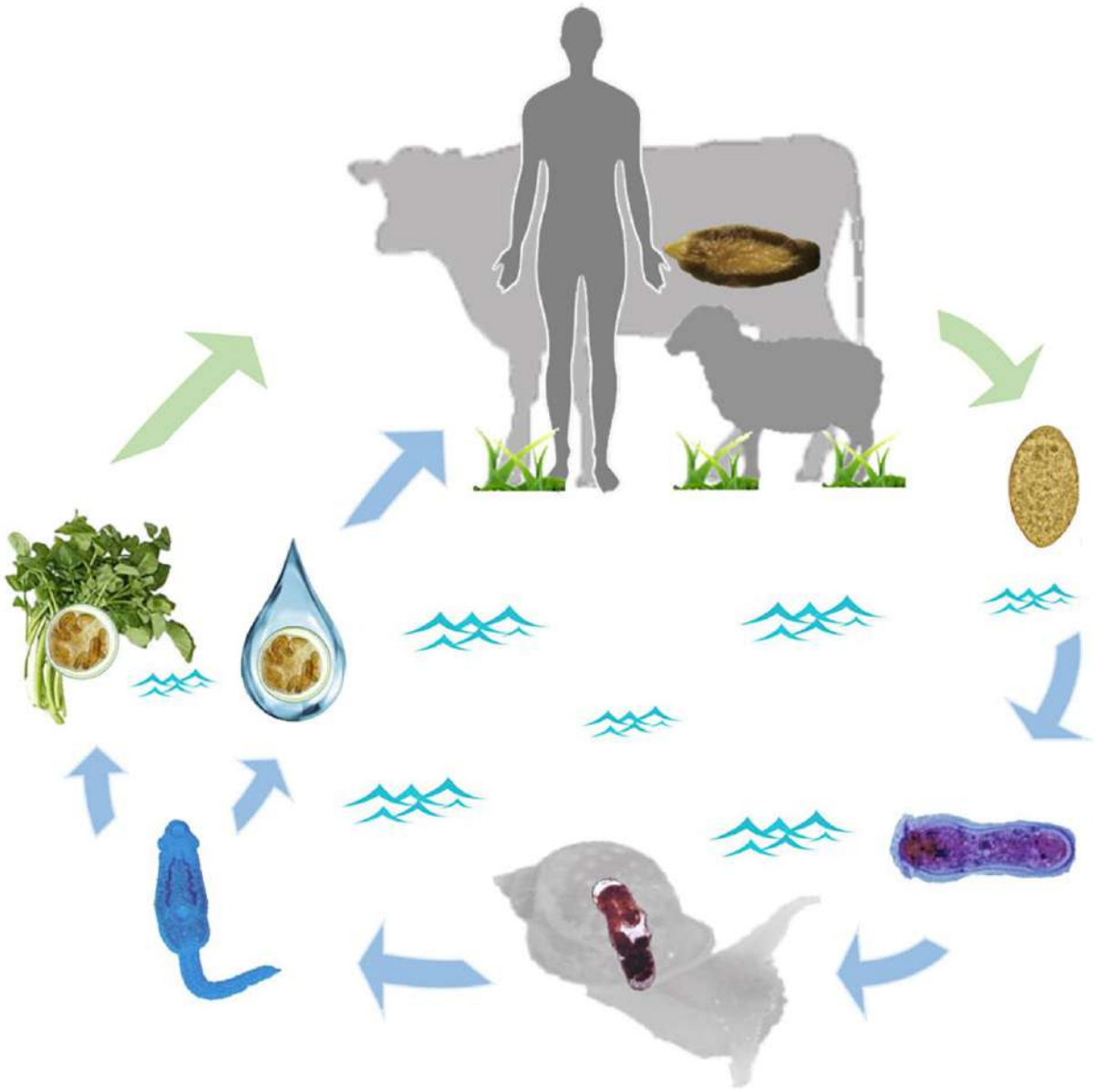

Figure 7. Generalized life cycle of *Fasciola* spp. (Fasciolidae): adult worms inhabiting the biliary ducts of several vertebrate hosts, including humans; eggs are voided with host feces; miracidia hatch in freshwater and actively infect the snail first intermediate host (Lymnaeidae); intramolluscan stages, that is, sporocysts and rediae, develop within the snail; cercariae are released by the first intermediate host and swim to locate freshwater plants, especially watercress, where cercariae encyst; and metacercariae are ingested by the definitive host and excyst to migrate through the intestinal wall, the peritoneal cavity, and the liver parenchyma into the biliary ducts, where they develop into adults. It has also been suggested that drinking water containing cercariae is a source of human infection. Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

infected people, accurate diagnoses to prevent new cases, and development of advanced morbidity can be adopted (WHO, 1995; Mas-Coma et al., 2018).

Human fasciolopsiasis.

Fasciolopsis buski is a species belonging to the subfamily Fasciolopsinae that causes intestinal infections in humans, referred to as human fasciolopsiasis. This is the largest trematode parasitizing humans (8–10 × 1–3 cm) and a common intestinal parasite of humans and pigs in Asia. Human infections are mainly found in East Asia and Southeast Asia (Toledo et al., 2012; 2014).

The life cycle of *Fasciolopsis buski* is similar to that of the above described for *Fasciola* spp., with several species

of the genera *Segmentina* and *Hippeutis* serving as intermediate hosts. Humans commonly become infected by eating raw or undercooked aquatic plants, but infection can be also contracted by the drinking or use of contaminated water or processing of the water-derived plants.

Clinical symptoms in *Fasciolopsis buski* infections in humans are related to parasite load and can be fatal in heavy infections. In light infections, symptomatology may include anemia, eosinophilia, dizziness, and gastrointestinal symptoms. In moderate and heavy infections there may be severe epigastric and abdominal pain, diarrhea or bowel obstruction, nausea, acute ileus, anasarca, and eosinophilia and leucocytosis. Moreover, the parasite can induce duodenal erosions, ulceration, hemorrhage, abscesses, and catarrhal inflammation. Eventually, it may cause intestinal perforation (Toledo et al., 2012; 2014).

Family Himasthlidae Odhner, 1910

Members of the family Himasthlidae are common parasites in birds and mammals worldwide, with some reported cases of human infections. This taxon was created as a subfamily, Himasthlinae, by Odhner (1910) on the basis of the length of the cirrus sac which extends beyond of the posterior border of the ventral sucker, the armed cirrus and the presence of a pars prostatica. However, Tkach and colleagues (2016), in their phylogenetic analysis based on the 28S rRNA gene sequences of several members of the group, concluded that Himasthlinae should be elevated to the family rank, accepting the boundaries proposed by Odening (1963) and Kostadinova (2005a), with the exceptions of the genera *Caballerotrema* (elevated to family rank) and *Artyfechinostomum* (allocated to Echinostomatidae). According to Tkach and colleagues (2016), Himasthlidae comprises 5 genera (*Acanthoparyphium, Aporchis, Cloeophora, Curtuteria*, and the type genus is *Himasthla*). These genera are differentiated on the basis of the extension of the vitelline fields, the morphology of the body and testes, and the structure of the spined collar.

Representatives of the family Himasthlidae have several interesting morphological and biological characteristic features. At the level of adult worms, they are characterized by exhibiting a very wide and dorsoventrally flattened body with finger-like processes on each ventrolateral edge, an intestinal bifurcation that is dorsal to a ventral sucker, and an extensive pars prostatica. Cercarial morphology is characterized by possessing collecting ducts forming numerous lateral branches in the forebody filled with excretory concretions. Moreover, Himasthlidae (with the exception of *Artyfechinostomum*) are among the few members of Echinostomatoidea with a marine life cycle. Interestingly, Himasthlidae, together with Psilostomidae and Philophthalmidae, are the only ones within the Echinostomatoidea using prosobranchs, such as littorinids, as the first intermediate host. They follow a 3-host life cycle with bivalves and clams as the second intermediate host and birds and mammals as the definitive hosts.

Only 2 species of Himasthlidae have been reported to infect humans, including *Acanthoparyphium tyosenense* which is known to have infected people who ate improperly cooked marine bivalves in South Korea and Japan. *Himasthla muehlensi* was reported in a German patient who had eaten raw clams (Toledo et al., 2014).

Family Philophthalmidae Looss, 1899

Digeneans of the family Philophthalmidae are parasites of the eyes, intestine, and bursa Fabricii of birds and, rarely, reptiles, and may accidentally infect humans. This group was established by Looss (1899) as a subfamily on the basis of the interrupted vitelline fields, a long cirrus sac, and embryonated eggs containing a fully developed miracidium. Adult worms may or may not present a cephalic collar of spines. Travassos (1918) elevated the group to full family rank. The status of rank at the family level was supported recently by Tkach and colleagues (2016) by molecular methods. These latest authors accepted 3 subfamilies (Philophthalminae, Parorchiinae, and Cloacitrematinae) in contrast to Kanev et al. (2005) who had recognized 5 subfamilies (Philophthalminae, Ommatobrephinae, Echinostephilinae, Cloacitrematinae, and Parorchiinae). Subfamilies are mainly differentiated on the basis of the site of the infection, the vertebrate host, and the morphology of the testes and esophagus. Kanev and colleagues (2005) accepted a total of 10 genera within Philophthalmidae, with *Philophthalmus* as the type genus.

Philophthalmids have a 2-host life cycle. Fully-embryonated eggs are shed into the water from the definitive host's eyes and miracidia hatch almost immediately in the water and penetrate the snail intermediate host, which commonly are prosobranch snails. Within the snail host, the miracidia undergo a series of stages and become cercariae. Cercariae are released from the snail and encyst on aquatic vegetation or other solid objects in the water. The definitive host, which is usually an aquatic bird, becomes infected upon ingestion of the metacercariae. Metacercariae excyst in the mouth and migrate to the eye where the adults reside. Humans rarely serve as incidental hosts but may do so when they ingest metacercariae on aquatic vegetation. Known human cases are from the United States, Central Europe, the Middle East, Southeast Asia, and Japan, and are caused by a species of the genus *Philophthalmus.*

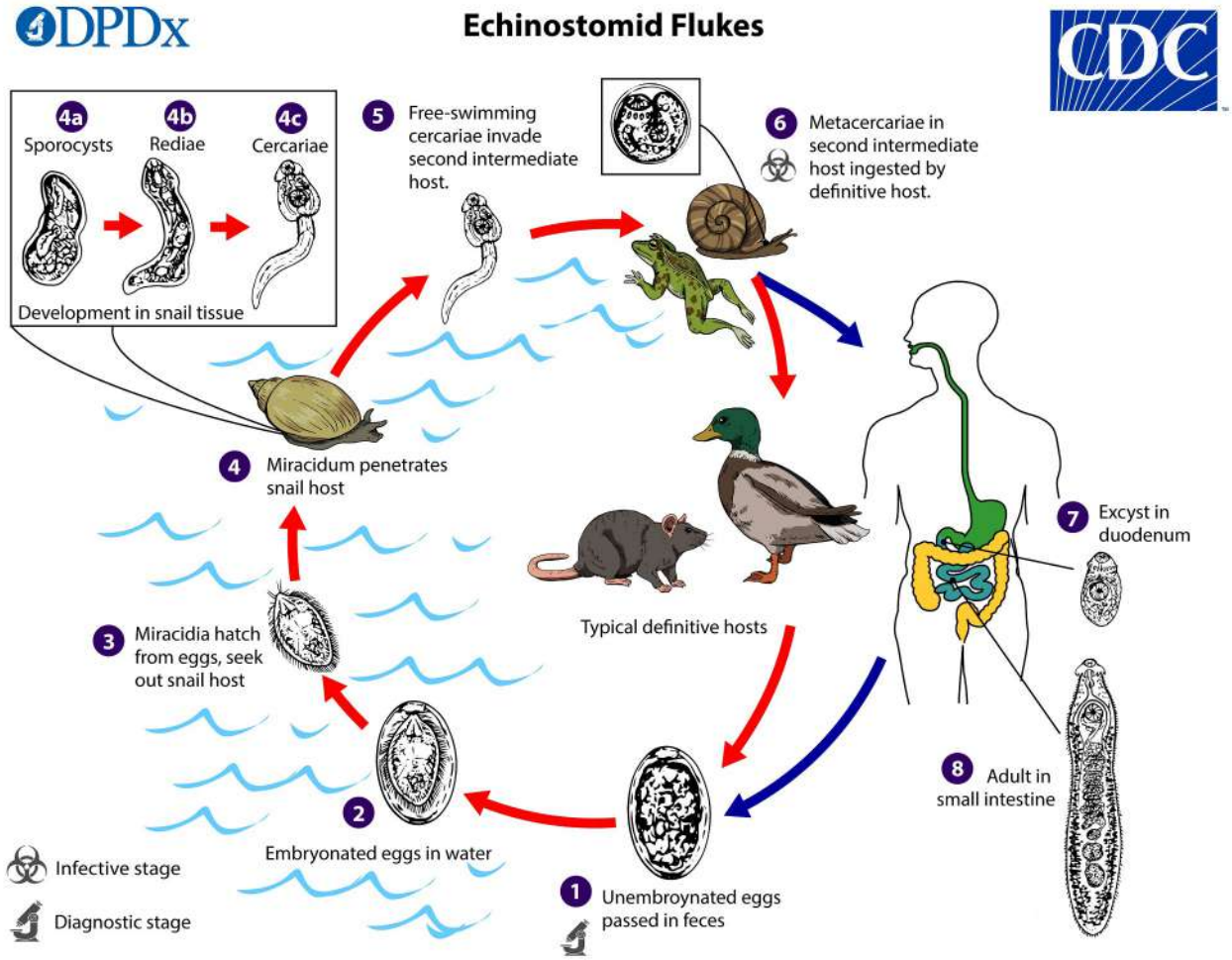

Figure 8. Life cycle of echinostomatid trematodes (flukes). Like many trematodes, echinostomid flukes undergo a multi-host (indirect) life cycle. Unembryonated eggs are passed in feces of infected definitive hosts (1) and develop in water (2). Miracidia usually take about 3 weeks to mature before hatching (3), after which they swim freely and penetrate the first intermediate host, a snail (4). The intramolluscan stages include a sporocyst stage (4a), 1 or 2 generations of rediae (4b), and cercariae (4c), which are released from the snail. The cercariae may encyst as metacercariae within the same first intermediate host or leave the host and penetrate a new second intermediate host (5). The definitive host becomes infected after eating metacercariae in infected second intermediate hosts (6). Metacercariae excyst in the duodenum (7) and adults reside in the small intestine (for some species, occasionally in the bile ducts or large intestine) (8). Source: United States Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria (DPDx), 2019. Public domain.

Family Psilostomidae Looss, 1900

The family Psilostomidae constitutes a small group of digeneans within the Echinsotomatoidea including gastrointestinal parasites of birds and mammals worldwide (Kostadinova, 2005b). The traditional systematics of this family has been based on the morphology of the sexually mature adult worms. Since its establishment by Looss (1900), the taxonomic structure of Psilostomidae has been the subject of several revisions (Odhner, 1910; Kostadinova, 2005b; Tkach et al., 2016; Kudlai et al., 2017). According to the revision by Kostadinova (2005b), the family contains 6 subfamilies and 13 genera: *Mehlisia, Psilochasmus, Psilorchis, Psilostomum*, and *Psilotrema* (Psilostominae); *Apopharynx* and *Psilotornus* (Apopharynginae); *Grysoma* (Grysominae); *Ribeiroia* and *Trifolium* (Ribeiroiinae); *Astacatrematula* and *Sphaeridiotrema* (Sphaeridiotrematinae); and *Stephanoproraoides* (Stephanoproraoidinae).

Tkach and colleagues (2016) assessed the phylogenetic position of Psilostomidae within Echinostomatoidea and proposed the allocation of *Ribeiroia* and *Trifolium* within Echinostomatidae, synonymizing the subfamily Ribeiroiinae. Thereafter, 3 new genera have been added to the Psilostomidae (*Neopsilotrema, Bydtrema*, and *Macracetabulum*) (Kudlai et al., 2016; 2017).

Morphologically, members of the Psilostomidae closely resemble those of the Echinostomatidae, except for the absence of a circumoral head-collar. The main features used for the differentiation at the subfamilial and generic levels are: The shape and size of the body, the position of the ventral sucker, the development of the pharynx, the structure of the male terminal genitalia and the vitellarium, the length of the post-testicular area, and the egg size (Kostadinova, 2005b).

The life cycle of the members of Psilostomidae is a 3-host life cycle similar to that of echinostomatids, using prosobranch gastropods as the first intermediate host. The second intermediate host commonly are amphibians or bivalves. The definitive host becomes infected as a result of ingestion of the second intermediate host harboring metacercariae.

Family Calycodidae Dollfus, 1929

Members of the family Calycodidae can be distinguished from other echinostomatoids by their prominent ventral and dorsal ridges at the level of the pharynx and an esophagus diverticulum (Bray, 2005). This family only comprises a single genus (*Calycodes*) and 2 species (*C. anthos* and *C. caborojoensis*) that are parasites of marine turtles (Bray, 2005).

Family Rhytidodidae Odhner, 1926

Members of Rhytidodidae are parasites of the intestine and gallbladder of marine turtles in tropical and subtropical seas. They are characterized by possessing a small lateral projection on each side of the oral sucker. They can be differentiated from Calycodidae by the absence of the ventral ridge at the anterior extremity and the lack of esophageal diverticulum (Blair, 2005). The family comprises 2 genera: *Rhytidodides* and *Rhytidodes*, which is the type genus. Both genera are differentiated on the basis on the location of the testes, ventral sucker, and vitelline follicles (Blair, 2005).

Life Cycle Diagram of Echinostomatid Trematodes

A life cycle diagram from the Division of Parasitic Diseases and Malaria of the United States Centers for Disease Control and Prevention (DPDx, 2019) is shown in Figure 8 and demonstrates how humans may become infected by echinostomatid trematodes (here referred to as flukes).

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39

Digenea, Plagiorchiida

Haplosplanchnata (Suborder): Two Hosts with Half the Guts

Daniel C. Huston

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Suborder Haplosplanchnata

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 39

Haplosplanchnata Olson et al., 2003 (Suborder): Two Hosts with Half the Guts

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Introduction

The suborder Haplosplanchnata Olson et al., 2003 represents a small, but distinct lineage within the Plagiorchiida. The suborder includes a single superfamily and family, the Haplosplanchnoidea Poche, 1925 and Haplosplanchnidae Poche, 1926, which encompass 9 genera and 59 species (Cribb, 2010).

Haplosplanchnids are intestinal parasites, mostly of marine herbivorous (grazing, scraping, and excavating) fishes, although a few species occur in predatory fishes (Nahhas, 1997; Huston et al., 2017; 2018a). As with most trematodes of wildlife, haplosplanchnids are not considered of medical, veterinary, or economic importance. For those interested in the evolution of the Digenea, however, haplosplanchnids present some intriguing morphological and life cycle adaptations. Some of these, such as a derived 2-host life cycle, add support to emerging evolutionary paradigms. Others, such as the specialized suckers possessed by some species, present new questions.

Identifying Haplosplanchnata

Haplosplanchnids gained their name from the type genus *Haplosplanchnus* Looss, 1902. The name means single-gut and is derived from Ancient Greek **haplo** (= single) and **splanch** (= intestine). The presence of a single intestinal cecum is the major feature uniting haplosplanchnids. All but 2 of the species currently recognized lack a cirrus sac and possess only a single testis. Species of 2 monotypic genera are problematic: *Prohaplosplanchnus diorchis* Tang & Lin, 1978 possesses 2 testes and *Parahaplosplanchnus cirrusaci* (Lü, 1995) possesses a cirrus sac. Neither has been evaluated with molecular data and it is likely that they will be found to belong elsewhere in the digenean phylogeny when such data become available. If these 2 species are ignored, haplosplanchnids can be recognized readily by the single intestinal cecum, lack of a cirrus sac, and a single testis. Other than in these features, haplosplanchnids have a typical digenean body plan with an oral sucker, ventral sucker, and a single ovary. In some groups the oral or ventral suckers may be specialized. Specialized glands are often visible in the ventral portion of the oral sucker, the so-called salivary glands. The vitelline follicles are highly restricted in some species, although in many they are profusely developed and often obscure the internal anatomy. Eggs are unembryonated in most species, but embryonated in utero in some.

Haplosplanchnid genera are readily differentiated morphologically (for example, Figure 1, and see the key in Huston et al., 2018a). Conversely, species-level identifications are far more difficult because of the simplified internal anatomy of most species. Thus, molecular data have become increasingly important for the taxonomy of the group (Huston et al., 2017; 2018a).

Haplosplanchnata in Relation to Other Organisms

Two haplosplanchnid life cycles have been elucidated, that of *Schikhobalotrema acutum* (Linton, 1910) and *Haplosplanchnus pachysomus* (Eysenhardt, 1829) (Cable, 1954; Fares and Maillard, 1975). In both, cercariae emerge from the intermediate gastropod host (families Cerithiidae and Hydrobiidae for *S. acutum* and *H. pachysomus*, respectively) and encyst as metacercariae on vegetation. In light of the derived position of the haplosplanchnids in the overall phylogeny of the Digenea (Olson et al., 2003; Littlewood et al., 2015), this form of metacercarial encystment suggests there was no second intermediate host in the evolution of the haplosplanchnid lineage (Cribb et al., 2003). External encystment has been demonstrated in multiple digenean lineages which exploit herbivorous fishes as definitive hosts, for example, the Atractotrematidae, Gorgocephalidae, Gyliauchenidae, and Microscaphidiidae (Al-Jahdali and Hassanine, 2012; Hassanine et al., 2016; Huston et al., 2016; 2018b).

Some species of the genus *Schikhobalotrema* infect predatory needlefishes (Beloniformes: Belonidae) (Nahhas et al., 1997; Huston et al., 2017). Although this might suggest that some haplosplanchnids have 3-host life cycles, a 2-host life cycle seems more likely, and can be inferred from the evolutionary relationships of their beloniform hosts. Some species of *Schikhobalotrema* are also known from the related halfbeaks (Beloniformes: Hyporhamphidae) (Nahhas et al., 1997). Halfbeaks are surface feeding omnivores which incorporate large amount of plant matter in their diet, thus representing a typical host group for haplosplanchnids. Cable (1954) elucidated the life cycle of *S. acutum* and found adults

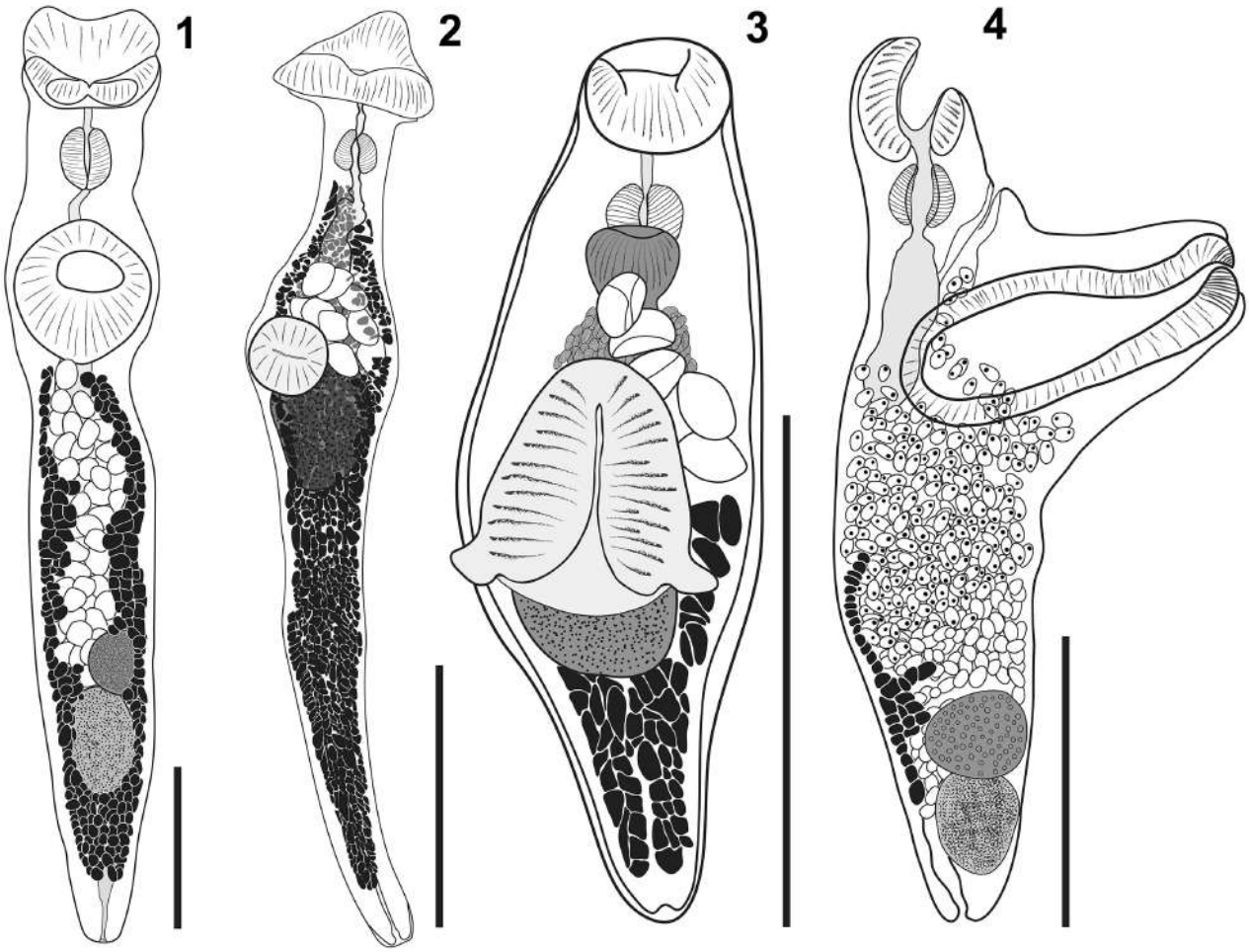

Figure 1. Representative species of the Haplosplanchnata. 1*) Hymenocotta mulli*. 2) *Trigonocephalotrema hipparchi*. 3) *Schikhobalotrema huffmani*. 4) *Haplosplanchnus pachysomus*. The single caecum is obscured in all except for *H. pachysomus*; this is frequently the case because of the densely packed vitellarium. Scale bars = 500 µm. Source: D. C. Huston. License: CC BY-NC-SA 4.0.

in both halfbeaks and needlefishes. Perhaps the haplosplanchnid species which have colonized belonids were parasites of halfbeaks which host-switched into the related belonids. Belonids likely consume metacercariae on vegetation incidentally when hunting, or perhaps *S. acutum* cercariae encyst on hard-bodied invertebrates on which the belonids feed. In either case, it seems likely that these species of *Schikhobalotrema* have 2-host life cycles, as do typical haplosplanchnids.

Haplosplanchnata in Relation to Others in Their Group

The evolutionary relationships between the haplosplanchnids and other lineages of the Plagiorchiida is still somewhat unclear. The family Haplosplanchnidae has been placed at times in either the Echinostomatoidae or Haploporoidea (see Madhavi, 2005). The molecular phylogenetic study of Olson et al. (2003) did not fully resolve the placement of the haplosplanchnids, but did demonstrate the lineage as distinct, warranting the erection of the Haplosplanchnata. Based on Olson et al. (2003) and other molecular phylogenies of the Digenea (for example, Littlewood et al., 2015), the haplosplanchnid lineage has the greatest affinity with the Paramphistomoidea Fischoeder, 1901, Pronocephaloidea Looss, 1899, and Echinostomatoidea Looss, 1902. It is significant that, like for the haplosplanchnids, cercariae of the Pronocephaloidea and Paramphistomoidea reach their definitive hosts by encysting on vegetation as metacercariae.

Most Important Groups

The most important haplosplanchnid genera are probably *Haplosplanchnus* and *Schikhobalotrema*, though important insights are to be gained from some of the smaller groups. Species of *Haplosplanchnus* are globally distributed and their definitive hosts are freshwater, brackish, and marine mullet (Mugilidae). Species of *Haplosplanchnus* have robust ventral suckers (often exceptionally so), which may be cannulated (for example, Figure 1.4). The function of such suckers

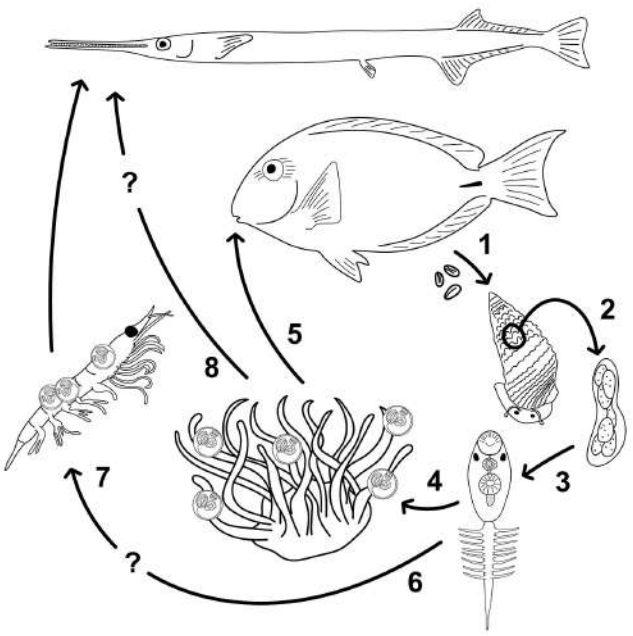

Figure 2. The haplosplanchnid life cycle. 1) Adult trematodes lay eggs, which pass into the environment with the host's waste. 2) Miracidium hatches from egg, infects snail intermediate host. Miracidium transforms to mother sporocyst; mother sporocyst produces daughter sporocysts. 3). Sporocysts produce cercariae; cercariae emerge from snail. 4) Cercariae encyst on vegetation as metacercariae. 5) Vegetation with metacercariae consumed by definitive host; metacercariae mature into adults. 6) It is not known how metacercariae reach predatory needlefish hosts, it is possible that cercariae 7) Encyst on the exterior of hard-bodied prey, or 8) Needlefishes consume plant matter incidentally, or purposefully. Source: D. C. Huston. License: CC BY-NC-SA 4.0.

is unclear. The eggs embryonate to miracidia with prominent eye spots *in utero*. The adaptive value of *in utero* embryonation is not known. The embryonated eggs in species of *Haplosplanchnus* form part of an unexplained pattern where multiple unrelated trematode lineages have embryonated eggs in species that parasitize mullets, and unembryonated eggs in species parasitizing other fish hosts.

Schikhobalotrema is the most species-rich genus in the Haplosplanchnidae. The most common host groups are the parrotfishes (Scaridae), surgeonfishes (Acanthuridae), and mullets (Mugilidae). Species parasitizing these fishes have unspecialized oral and ventral suckers. The species that parasitize predatory belonids, however (as discussed above), have unusual lateral lobes extending from the posterior part of their ventral sucker, and the ventral sucker has a longitudinal slit aperture. The function of this strange ventral sucker is unknown, but perhaps it is an adaptation for life in the very short gut of the belonid host.

Species of the genera *Discocephalotrema* Machida, 1993, *Hymenocotta* Manter, 1961, and *Trigonocephalotrema* Huston, Cutmore & Cribb, 2018 are all interesting with respect to their oral suckers. In species of these 3 genera the ventral suckers are unspecialized, but the oral suckers form specialized lobed or flattened plates, with small oral openings. Again, the adaptive significance of these specializations is not known. Although molecular data are not available for *Discocephalotrema*, molecular phylogenetic analyses of the Haplosplanchnidae (Huston et al., 2017; 2018a), support *Hymenocotta* as basal, and *Trigonocephalotrema* as sister to *Schikhobalotrema* + *Haplosplanchnus*. Thus, specialized and unspecialized suckers are distributed throughout the haplosplanchnid phylogeny. The molecular data available for the Haplosplanchnata are not yet comprehensive enough to make deep evolutionary inferences into the origin of sucker specialization in this group. However, it seems that the standard sucker template has been modified repeatedly, allowing for the exploitation of a wide variety of hosts and niches.

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Digenea, Plagiorchiida

Hemiurata Skrjabin & Guschanskaja, 1954 (Suborder)

Lucrecia Acosta Soto, Bernard Fried, and Rafael Toledo

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Suborder Hemiurata

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Chapter 40

Hemiurata Skrjabin & Guschanskaja, 1954 (Suborder)

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Suborder Hemiurata Skrjabin & Guschanskaja, 1954

The suborder Hemiurata represents one of the most diverse groups of digeneans, which usually occurs in the stomach and intestine mainly of marine teleost fishes but also occurs in freshwater teleosts, elasmobranchs, amphibians, and reptiles (Gibson and Bray, 1979). This group has a wide geographical distribution as it is found in the Great Barrier Reef of Australia, the Indian Ocean, and the Atlantic Ocean (Gibson and Bray, 1986).

Typically, members of Hemiurata have a 2- or 3-host life cycle in which marine gastropods act as the first intermediate host, crustaceans or other invertebrates as the second intermediate host (in the 3-host life cycles), and fishes as the final host. These life cycles are characterized by the fact that eggs are eaten by the first intermediate host.

Although, the systematic status of Hemiurata has been somewhat controversial, its taxonomic position within Plagiorchiida was well-supported by Olson et al. (2003), except for 2 superfamilies (Azygioidea and Hemiuroidea) in this suborder.

Superfamily Azygioidea Lühe, 1909

This group was erected by Lühe (1909) as a subfamily (Azyigiinae) and used at the family rank by Odhner (1911). La Rue (1957) included this group of digeneans in the superfamily Azygioidea. The superfamily was, thereafter, recognized by Gibson (2002l).

Members of this superfamily are parasitic in the stomach or body cavity of freshwater and marine fishes, mainly elasmobranchs, teleosts, and holosteans (Cribb et al., 2003; Gibson, 2002l). Eggs of azygioids have to be ingested by the first intermediate host (which are gastropods). Fork-tailed cercariae are produced in rediae in the gastropod. The cercarial body is withdrawn into the tail after emergence and the definitive host becomes infected by ingesting the cercariae directly. In some cycles, cercariae emerge with an egg already formed in the uterus. In another cycle, a second intermediate host is intercalated (Cribb et al., 2003).

The superfamily Azygioidea is monotypic and contains only 1 family: Azygiidae.

Family Azygiidae Lühe, 1909

This family contains 2 subfamilies and 4 genera, *Azygia* being the type genus. Subfamilies are differentiated on the basis of the position of the testes, specifically, whether they are post-ovarian (Azygiinae) or pre-ovarian (Leucerothrinae). The subfamily Azygiinae includes 3 genera (*Proterometra, Otodistomum*, and *Azygia*) which are differentiated by the structure of the testes, uterus, and vitelline follicles (Gibson 2002m). Subfamily Leucerothrinae only includes 1 genus (*Leuceruthus*) characterized as mentioned above, by the pre-ovarian position of the testes.

Superfamily Hemiuroidea Lühe, 1909

The Hemiuroidea, with a somewhat controversial taxonomy, constitute a huge and diverse group of digeneans that are commonly parasites the gut—mainly the stomach—of fishes. They are especially found in marine teleosts, but also occur in freshwater teleosts, elasmobranchs, and occasionally in amphibians and reptiles (Gibson, 2002m). In addition to the gut, species or entire groups are known from tissues, gallbladder, swimbladder, body cavity, lungs, and skin.

The life cycles of Hemiuroidea present specialized fork-tailed cercariae known as cystophorous cercariae, which are peculiar and highly modified forms possessing a tail with a caudal cyst into which the body of the worm can be withdrawn and a delivery tube through which the cercarial body is injected into the second intermediate host after the cercaria is released from the mollusc (Figure 1). Cercariae are produced in sporocysts or rediae in the first intermediate host gastropods or, rarely, scaphopods or bivalves. After ingestion of cercariae by the second intermediate host, a specialized structure

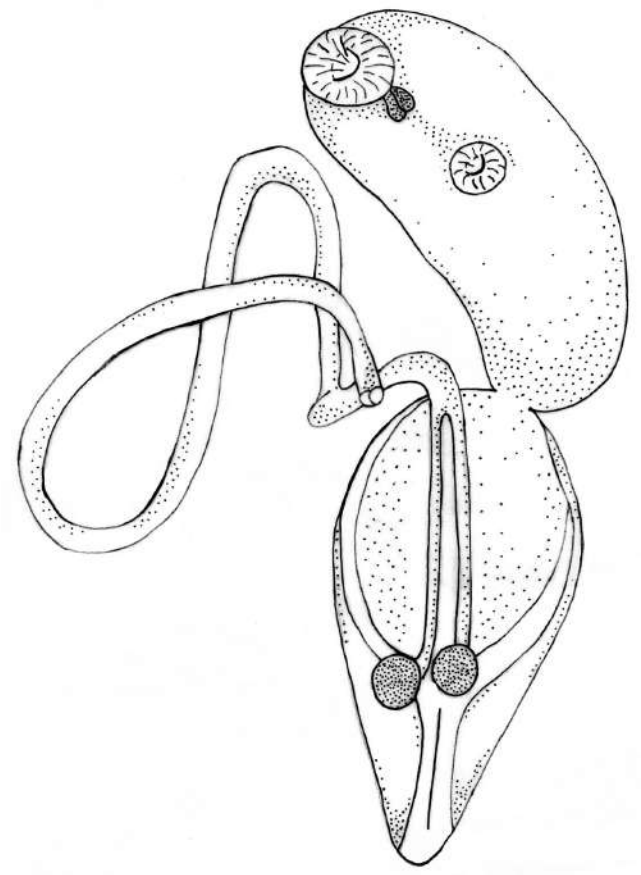

Figure 1. General scheme of a cystophorous cercariae. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

(the delivery tube) everts, penetrating the host gut where unencysted metacercariae are formed. The final host becomes infected after ingestion of metacercariae (Cribb et al., 2003). In some life cycles of hemiuroids, a fourth host can be included, by intercalation of this extra host between the second intermediate and the definitive host. In contrast, other life cycles can be abbreviated with all larval stages occurring within the gastropod (Cribb et al., 2003).

The main morphological features of this family are a genital pore that is mid-ventral, male and female terminal ducts that normally fuse to form a hermaphroditic duct with, commonly, a hermaphroditic intromittent organ (sinus organ) and a surrounding muscular sac, and characterized by the absence of a pre-pharynx, a tegument devoid of spines, and a Y-shaped excretory vesicle with arms bonding dorsally in the forebody (Gibson, 2002m). Moreover, there are a number of other specialized structures in certain families of Hemiuroidea (Cribb et al., 2003), including:

- **Ecsoma**: Name given to the posterior region of the body of a digenean when it is capable of being retracted within the body, which appears to be associated with the inhospitable environment (the cardiac stomach, which these worms inhabit) in that it is believed to be a feeding organ protruded only when the pH and/or osmolarity are suitable (Gibson and Bray, 1979)
- **Plications**: Regular backwardly directed thickenings of the tegument which surround, partly or completely, the body transversally.
- **Juel's organ**: A sac containing an amorphous granular material on which Laurer's canal opens dorsally.
- **Manter's organ**: A tubular vesicle lined with an epithelium and usually surrounded by bundles of muscle, occurring dorsally to the excretory vesicle into which it opens close to the excretory pore.
- **Fistchal's organ**: A round vesicle of unknown function, lined with epithelial cells and surrounded by a mass of gland cells opening dorsally to the right of Mehlis' gland.

Using these morphological features, (Gibson and Bray, 1979) classified the Hemiuroidea on the basis of 3 transformational series: 1) The seminal storage and disposal apparatus in the female reproductive system, with emphasis on the presence or absence of Juel's organ; 2) the form of the vitellarium; and 3) the structure of the terminal genitalia (Figure 2). On this basis, a total of 14 families were admitted. Blair et al. (1998) first used molecular and morphological matrices for phylogenetic reconstructions of Hemiuroidea. The main conclusion of this study was that molecular and morphological matrices for a large group of digeneans are not incongruent, leading to the belief that both kinds of data are of value in inferring relationships within this group. Based on the data reported by Blair et al. (1998), Gibson (2002m) admitted a total of 12 families within the Hemiuroidea.

Family Hemiuridae Looss, 1899

The Hemiuridae is a group of digeneans which usually occur in the stomach of marine teleosts, although forms are known from freshwater teleosts and the lung of piscivorous sea snakes. Gibson and Bray (1979) characterized the members of this family by their possession of a terminal ecsoma or "tail," which is capable of being retracted within the body. However, in later works, the ecsoma has not been considered to be the primary apomorphy of the group (Gibson and Bray, 1986; Gibson, 2002e; Atopkin et al., 2017). This structure is thought to be associated with the inhospitable environment (the cardiac stomach, which these worms inhabit) in that it is believed to be a feeding organ protruded only when the pH and/or osmolarity are suitable (Gibson and Bray, 1979).

Figure 2. General scheme of an adult specimen of *Accacoelium* sp. (Accacoeliidae). Source: L. Acosta Soto, B. Fried, and R. Toledo.

The life cycle of most hemiurids is poorly known, but it is likely that they follow the typical hemiuroid pattern (Gibson and Bray, 1986), which includes the following stages. Embryonated eggs passed by the fish in its feces are swallowed by gastropod molluscs and hatch in the gut, releasing the miracidium. Within the tissues of the mollusc the miracidium is transformed into a mother-sporocyst which normally gives rise to a generation of rediae (on rare occasions daughter-sporocysts). Within these parthenitae develop cystophorous cercariae. The metacercaria, which is unencysted, usually occurs in the hemocoel of planktonic organisms, such as copepods and chaetognaths. Chaetognaths acquire the parasites by feeding upon infested copepods, but it is not known for certain that hemiurids cannot be acquired directly by these hosts. The definitive hosts become infected either directly, in the case of young fish, small fish, and filter feeders, or indirectly by feeding upon small, infected fishes. In some cases, such as some lecithochiriines, immature forms may occur encapsulated in the body cavity of fishes which appear to act as obligate third intermediate hosts.

Although the composition of taxa within hemiuridae is somewhat confusing, Gibson (2002e) accepted a total of 12 subfamilies (Glomeriricirrinae, Lecithochiriinae, Plerurinae, Pulmoverminae, Lethadeninae, Hemiurinae, Elytrophallinae, Dinurinae, Ophasthadeninae, Theletrinae, Bunocotylinae, and Aphanurinae) and a total of 53 nominal genera, with *Hemiurus* being the type genus. Subfamilies are mainly differentiated on the basis of the presence or absence of an ecsoma, ejaculatory vesicle, sinus sac, and uterine seminal receptacle and the structure of the seminal vesicle and body surface (Gibson, 2002e).

Family Accacoeliidae Odhner, 1911

Members of the family Accacolediiae are easily recognized by the presence of an anterior extension to the pharynx, which penetrates the base of the oral sucker. Although several species of fishes can be infected by accacoelids, most of the taxa occur in a single fish species, *Mola mola*. This is related to the fact that *M. mola* is medusaophagus and the metacercariae of this family occurs in nektonic organisms, especially cnidarians and ctenophores (Gibson, 2002a). Accacoelids are commonly parasites in the gut but, occasionally, they can inhabit the gill of fishes as monogeneans. Various classifications have been proposed for the family Accacoelediiae. Gibson (2002a) included 2 subfamilies: Accacoelinae including 7 genera (*Accacoelium, Rhynchopharynx, Accacladium, Accacladocoelium, Odhnerium, Tetrotechtus*, and *Orophocotyle*); and the monotypic subfamily Paraccacladiinae that only comprises the genus *Paraccacladium*. *Accacoelium* is the type genus.

Family Bathycotylidae Dollfus, 1932

Bathycotylidae is a monotypic family that includes only 1 genus (*Bathycotyle*). They are parasites on gills and probably the stomach of pelagic marine teleosts. The most relevant features of this family are the presence of an intertesticular ovary, the absence of a sinus sac, and that they inhabit the gills (Gibson, 2002b).

Family Derogenidae Nicoll, 1910

This family includes parasites usually in the intestinal system (normally the stomach) of freshwater and marine teleosts, but occasionally recorded from amphibians, reptiles, and freshwater shrimp. Members of the family Derogenidae are characterized by the absence of constant seminal storage and the presence of a disposal apparatus in the female (Gibson and Bray, 1979). Gibson and Bray considered that these morphological variations were related to the fact that derogenids have evolved around the time the first modifications of the primitive arrangement of the seminal storage and disposal apparatus began to occur.

Gibson (2002c) accepted the previous classification of the family by Gibson and Bray (1979) including 3 subfamilies: Gonocercinae (including 2 genera), Halipeginae (including 16 genera), and Derogeninae (including 5 genera). Subfamilies are differentiated on the basis of the position of the testes with respect the ovary and vitellarium and the character of

the life cycle (marine or freshwater) (Gibson, 2002c). *Derogenes* is the type genus.

Family Dictysarcidae Skrjabin & Guschanskaja, 1955

The family Dictysarcidae comprises parasites in the swimbladder of marine physostomatous teleosts. Gibson (2002d) included 3 subfamilies within the Dictysarcidae: Albulatrematinae, comprising the genera *Albulatrema* and *Elongoparorchis*, Dictysarcinae, including the genera *Dictysarca* and *Aerobiotrema*, and the monotypic Cylindrorchiinae, comprising only the genus *Cylindrorchis*. Differentiation of the subfamilies is based on position of the uterus (whether it is pre- or post-ovarian) and the structure and shape of the ovary, vitellarium, and hermaphroditic duct. The type genus is *Dictysarca*.

Family Hirudinellidae Dollfus, 1932

Members of the family Hirudinellidae are very large hemiuroids that parasitize the stomach of large marine teleosts; and immature forms are occasionally present in salmonids (Gibson and Bray, 1979). They can be differentiated from other hemiuroids by the absence of a hermaphroditic duct, and the possession of a form of cirrus and cirrus sac that are different from other digeneans. This structure almost certainly developed independently of the sinus sac, but it does appear to be analogous and not homologous. (The sinus sac is a muscular sac that surrounds the base of the genital atrium.) Such a structure probably developed in this group because its ancestors lost, or did not develop, a hermaphroditic duct, with the result that the copulatory organ (the cirrus) did not contain the female duct. In this group, therefore, both the male and the female ducts have developed their own finger-like projections from the wall of the genital atrium (Gibson and Bray, 1979).

The Family Hirudinellidae contains 1 subfamily (Hirudinellinae) and 3 genera (*Lampitrema*, *Hirudinella*, and *Botulus*) that are differentiated by the body shape and the position of the uterus and the vitellarium (Gibson, 2002f).

Family Isoparorchiidae Travassos, 1922

This is a monotypic family that only contains 1 genus, *Isoparorchis*, that parasitize the swimbladder of physostomatous freshwater teleosts in Asia and Australia. The species of *Isoparorchis* differ from other parasites of the swimbladder in that they occur in freshwater rather than marine environments, and due to the possession of Laurer's canal, a tubular vitellarium, and an ovary and well-developed muscular sinus sac (Figure 3) (Gibson and Bray, 1979; Gibson, 2002g).

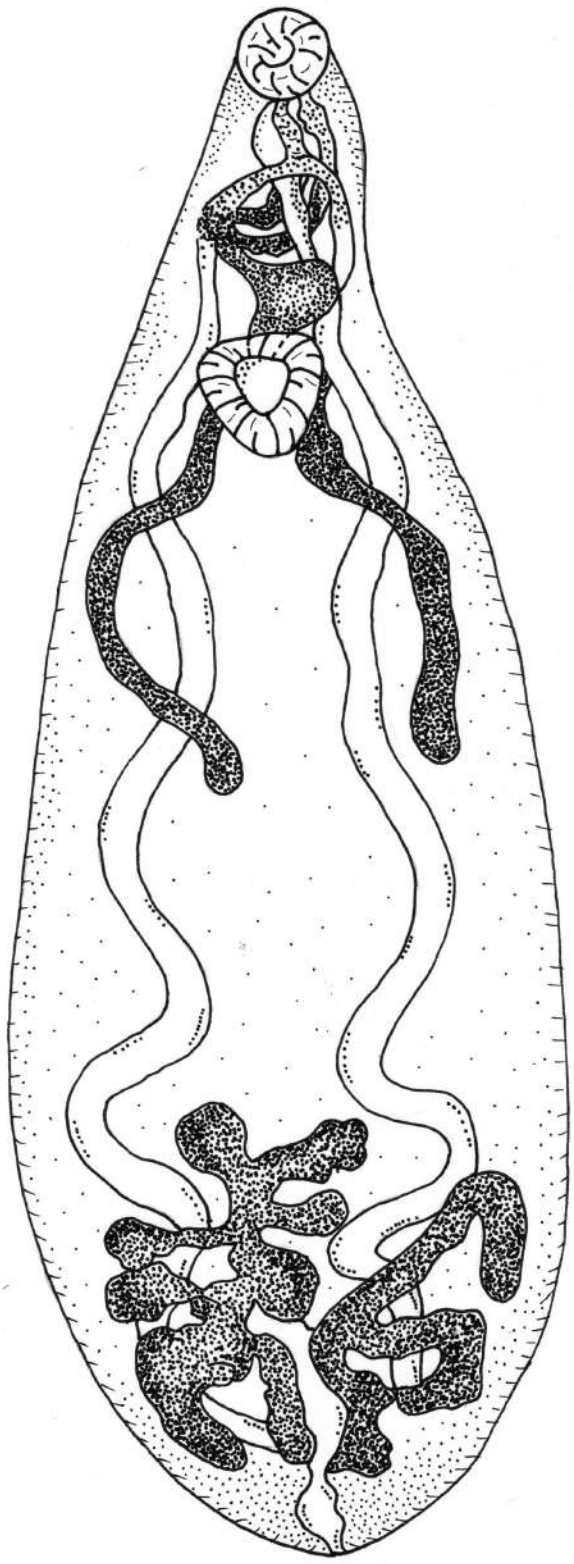

Figure 3. General scheme of an adult specimen of *Isoparorchis* sp. (Isoparorchiidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

Family Lecithasteridae Odhner, 1905

This family contains parasites in pyloric cecae and the anterior intestines of marine teleosts, mainly in the waters of the Great Barrier Reef off the coast of Australia. This group is characterized by a hermaphroditic duct that is relatively tubular, with a distinct gap, usually filled with fibrous connective tissue and gland cells between the wall of the hermaphroditic duct and the wall of the sinus sac. Species of Lecithasteridae retain both a uterine seminal receptacle and a rudimentary seminal receptacle. Moreover, the uterine distribution and structure of the vitellarium has taxonomic value (Gibson and Bray, 1979; Gibson, 2002h). Gibson (2002h) included a total of 5 subfamilies and 19 genera within the Lecithasteridae: Lecithasterinae (with the genera *Monorchiaponorus, Lecithaster, Lecithophyllum*, and *Aponorus*), Hysterolecithinae (including *Thulinia, Hysterolecitha, Hysterolecithoides*, and *Machidatrema*), Macradenininae (*Monorchimacradena, Dichadena, Neodichadena, Acanthuritrama, Macradenina*, and *Macradena*), Quadrifoliovariinae (comprising the genera *Unilacinia, Quadrifoliovarium*, and *Bilacinia*), and Trifoliovariinae (including *Trifoliovarium* and *Assitrema*), with *Lecithaster* serving as the type genus. Subfamilies are mainly differentiated by characters such a as the presence or absence of a uterine vesicle, blind seminal vesicle, and sinus sac, the structure of the uterus and the hermaphroditic duct, or the position of the seminal vesicle (Gibson, 2002h).

Family Ptychogononimidae Dollfus, 1937

This is a small family of hemiuroids that contains only 2 genera, *Ptychogononimus* and *Melagonimus*, which are differentiated by the presence of a uroproct, the position of the uterus, and the structure of the wall of the genital atrium (Gibson, 2002h). Ptychogononimidae is characterized by an unusual life cycle in that its members use a scaphopod as the first intermediate host and transmission to the second intermediate host is affected by means of a motile parthenita (a unisexual stage in an intermediate host).

Family Sclerodistomidae Odhner, 1927

The family Sclerodistomidae is a controversial and small group of trematodes that are generally parasites of the gut (mainly the stomach) and are occasionally found in the body cavities of marine teleosts. This family only contains 4 subfamilies and 5 genera: Sclerodistominae (with the genus *Sclerodistomum*), Kenmackenziinae (including *Kenmackenzia*), Prosogonotrematinae (containing the genus *Prosogonotrema*), and Prosorchiinae (comprising *Prosorchis* and *Prosorchipsis*). Probably the most relevant feature of the members of this family is the presence of 1 or 2 Manter's organs (an accessory excretory organ) which occurs dorsal to the excretory vesicle, into which it opens posteriorly (Gibson, 2002i). Subfamilies are differentiated on the basis of the structure of Manter's organ, and the position of the testes and ovary. The type genus is *Sclerodistomum* (Gibson, 2002i).

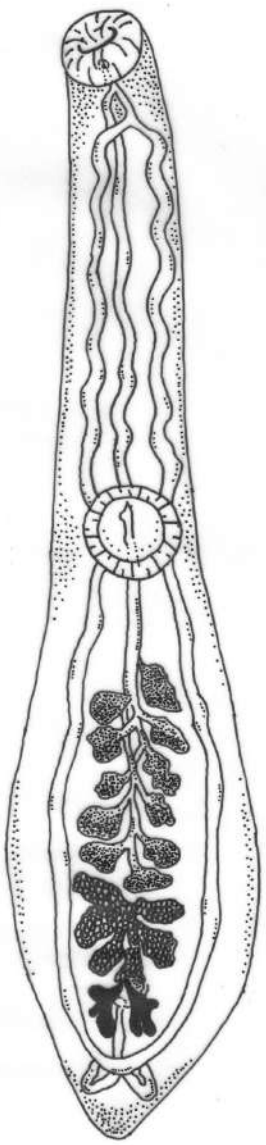

Figure 4. General scheme of an adult specimen of *Syncoelium* sp. (Syncoeliidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

Family Sclerodistomodiidae Gibson and Bray, 1979

The family Sclerodistomodiidae was established by Gibson and Bray (1979) to allocate *Sclerodistomoides pacificus*, which is currently the only species in the family. These authors considered the genus *Sclerodistomoides* to be different from other hemiuroids based on the structure of the pharynx, the absence of Manter's organ, and the orientation of the main collecting ducts of the vitelline system (Gibson and Bray, 1979; Gibson, 2002i).

Family Syncoeliidae Looss, 1899

The Syncoeliidae is a marine family of robust digeneans with 11 species distributed across 2 subfamilies (Syncoeliinae and Otiotrematinae) and 4 genera, including: *Syncoelium* and *Copiatestes* (belonging to Syncoeliinae) and *Otiotrema* and *Paronatrema* (included in Otiotrematinae), with *Syncoelium* which is the type genus (Figure 4). Subfamilies are differentiated on the basis of the structure of the ovary and vitellarium (Gibson, 2002k). This family is closely related to Hirudinellidae (Calhoun et al., 2013). Adults of Syncoeliidae are usually found associated with the gills, stomach, or buccal cavity of elasmobranchs or teleosts (Gibson and Bray, 1979; Gibson, 2002k; Curran and Overstreet, 2000). Pelagic, benthopelagic, and benthic fishes serve as definitive hosts and the metacercaria for a syncoeliid species (Calhoun et al., 2013). Since many of the 11 species of syncoelliids use definitive hosts that occur in benthic or bentho-pelagic habitats, it is likely that vertical migration of crustaceans or the use of paratenic hosts may play a role in the life history of the Syncoeliidae.

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41

Digenea, Plagiorchiida

Monorchiata (Suborder): Two Families Separated by Salinity

Nicholas Q.-X. Wee

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Suborder Monorchiata

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 41

Monorchiata Olson et al., 2003 (Suborder): Two Families Separated by Salinity

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Introduction

The Monorchiata Olson et al., 2003 is a speciose suborder of digenean trematodes parasitizing fishes as adults. It contains only 1 superfamily, the Monorchioidea Odhner, 1911, which comprises the families Monorchiidae Odhner, 1911 and Lissorchiidae Magath, 1917. Most species from the 2 groups have a single testis, which separates them from many other trematode groups, and they also generally have a spinous tegument and restricted vitellaria. The Monorchiidae was established for *Monorchis monorchis* (Stossich, 1890) and presently comprises 258 species in 48 genera (Gibson and Cribb, 2010). Magath (1917) proposed the subfamily Lissorchiinae for *Lissorchis fairporti* Magath, 1917, under the family Plagiorchiidae Lühe, 1901. Poche (1926) subsequently elevated the group to family status. The Lissorchiidae comprises only 43 species in 8 genera (Bray, 2008).

Monorchiids and lissorchiids are differentiated by 6 key traits: 1) Infection of marine versus freshwater fishes; 2) oculate (with eye spots) versus non-oculate cercariae; 3) infection of bivalve versus gastropod first intermediate hosts; 4) cercariae development in sporocysts versus rediae; 5) having a median versus lateral genital pore; and 6) having a complex metraterm with a specialized terminal organ versus simple metraterm (Shimazu, 1992). Although phylogenetic analyses clearly indicate that they are sister taxa, the 2 families are so distinct that they are dealt with separately here.

Family Monorchiidae

Monorchiids infect marine bony fishes from over 70 families. They normally have a spinous tegument, complex and spined male (cirrus sac) and female (terminal organ) terminal genitalia, and restricted fields of vitelline follicles (Madhavi, 2008) (Figure 1). In many monorchiids, the female terminal organ is bipartite, comprising an unspined posterior chamber and a spined anterior tubular section. The posterior chamber usually contains a fibrous mass that has been suggested to be remnants of prostatic secretions (Dove and Cribb, 1998). The genus *Cableia* Sogandares-Bernal, 1959, is exceptional; species of this group lack the major morphological characters of the family and it may be a basal monorchiid genus. Despite the name of the family referring to a single testis, monorchiids can also have 2 or 8 testes.

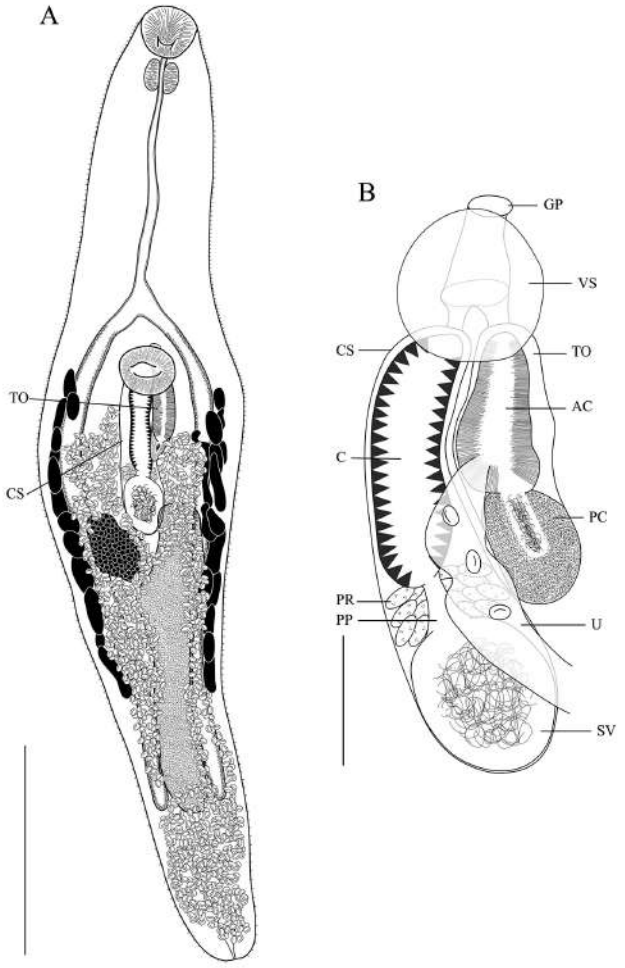

Figure 1. A typical monorchiid, *Parachrisomon delicatus* (Manter & Pritchard, 1964) Madhavi, 2008. A) Whole worm, ventral view; B) Terminal genitalia, ventral view. Abbreviations: AC: Anterior chamber; C: Cirrus; CS: Cirrus sac; GP: Genital pore; PC: Posterior chamber; PP: Pars prostatica; PR: Prostatic cells; SV: Seminal vesicle; TO: Terminal organ; U: Uterus; VS: Ventral sucker. Scale bars: A) 500 µm; B) 100 µm. Source: N. Q.-X. Wee. License: CC BY-NC-SA 4.0.

Systematics and Taxonomy

Uncertainty about which morphological features are useful in differentiating monorchiid taxa has led to issues with

the systematics of the family. The composition of some large genera, such as *Lasiotocus* Looss, 1907 and *Genolopa* Linton, 1910, is doubtful, given the broad morphological variation in features such as body shape and the shape of the oral sucker among species. The need for revision of such genera and has been demonstrated by recent phylogenetic analyses (Cribb et al., 2018; Wee et al., 2018), showing that sequenced species of *Lasiotocus* are not monophyletic. Phylogenetic analyses also demonstrate the need to revise the subfamilial characterization of the family. Sequenced representatives of *Helicometroides* Yamaguti, 1934, *Hurleytrematoides* Yamaguti, 1953 and *Provitellus* Dove & Cribb, 1998, which are united in having filamented eggs (eggs with filaments on the polar parts of the egg) and as such putatively belong to the subfamily Hurleytrematinae Yamaguti, 1958, according to Madhavi (2008), are only distantly related to each other.

Life Cycles

Most monorchiids have 3-host life cycles (Figure 2). As presently known, complete monorchiid life cycles incorporate bivalves (specifically, pelecypod molluscs) as the first intermediate hosts (Cremonte et al., 2001). Mother sporocysts develop in the visceral mass of the bivalve as simple sacs. They produce daughter sporocysts, which in turn produce cercariae. The cercariae usually leave the first intermediate host in search of the second intermediate host (Gilardoni et al., 2013). Monorchiid cercariae all have a spinous tegument but otherwise are highly varied, with 4 distinct forms: 1) Fusiform body, short tail; 2) elongate body, short, bifurcated tail; 3) fusiform to elongate body, long tail; and 4) elongate body, minute tail (Figure 3). Cercariae with long tails swim in search of a second intermediate host, whereas cercariae with short tails, especially those with minute tails, crawl on the substrate or adhere to a particle, to be picked up by the next intermediate host (Stunkard, 1981a; 1981b).

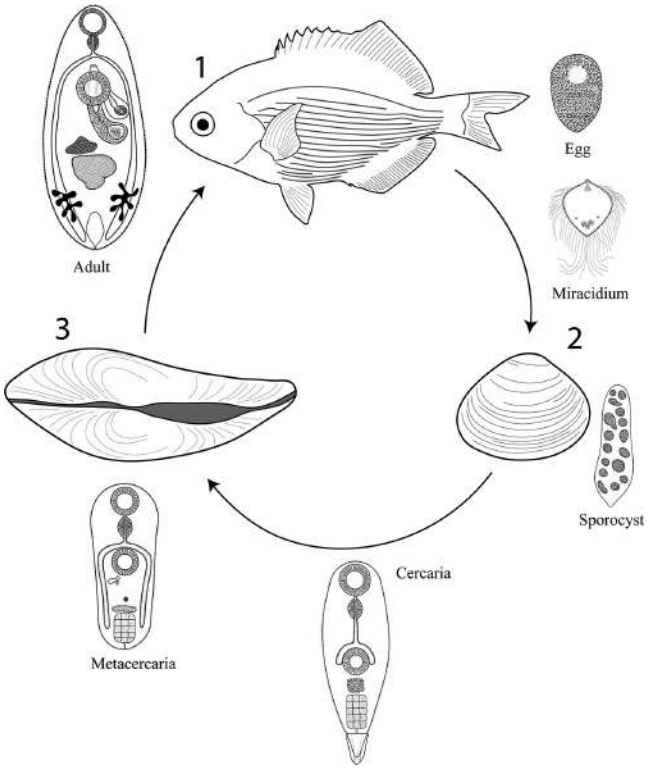

Figure 2. Life cycle of *Telolecithus pugetensis* Lloyd & Guberlet, 1932 (Monorchiidae). Source: modified from DeMartini and Pratt, 1964. License: CC BY-NC-SA 4.0.

All known second intermediate hosts are also bivalves. Once in contact with an exposed part (such as a foot, or mantle) of a bivalve, the cercariae adhere via their tails, penetrate, shed their tail, encyst in the host tissue, and await ingestion by the definitive fish host.

Although all known second intermediate hosts are bivalves, the broad range of diets of fishes that harbor monorchiids, suggesting that other groups might also be exploited. For example, species of *Hurleytrematoides* mainly infect chaetodontids (butterflyfishes), which rarely consume bivalves. Instead, chaetodontids eat a broad range of organisms such as copepods, sponges, polychaetes, and corals (Sado, 1989). Thus, their metacercariae probably infect 1 of these organisms as second intermediate hosts.

It has also been suggested and shown experimentally shown that some monorchiids might use a carnivorous invertebrate as the second intermediate host (Stunkard, 1981a; Gilardoni et al., 2013). Stunkard (1981a; 1981b) described the metacercariae of some monorchiid species as being embedded in a thick-walled cyst or jelly-like matrix that can be shed into the environment. The cyst is then suspended and floats in seawater or sinks to the bottom, awaiting ingestion by the second intermediate host.

Some monorchiids have abbreviated life cycles that include only 2 hosts. For these species, cercariae encyst and develop into metacercariae inside the sporocyst within the first intermediate host (Stunkard, 1981b; 1981a; Cremonte et al., 2001; Gilardoni et al., 2013; Bagnato et al., 2016). It has been suggested that environmental stresses drive the evolution of an abbreviated life cycle that ensures that transmission of the parasite (Poulin and Cribb, 2002; Bagnato et al., 2016).

Biogeography

The biogeographical patterns of monorchiids are poorly understood. Only 1 study (McNamara et al., 2012) has explored monorchiid distributions in detail, examining 18 species of *Hurleytrematoides* infecting 45 species of chaetodontid fishes from 6 sites across the tropical Indo-West Pacific. Seven of these species were found at just 1 locality, 11 were

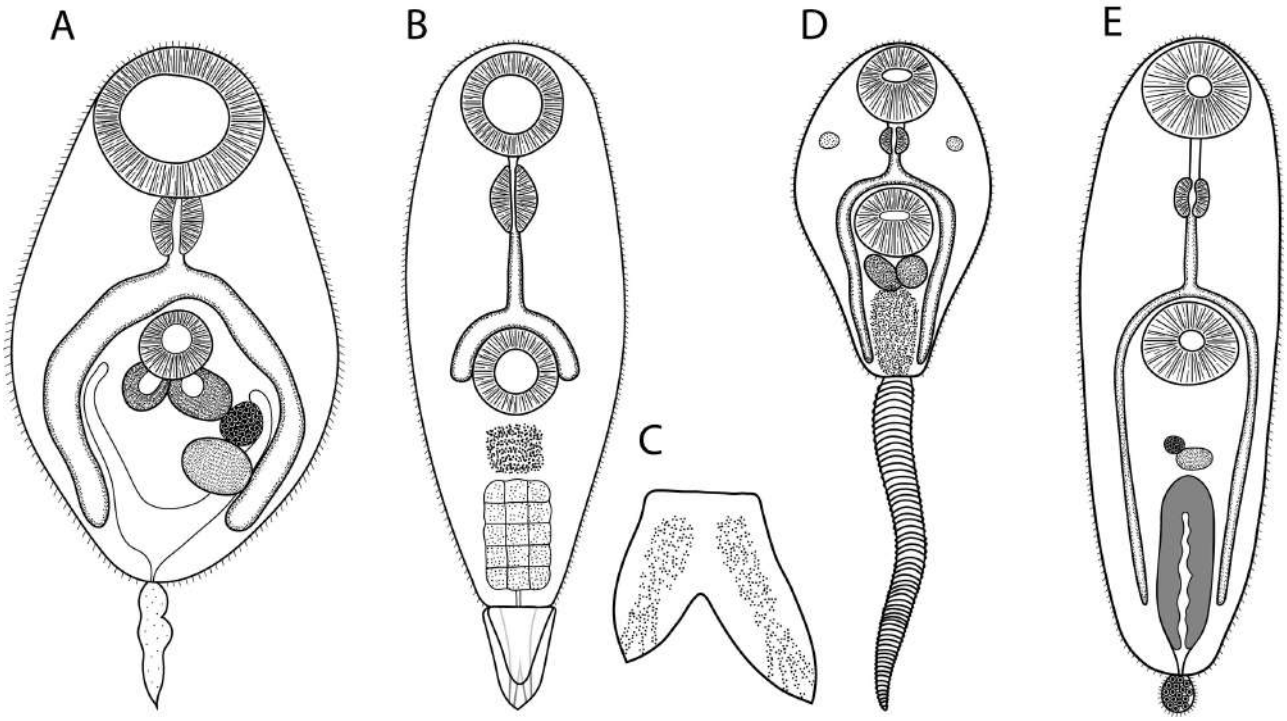

Figure 3. Monorchiid cercariae showing different body forms. A) *Monorchis parvus* Looss, 1902; B) *Telolecithus pugetensis* Lloyd & Guberlet, 1932; C) Bifurcated tail of *T. pugetensis* cercariae; D) *Paratimonia gobii* Prévot & Bartoli, 1967; E) *Proctotrema bartolii* Carballo, Laurenti & Cremonte, 2011. Source: Adapted from Bartoli et al., 2000; DeMartini and Pratt, 1964; Maillard, 1975; Gilardoni et al., 2013.

found at multiple locations, and just 1 was found at all 6 sites. They suggested that species of monorchiids were not as widespread as their hosts due to their limited dispersal capabilities. Adult monorchiids parasitize adult fishes, which are site-attached to reefs but have long-lived pelagic larvae that enable widespread distribution. In contrast, the larval stages of monorchiids are unable to survive for an extended period outside their hosts. This discrepancy in dispersal ability probably plays a role in the unequal distribution of these parasites and their hosts.

Family Lissorchiidae

Species of Lissorchiidae infect freshwater fishes, most of which are cypriniforms (carp, loaches, minnows, and relatives). They are known from the Nearctic, Palaearctic, India, and Southeast Asia. Lissorchiids resemble monorchiids in having a spinous tegument, a spined ejaculatory duct, and restricted fields of vitelline follicles. However, lissorchiids have a laterally orientated genital pore and have a simple spined or unspined metraterm, but they lack a complex terminal organ (Bray, 2008) (Figure 4).

Systematics and Taxonomy

Magath (1917) proposed the subfamily Lissorchiinae for *Lissorchis*, stating that the group either belonged in the Plagiorchiidae Lühe, 1901, or that it required full family status. Subsequently, Poche (1926) found evidence that the Lissorchiinae does not belong to the Plagiorchiidae and raised it to the family level.

Classification within the family has been primarily based on morphology. Some species previously thought to be lissorchiids now belong to other families; for instance, species of *Anarchichotrema* Shimazu, 1973 and *Neolissorchis* Machida, 1985 are now considered to belong to the Zoogonidae. Additionally, some lissorchiid genera, such as *Asymphylodora* Looss, 1899 and *Palaeorchis* Szidat, 1943, generally resemble monorchiids, which hindered their recognition as lissorchiids. Finally, the validity of some lissorchiid species such as *Tigrotrema gwailiorense* Bhadauria & Dandotia, 1984 are doubtful as their morphological characters are not typical for the family (Bray, 2008). It appears that molecular sequencing will be necessary to resolve the classification within the family. However, few sequences are currently available.

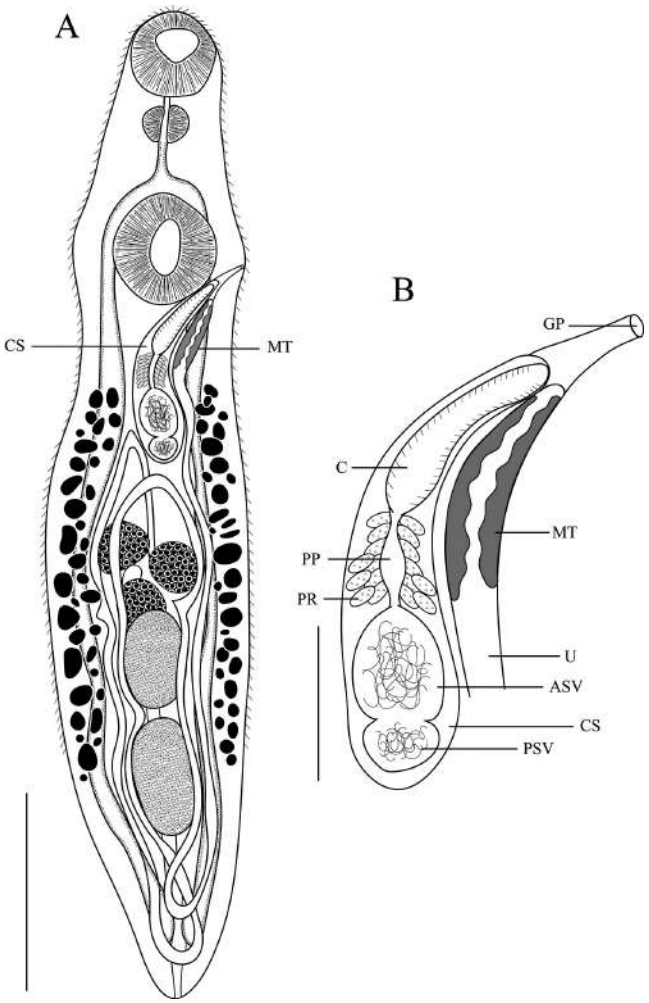

Figure 4. A typical lissorchiid, *Lissorchis hypentelii* (Fischthal, 1942). A) Whole worm, ventral view; B) Terminal genitalia, ventral view. Abbreviations: ASV: Anterior seminal vesicle; C: Cirrus; CS: Cirrus sac; GP: Genital pore; MT: Metraterm; PP: Pars prostatica; PR: Prostatic cells; PSV: Posterior seminal vesicle; U: Uterus. Scale bars: A) 400 µm; B) 200 µm. Source: Adapted from Fischthal, 1942. License: CC BY-NC-SA 4.0.

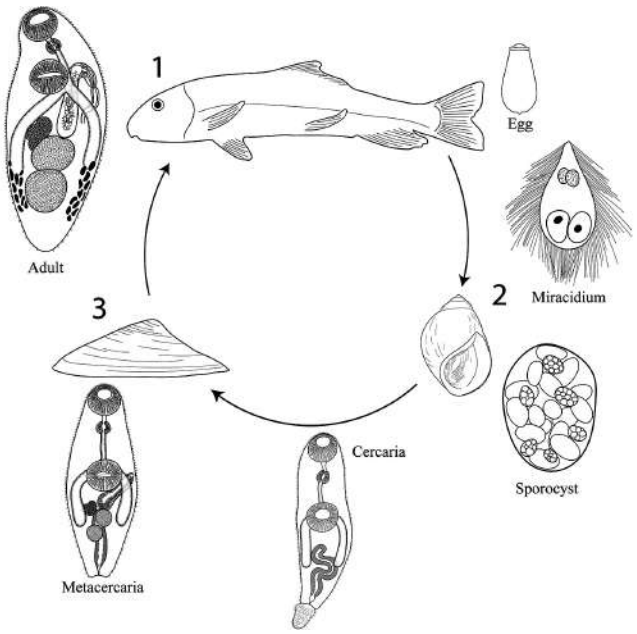

Figure 5. Life cycle of *Neopaleorchis catostomi* Schell, 1973 (Lissorchiidae). Source: Adapted from Schell, 1973. License: CC BY-NC-SA 4.0.

Life Cycles

Lissorchiids have a 3-host life cycle (Figure 5). To date, in 6 studies complete life cycles have been elucidated, all of which report gastropods as the first intermediate hosts (Wallace, 1941; Stunkard, 1959; Schell, 1973; Macy and English, 1975; Našincová and Scholz, 1994; Besprozvannykh et al., 2012).

Mother sporocysts in the first intermediate host produce rediae in which cercariae develop. Gastropods, insect larvae, planarians, oligochaetes, and fishes have all been reported as second intermediate hosts. The cercariae penetrate the second intermediate host and encyst in thin-walled membranes. Similar to the Monorchiidae, lissorchiid cercariae have a spinous

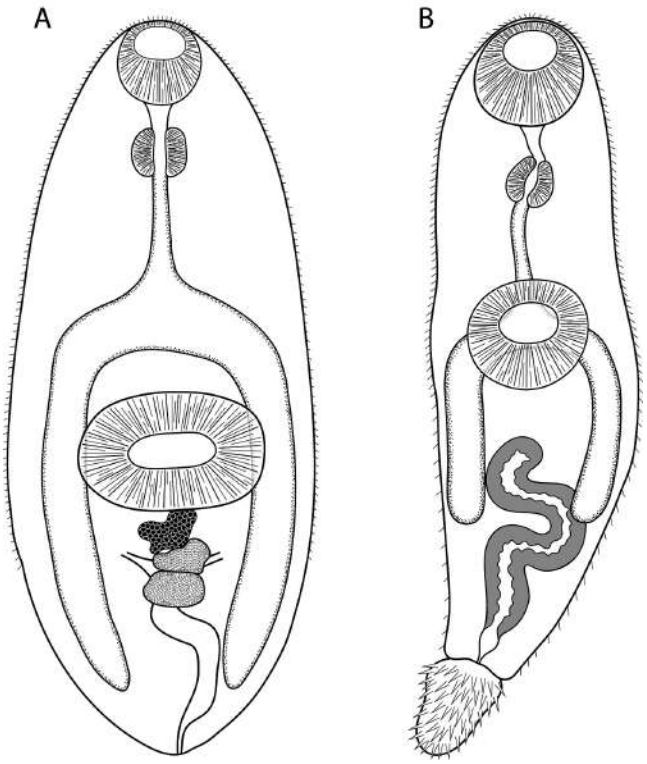

Figure 6. Lissorchiid cercariae showing different body shapes. A) *Lissorchis mutabile* (Cort, 1918); B) *Neopaleorchis catostomi* Schell, 1973. Source: Adapted from Wallace, 1941; Schell, 1973. License: CC BY-NC-SA 4.0.

tegument and also exhibit variation in overall tail morphology; some lack a tail completely, whereas others have a short, spined and knobbed tail (Figure 6).

Some lissorchiids have a truncated life cycle. *Palaeorchis problematicus* (Macy and Berntzen, 1970) and *Asymphylodora tincae* (Modeer, 1970) have a 2-host life cycle, infecting only a freshwater snail and a cyprinid (Macy and English, 1975; Našincová and Scholz, 1994). Experimental infections of *A. tincae* showed that cercariae harbored by gastropods that were fed to fishes developed into adults without a metacercarial stage (Našincová and Scholz, 1994).

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Digenea, Plagiorchiida

Opisthorchis (Genus)

US CDC, Division of Parasitic Diseases and Malaria

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Family Opisthorchiidae

Genus *Opisthorchis*

doi:10.32873/unl.dc.ciap042
2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 42

Opisthorchis (Genus)

United States Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria

Introduction

Opisthorchis sp. are liver fluke parasites (trematodes) that humans can get by eating raw or undercooked fish from areas in Asia and Europe where the parasite is found, including Thailand, Laos, Cambodia, Vietnam, Germany, Italy, Belarus, Russia, Kazakhstan, and Ukraine. *Opisthorchis viverrini* is known as the Southeast Asian liver fluke and *O. felineus* as the cat liver fluke.

Original Description and Taxonomy

Class Trematoda Rudolphi, 1808
Subclass Digenea Caru, 1863
Order Plagiorchiida La Rue, 1957
Suborder Opistorchiata La Rue, 1957
Superfamily Opistorchioidea Looss, 1899
Family Opisthorchiidae Looss 1899
Subfamily Opisthorchiinae Looss, 1899
Genus *Opisthorchis*

The original description of the genus may be found in Blanchard (1895). See King and Scholz (2001) for a detailed discussion of the classification and taxonomy of the family Opisthorchiidae, and Scholz (2008) for a presentation of

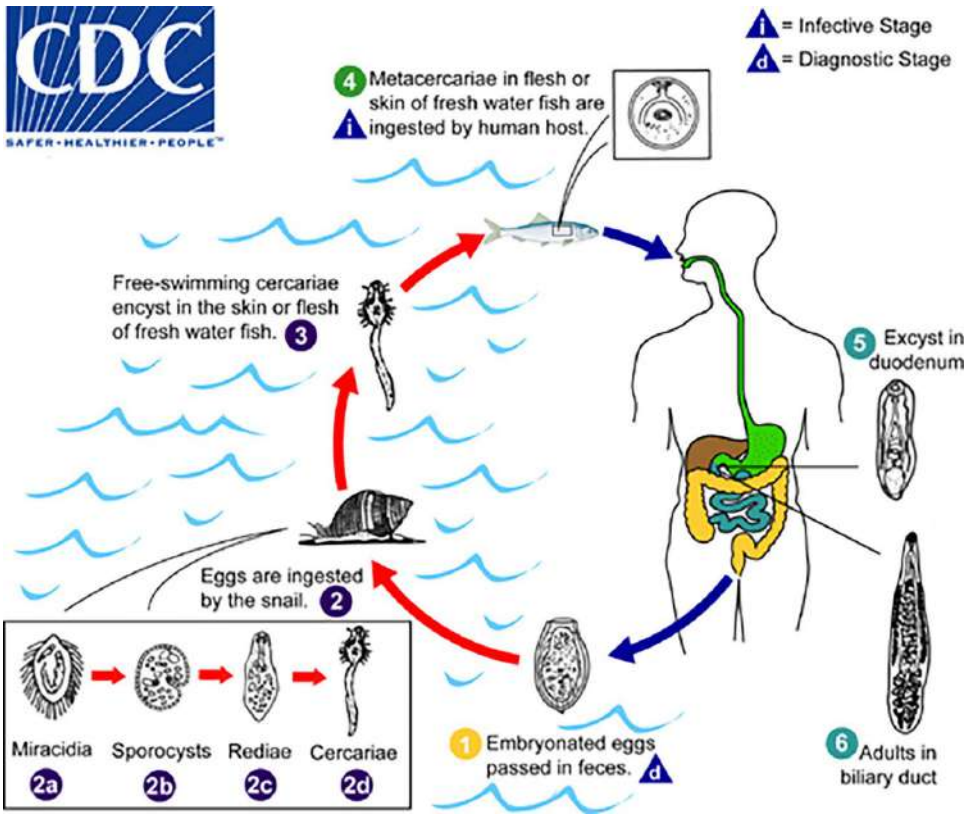

Figure 1. The adult flukes deposit fully developed eggs that are passed in the feces (1). After ingestion by a suitable snail (first intermediate host) (2), the eggs release miracidia (2a), which undergo in the snail several developmental stages (sporocysts (2b), rediae (2c), cercariae (2d)). Cercariae are released from the snail (3) and penetrate freshwater fish (second intermediate host), encysting as metacercariae in the muscles or under the scales (4). The mammalian definitive host (cats, dogs, and various fish-eating mammals including humans) become infected by ingesting undercooked fish containing metacercariae. After ingestion, the metacercariae excyst in the duodenum (5) and ascend through the ampulla of Vater into the biliary ducts, where they attach and develop into adults, which lay eggs after 3 to 4 weeks (6). The adult flukes (*Opisthorchis viverrini*: 5 mm to 10 mm by 1 mm to 2 mm; *O. felineus*: 7 mm to 12 mm by 2 mm to 3 mm) reside in the biliary and pancreatic ducts of the mammalian host, where they attach to the mucosa. United States Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria (DPDx), 2018. Public domain.

the classification of subfamilies, including Opisthorchiinae Looss, 1899 (which includes the genus *Opisthorchis*; Gibson et al. 2021), as well as the others in the family: Allogomtiotrematinae, Delphinicolinae, Diasiellinae, Metorchiinae, Microtrematinae, Oesophagicolinae, Pachytrematinae, Plotnikoviinae, Pseudamphistominae, Ratziinae, Tubangorchiinae, and Witenbergiinae. A phylogenetic tree for some of these groups may be found in Waikagul and Thaenkham (2014).

Medical Importance

Members of the family Opisthorchiidae are known parasites of mammals, birds, fish, and reptiles. Liver flukes of the genus *Opisthorchis* may infect the liver, gallbladder, and bile duct in humans. While most infected persons do not show any symptoms, infections that last a long time can result in severe symptoms and serious illness, including cancers.

Untreated, infections may persist in humans for up to 25–30 years, the lifespan of the parasite. Typical symptoms include indigestion, abdominal pain, diarrhea, or constipation. In severe cases, abdominal pain, nausea, and diarrhea can occur. *Opisthorchis felineus*, in addition to presenting with the typical symptoms also seen in *O. viverrini* infections, can present with fever, facial swelling, swollen lymph glands, sore joints, and rash—similar to the signs and symptoms of schistosomiasis. Chronic *O. felineus* infections may also involve the pancreatic ducts.

Diagnosis of *Opisthorchis* infection is based on microscopic identification of parasite eggs in stool specimens. Safe and effective medication is available to treat *Opisthorchis* infections. Adequately freezing or cooking fish will kill the parasite.

Life Cycle

The eggs of *Opisthorchis viverrini* are ingested by snails in fresh water. After the eggs hatch, infected snails will release microscopic larvae that can enter freshwater fish. People become infected when eating raw or undercooked fish that contains the parasite. After ingestion, the liver flukes grow to adult worms that live inside the human bile duct system. The life cycle takes 3 months to complete in humans. Infected people will then pass eggs in their stool or may cough them up (see Figure 1 for a life cycle diagram).

Acknowledgement

This section includes a very brief introduction to the genus *Opisthorchis*, adapted from material in the public domain on the United States Centers for Disease Control and Prevention website as well as the other cited sources.

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Digenea, Plagiorchiida

Xiphidiata

Allocreadiidae Looss, 1902 (Family)

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Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Family Allocreadiidae

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Chapter 43

Allocreadiidae Looss, 1902 (Family)

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Introduction

Allocreadiids are digeneans mainly found as parasites of the digestive tracts of freshwater fishes. The taxonomy and classification system of the family Allocreadiidae has been controversial. The detailed taxonomic history of the family within the Digenea was revised in great detail by Caira and Bogéa (2005). Due to work by helminthologists interested in top-down systematics of these trematodes, species composition, the validity of the genera, and the taxonomic arrangement within subfamilies have fluctuated over time. Some species included in the genera *Bunodera, Bunoderella, Crepidostomum, Megalogonia, Creptotrema, Creptotrematina*, and *Auriculostoma* possess muscular papillae associated with the oral sucker, which led Sewell Hopkins (1934) to coin the term "papillose allocreadiids." This concept, of synapomorphies in the group was developed by Hopkins (without him knowing the term synapomorphy) was modified much later by Caira (1989) who referred only to the North American forms and did not consider other allocreadiids such as species of *Creptotrema* and *Creptotrematina* occurring mainly in South American freshwater fishes, which also possess these structures. It is not known if these muscular papillae, more correctly called oral lobes, are homologous in all allocreadiids, since oral lobes are also found in other unrelated genera of digeneans; for example, in species of the lepocreadiid genus *Enenterum*. Furthermore, it is now widely accepted that species of the Allocreadiidae comprise forms with and without these muscular oral lobes which may show up with scanning electron microscopy (Figures 1 and 2). The currently accepted classification of the family does not consider subfamilies as a taxonomic category based on the presence or absence of these traits because they do not represent natural groups (Gibson, 1996; Caira, 1989).

Members of the Allocreadiidae include digeneans commonly found, as adults, in the digestive tract of freshwater fishes, and only 2 species (allocated in monotypic genera: *Caudouterina rhyacotritoni* and *Bunoderella metteri*) are found, respectively, in salamanders or frogs in the United States (Schell, 1964; Martin, 1966). After their description in the 1960s, these 2 species have not been reported again. The main morphological traits of allocreadiids are well described in the diagnoses of Yamaguti (1971) and Caira and Bogéa (2005), although the species and groups differ between these 2 taxonomic treatments. The reader must refer to these 2 references for a detailed taxonomic description of the family. Following is a brief morphological characterization of the Allocreadiidae.

Main Morphological Characteristics

Allocreadiids are digeneans with an elongate or oval body, lacking spines on the tegument. Eye spots, fully developed or as remnants, might be observed on the ventral surface of some species. Muscular oral lobes, variable in number and shape are present in some species (Figure 2).

Most species of allocreadiids possess long cecae extending to the level of the posterior testis, or to the posterior end of body; they possess 2 testes, smooth, slightly, or deeply lobated, situated in tandem, oblique, or symmetrical. The well-developed cirrus sac contains an internal seminal vesicle; they lack an external seminal vesicle. The genital pore is located anterior to the ventral sucker, immediately posterior to or at level of, intestinal bifurcation, occasionally pre-bifurcal, between the intestinal bifurcation and the pharynx. The ovary is smooth, round to pyriform, and pre-testicular. The uterus is entirely pre-testicular in most species of allocreadiids (Figure 3), but in some species, uterine coils can extend to the posterior extremity of the body. The eggs are variable in size and number, and they lack spines or filaments. The vitelline follicles are located in the lateral fields of the body, and their

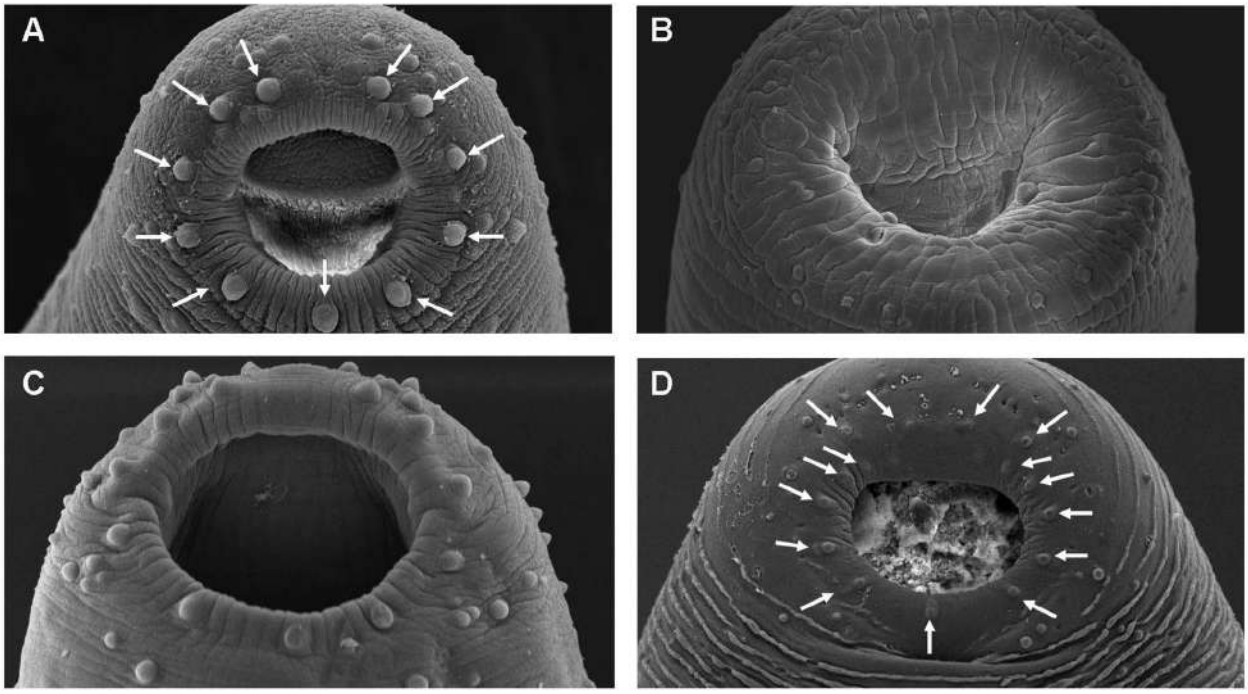

Figure 1. Scanning electron microscopy microphotographs of 4 species of allocreadiids lacking muscular oral lobes on the oral sucker. A) *Margotrema resolanae* from *Xenotaenia resolanae*. Note the arrows pointing to 15 dome-like papillae; B) *Wallinia mexicana* from *Astyanax mexicanus*; C) *Pseudoparacreptotrema macroacetabulata*; D) *Allocreadium isoporum* from *Capoeta* sp. Note the arrows pointing to 11 well-developed dome-like papillae. Sources: A) Adapted from Aydogdu et al., 2018. B) G. Pérez-Ponce de León, D. I. Hernández-Mena, and B. Solórzano-García. C) Adapted from Pérez-Ponce de León et al., 2016. D) Adapted from Pérez-Ponce de León et al., 2013. License for all: CC BY-NC-SA 4.0.

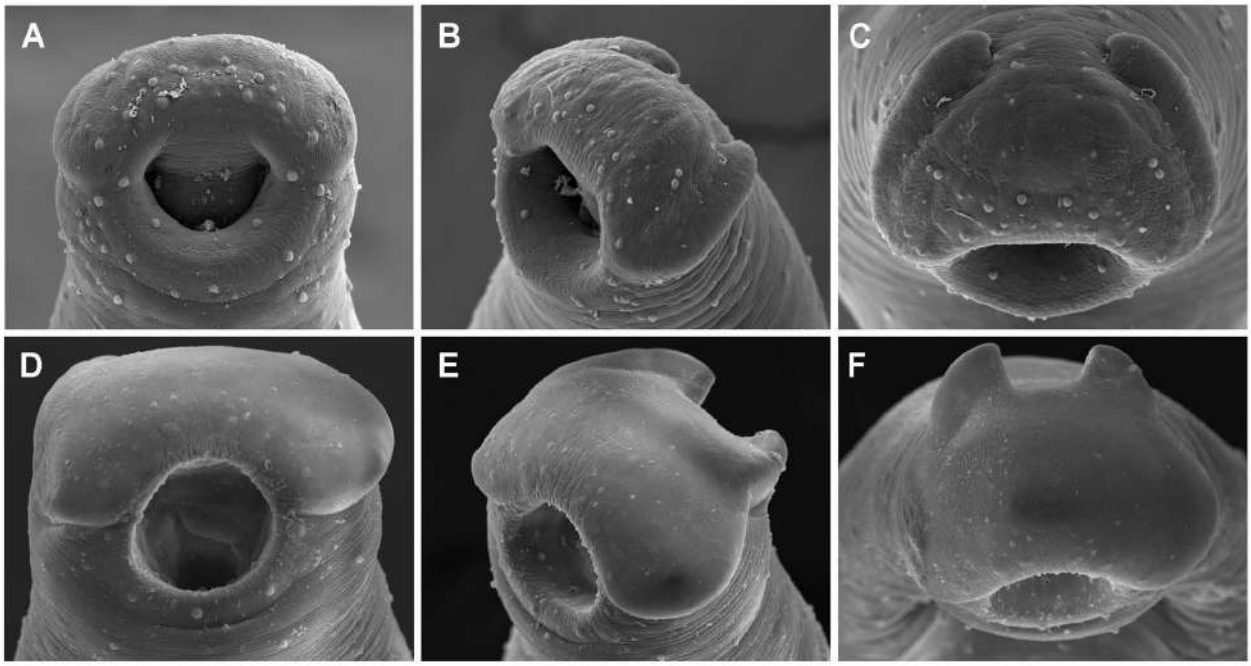

Figure 2. Scanning electron microscopy microphotographs of 2 species of allocreadiids possessing muscular oral lobes on the oral sucker (frontal, lateral, and anterior views); A–C) *Auriculostoma totonacapenensis* from *Astyanax mexicanus*; D–F) *A. lobata* from *Brycon guatemalensis*. Sources: A–C) Adapted from Razo-Mendivil et al., 2014; D–F) Adapted from Hernández-Mena et al., 2014. License for all: CC BY-NC-SA 4.0.

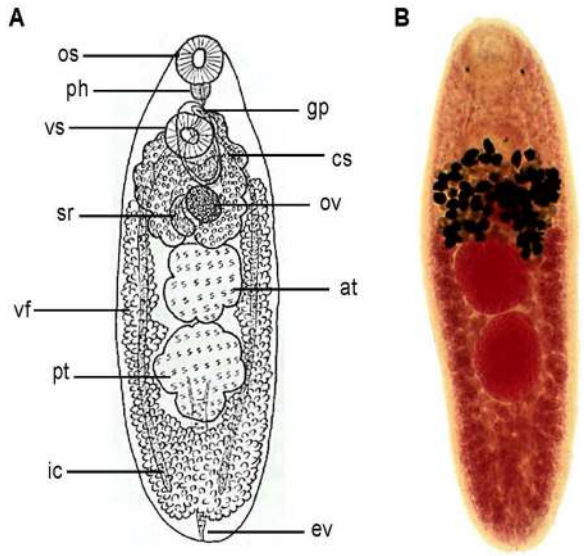

Figure 3. *Allocreadium lobatum*, ventral view. A). Line drawing; B) Microphotograph of a stained specimen from *Semotilus atromaculatus*. Note the pretesticular position of the uterus (eggs hydrated), and the eye spots on the posterior border of the oral sucker. Sources: A) Adapted from Hoffman, 1999; B) A. Choudhury. License: CC BY-NC-SA 4.0.

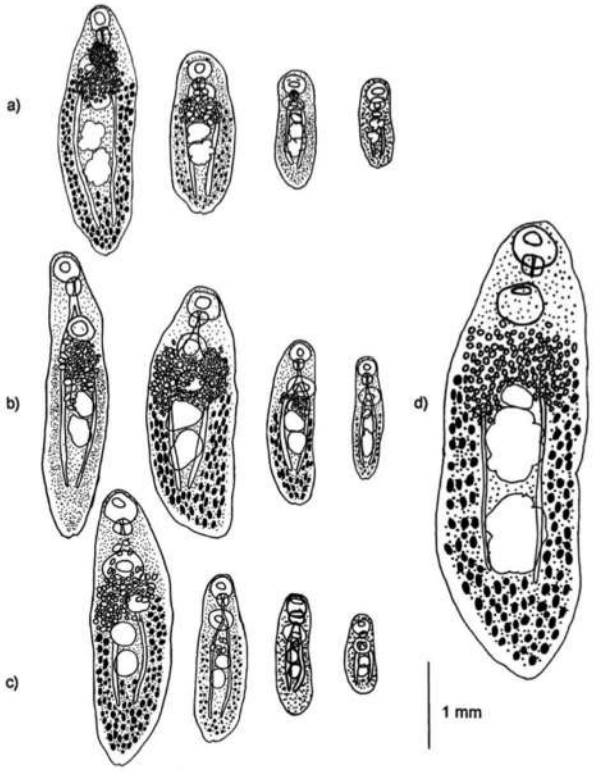

Figure 4. Morphological types in descending size classes of *Allocreadium lobatum*: a) lobate; b) asymmetrical; c) round; and d) syntype. Sources: Willis, 2002: a) Left to right, HWML slides 35119 (37-5), 35119 (37-4), 35117 (24-3), 35120 (53-8); b) Left to right, slides 35126 (79-2), 35114 (1-10), 35127 (84-2), 35139 (139-3); c) Left to right, slides 35117 (20-1), 35139 (140-1), 35117 (21-13), 35139 (151-7); d) slide 35114 (1-5). License: CC BY-NC-SA 4.0.

distribution is variable, sometimes extending the full length of the body, or sometimes being restricted anteriorly by the ventral sucker or posteriorly by the testes. The excretory vesicle is I-shaped. The type genus of the family is *Allocreadium*, with around 70 species described worldwide associated mainly with cyprinid fishes.

Morphological Variability

Some species of allocreadiids exhibit a wide geographical and host range, and some are endemic to certain localities and/or host species. This has led to the recognition of polymorphic species in this group. For example, one of the North American species, *Allocreadium lobatum*, was originally described by Wallin (1909) as a parasite of the fallfish *Semotilus corporalis* (Cyprinidae) at Sebago Lake, Maine, United States. In the original description, the presence of lobed testes was the diagnostic character that differentiated it from the European species, *A. isoporum* (the type species of the genus, commonly found in cyprinids). Willis (2002) studied 636 individuals of *A. lobatum* from the creek chub, *S. atromaculatus* collected in Nebraska, United States, and demonstrated that the shape of the testes in this digenean lies in a continuum from round to lobate and represents intraspecific morphological variation (Figure 4). Not only did Willis (2002) demonstrate the intraspecific variability of this morphological trait, he also discussed 3 possible reasons for this morphological variation in the testes including: Differences in mounting technique, species polymorphism, and the possible existence of new species. Because of this polymorphism, it is important to note that when workers identify specimens of *Allocreadium* from North America, the presence of lobated testes may or may not be diagnostic to the level of the species. Thus far, only 2 species are considered valid as parasites of freshwater fishes (Choudhury et al., 2016). *Allocreadium lobatum* is widely distributed throughout at least 12 states of the United States, and in 3 provinces of Canada (McDonald and Margolis, 1995; Hoffman, 1999; McAllister et al., 2014). The other species, *A. lucyae*, has only been recorded from cyprinids in Alabama, United States (Williams and Dyer, 1992; Hoffman, 1999).

Life Cycle

The life cycles of several species of allocreadiids included in the genera *Allocreadium, Crepidostomum, Bunoderella*, and *Bunodera* have been elucidated either by looking at the

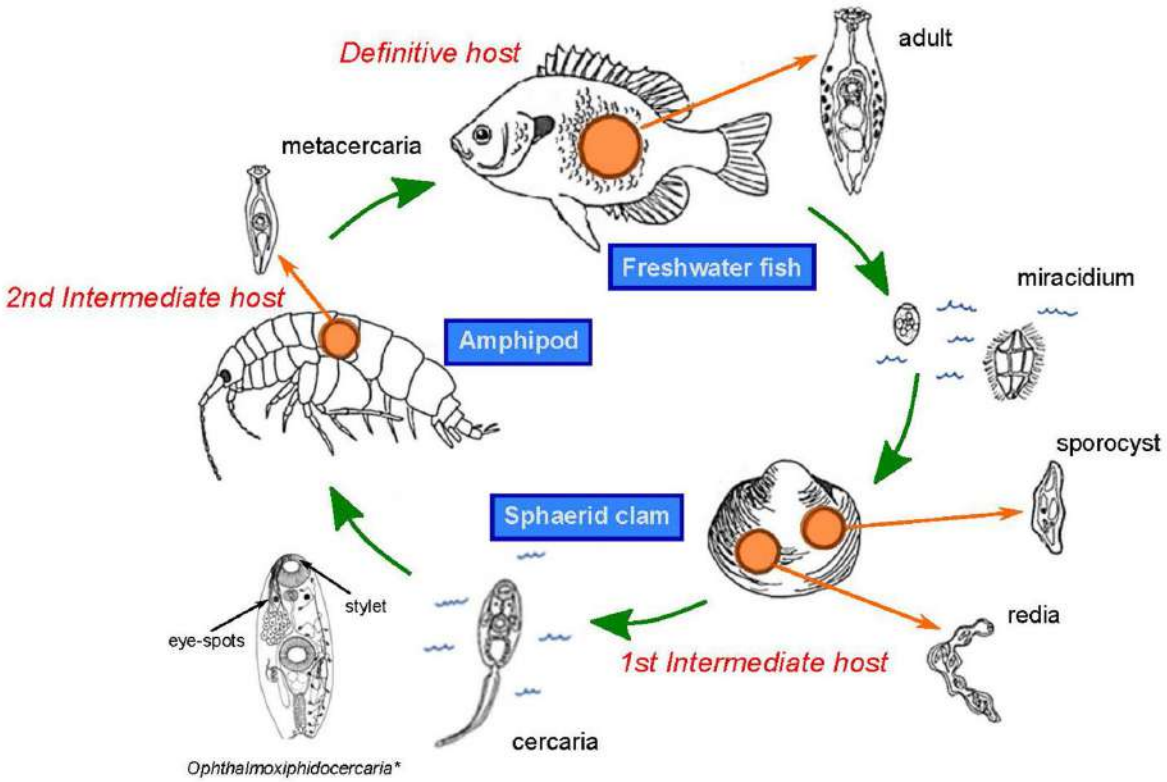

Figure 5. Generalized 3-host life cycle of an allocreadiid trematode. Sources: Adapted from Caira, 1989; Niewiadomska and Valtonen, 2007. License: CC BY-NC-SA 4.0.

natural infections of intermediate and definitive hosts or through experiments (see Yamaguti, 1975; Caira, 1989 and references therein). The general life cycle pattern for allocreadiids involves 3 hosts (Figure 5). The first intermediate host is usually a clam of the family Sphaeridae where the free-living miracidium penetrates to form either a sporocyst or a redia, or in some cases both. In some species, lymnaeid gastropods are the first intermediate host, as in *Crepidostomum metoecus* (see Awachie, 1968). Cercariae are characterized by having eye spots and a stylet, and because of that they are known as ophthalmoxiphidocercariae. They are released into the mantle cavity of the clams. Free-swimming cercariae exit the clam, search for, and penetrate the second intermediate hosts, usually an aquatic arthropod, where the cercariae loses their tails, encyst, and develop into a metacercariae. The definitive hosts, which are freshwater fishes, and in a few cases amphibians, are infected when they feed on infected arthropods; the metacercariae excyst in the digestive tract of the definitive host and the adult forms develop in the intestine. In some cases, the life cycle is truncated and the metacercariae reach maturity in the second intermediate hosts through a process known as progenesis. This phenomenon has been documented at least in 4 species of allocreadiids (Bray et al., 2012), 3 of *Allocreadium*, and 1 of *Crepidostomum* in which progenesis occurs in either crustaceans (decapods or amphipods) or in insects (coleopterans or ephemeropterans) (Lefebvre and Poulin, 2005).

The life cycles of 3 species of *Allocreadium* are relatively well-known, including that of the type species of the genus, *A. isoporum*, and also for those of both *A. lobatum* and *A. alloneotenicum* (see Yamaguti, 1975; Bray et al., 2012). In case of the type species, the mollusc acts as both the first and second intermediate host. The rediae develop in clams (*Sphaerium rivicola*), as the first intermediate host. Cercariae are released from the clam and encyst on the same clam where they develop into metacercariae. Cyprinids are infected when they feed on clams, and the adults develop in the intestine. In the life cycle of *A. lobatum*, commonly found in cyprinids in the United States and Canada, species of clams in the genus *Pisidium* act as the first intermediate host; in this case, cercariae are released from the clam into the water (DeGiusti, 1962). The second intermediate hosts are amphipods and isopods (see McAllister et al., 2014 and references therein). Fish are infected when they feed on these crustaceans (Yamaguti, 1975). The adults of *A. lobatum* also may develop progenetically in the haemocoel of amphipods (*Gammarus pseudolimneus* and *Crangonyx gracilis*).

Population Biology

The ecology of some species of allocreadiids has been studied to a certain extent in freshwater systems of the United States and Europe, particularly those of *Allocreadium* and *Crepidostomum*. For example, in Europe, the population dynamics of *A. isoporum* was studied by Moravec (1992), and more recently by Koyun and colleagues (2016) and Aydogdu and colleagues (2018). In the Danube River basin, the most important definitive host is the chub, *Leuciscus cephalus*, where *A. isoporum* exhibits a seasonal cycle of maturation characterized by quantitative changes in the abundance of young to mature worms having eggs in the uterus throughout the year (Moravec, 1992). This pattern is determined by ecological factors such as the temperature fluctuations in the locality. Aydogdu and colleagues (2018) discovered a seasonal dynamic throughout the year, when looking at the presence of *A. isoporum* in several species of cyprinids (*Capoeta* spp.) in Turkey, and found that the prevalence of infection varied with respect to host size and sex; prevalence was higher in males than females. In this case, changes in food composition and different biological characteristics between the sex of the hosts explains the difference (Koyun et al., 2016). In North America, the population dynamics of *A. lobatum* in the creek chub, *Semotilus atromaculatus,* was studied by several authors, for example, Camp (1989) and Willis (2001). In these studies, seasonal changes in the prevalence and/or mean intensity were reported in association with changes in parasite maturity. However, Willis (2001) reported seasonal changes in prevalence but not mean intensity of *A. lobatum*; the opposite pattern was found by Camp (1992). Also, an increase of mean intensity of *A. lobatum* as a function of host size was described. These studies demonstrate the complexity and diverse patterns in the population biology of allocreadiids, not only among species of the same genus, but also among populations within species. The population dynamics of some species of *Crepidostomum* have also been studied in Europe and North America, for example, *C. metoecus* and *C. farionis* in salmonids of north Wales (Awachie, 1968), *C. cooperi* in their second intermediate hosts, the burrowing mayfly, *Hexegenia limbata* in Michigan, United States (Esch et al., 1986), and *Crepidostomum* spp. in *Hexagenia* spp. in the Great Lakes in the United States and Canada (Scholoesser, 2005).

Phylogenetic Relationships

A taxonomic assessment of the family Allocreadiidae was conducted by Caira and Bogéa (2005). A cladistics analysis using morphological characters was conducted earlier by Caira (1989) and Caira and Bogéa (2005). Morphology was not very useful to assess the interrelationships among members of the family, since unresolved polytomies were recovered after the parsimony analyses. Significant progress has been made on the evolutionary history, classification, and historical biogeography of this group of digeneans. Molecular tools and scanning electron microscopy have provided useful information to expand the knowledge about the family. For the purposes of contributing data to this chapter of the textbook, the authors conducted a new molecular phylogenetic analysis of the Allocreadiidae based on 28S rDNA sequences; 1 representative sequence of each allocreadiid species allocated in 12 of the 14 genera was used. The genetic library for this molecular marker in allocreadiids has increased steadily in the last decade, a tendency observed for all digeneans (Pérez-Ponce de León and Hernández-Mena, 2019). Nevertheless, no sequence data have been produced for 2 allocreadiids, *Caudouterina rhyacothritoni*, and *Bunoderella metteri*; actually, no records of these species have been published after the original descriptions were made. The new phylogenetic analysis is based on Maximum Likelihood (ML) (Figure 6). The complete alignment consists of 1,409 base pairs. ML analyses were run in RAxML version 7.0.4 (Stamatakis, 2006). The reliability of clade support was estimated through a bootstrap with 1,000 replicates.

According to the currently accepted classification scheme of the Digenea using 28S rDNA sequences (Pérez-Ponce de León and Hernández-Mena, 2019), the family Allocreadiidae belongs to the superfamily Gorgoderoidea, within the suborder Xiphidiata, in the order Plagiorchiida. Figure 6 depicts the interrelationships among allocreadiids and other members of the Xiphidiata, some of them used as outgroups for rooting the tree. The Allocreadiidae is a monophyletic group, with high bootstrap support values (blue shadow in Figure 6). The sister group of allocreadiids is represented by the callodistomids, that is, the genus *Prosthenhystera*. Two groups of digeneans considered in the past to be allocreadiids, specifically, *Polylekithum* spp. and *Paracreptotrematina limi* (see Caira and Bogéa, 2005; Platta and Choudhury, 2006) are not members of the family (purple and brown shadows in Figure 6, respectively). Molecular evidence has demonstrated that both species are sister taxa to other groups of xiphidiatans (Choudhury et al., 2007; Curran et al., 2011).

Three additional facts are also evident in Figure 6 regarding the evolutionary and biogeographical history of allocreadiids.

1) A clear pattern of geographical association among members of Allocreadiidae is not observed, probably other than the clade composed by species of the genera *Wallinia, Auriculostoma, Creptotrematina, Paracreptotrema, Paracreptotrematoides*, and *Pseudoparacreptotrema*, which is distributed in

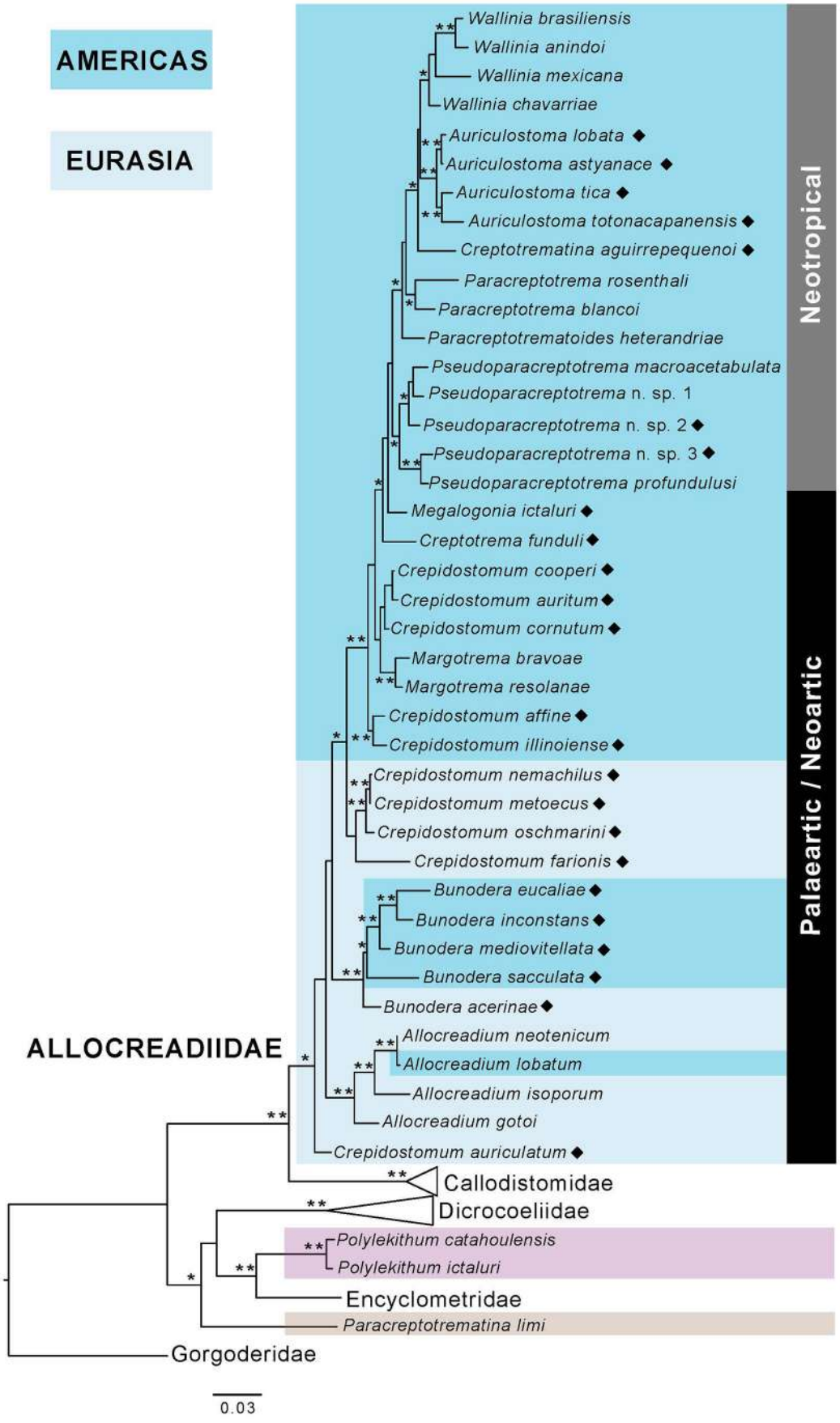

Figure 6. Phylogenetic tree of 28S rDNA sequences of members of Allocreadiidae based on Maximum Likelihood analysis. Blue shadow denotes monophyly of Allocreadiiidae. Diamond marks indicate species possessing muscular oral lobes. One asterisk refers to bootstrap support values from 70 to 90; 2 asterisks refer to bootstrap values from 90 to 100. Source: G. Pérez-Ponce de León, D. I. Hernández-Mena, and B. Solórzano-García. License: CC BY-NC-SA 4.0.

the Neotropical biogeographical region. The other species are distributed in the Nearctic or Palaearctic biogeographical regions. Species labeled with an intense blue shadow are found in the Americas and those labeled with a light blue shadow occur in Eurasia. There is no question that a former continuity occurred between North America and Eurasia, in a landmass known as Laurasia. The American parasitologist Harold W. Manter pointed out that the trematodes of fishes of these continents were related and he used the allocreadiid genus *Crepidostomum* as one of the examples of such connection (Manter, 1963). *Crepidostomum farionis* occurs widely in both North America and Eurasia. This is the result of an ancient connection and the breakup of Pangaea into northern and southern landmasses, namely Laurasia and Gondwana. North America separated from Europe and later became closer to northeast Asia (see Choudhury et al., 2016 and references therein).

Allocreadium is by far the most speciose genus of allocreadiids. Their species are distributed worldwide except Australia and the species distribution pattern might also be the result of the breakup of Pangaea, following the diversification and dispersal of their main host group, represented by cyprinids. Also, according to Manter, species of *Allocreadium* are predominately parasites of cyprinids but adaptable to several other species of hosts, and he hypothesized that the genus may have followed cyprinids from an Asiatic origin to Europe, North America, and a few into Africa; however, no phylogenetic evidence is currently available to test such a hypothesis, mainly because very few representative species of the genus *Allocreadium* have been sequenced thus far (Figure 6).

2) As seen in Figure 6, even though allocreadiids tend to be very host specific and many of them are part of the biogeographical core fauna of their hosts (see Pérez-Ponce de León and Choudhury, 2005), an overall host association pattern is not evident. Most of the Neotropical species are found either in characiforms or siluriforms, with some species infecting cyprinodontiforms. These groups are highly diverse components of the Neotropical freshwater fish fauna.

3) From the data presented in Figure 6 relative to the presence or absence of muscular lobes on the oral sucker, the molecular phylogenetic tree of allocreadiids corroborates the fact that the species possessing these structures do not form a monophyletic group, and the presence of oral lobes arose several times during the evolutionary history of the family (see the diamond-shaped symbols in Figure 6). A formal test of this hypothesis is required through a comprehensive analysis of xiphidiatan digeneans to determine if the lack of oral lobes is the plesiomorphic condition.

Species Diversity among the Allocreadiidae

Caira and Bogéa (2005) recognized 15 valid genera within the family and presented an identification key to recognize them. Later on, of the 15 genera, 3 were synonymized with *Bunodera*, that is, *Bunoderina* (as *Bunodera eucaliae*), *Allobunodera* (as *Bunodera mediovitellata*), and *Culeatrema* (as *Bunodera inconstans*). Furthermore, the genus *Pseudoallocreadium* was synonymized with *Allocreadium*, as *A. neotenicum* and *A. alloneotenicum*. Finally, molecular evidence demonstrated that 2 genera, *Paracreptotrematina* and *Polylekithum,* do not belong to Allocreadiidae (see Curran et al., 2006; 2011; Platta and Choudhury, 2006; Choudhury et al., 2007).

The current classification of the Allocreadiidae includes 14 genera and approximately 130 species. Twelve of these genera for which 28S rDNA sequences have been generated are depicted in Figure 6. No sequences are available for *Caudouterina* and *Bunoderella*, both amphibian allocreadiids. *Allocreadium* is the richest genus, with approximately 71 species. The other genera include between 1 and about 24 species, and 3 genera are monotypic. An account of the species richness and geographical location of species of Allocreadiiidae is presented below in alphabetical order. Representative species of some of the genera are shown in Figure 7(A–F).

***Allocreadium* Looss, 1900**

***Allocreadium isoporum* (Looss, 1894) Looss, 1900; type host; Cyprinidae; Europe**

Species of this genus are mainly found in cyprinids around the world, with isolated records in other fish families. The list of congeneric species validated in the synopsis of Yamaguti (1971) includes 25 species. At least 21 species were described after that; however, according to Decock and colleagues (2020), the genus contains over 70 described species, although that list does not include several additional species, including, *Allocreadium alloneotenicum* from trichopterans in the United States and Canada, *A. lucyae* from cyprinids in the United States, and *A. mexicanum* from atherinopsids and goodeids in central Mexico (although the authors have gathered molecular evidence showing that this species does not belong in *Allocreadium*); and 4 species from cyprinids in Japan: *A. aburahaya*, *A. brevitellatum A. tosai*, and *A. tribolodontis* (see Margolis and Arthur, 1979; McDonald and Margolis, 1995; Hoffman, 1999; Shimazu et al., 2016a; Ostrowski de Nuñez et al., 2017; Kudlai et al., 2018, among others). Another congeneric species is *A. danjiangensis*, described from 5 species of cyprinid fishes in China (Gao, 2018).

The species composition in the genus is clearly in need of revision. The list of species of *Allocreadium* includes those

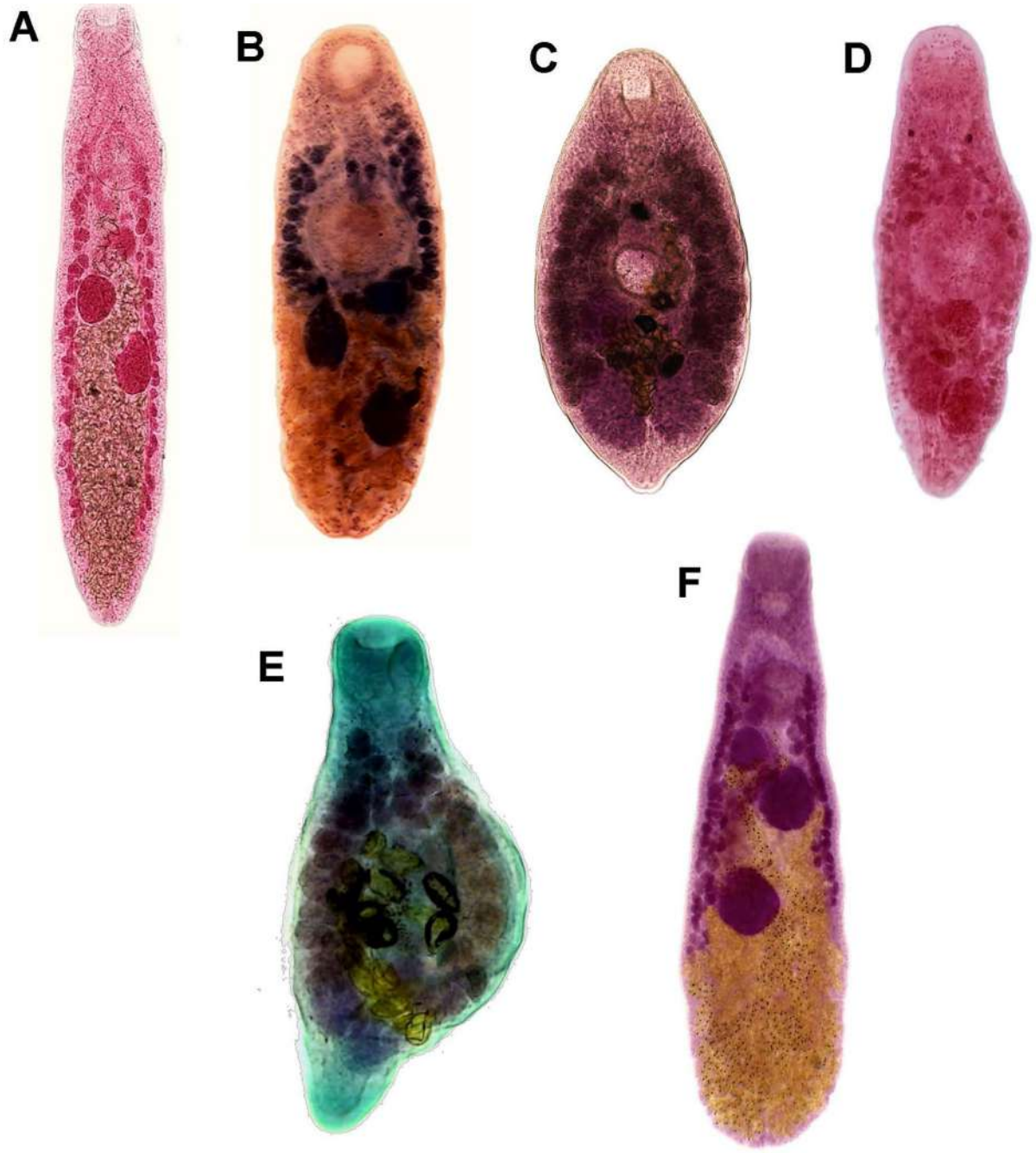

Figure 7. Microphotographs of stained specimens of allocreadiids representing some of the genera in the family. A) *Creptotrematina aguirrepequenoi,* B) *Margotrema resolanae,* C) *Paracreptotrema blancoi,* D) *Paracreptotrematoides heterandriae,* E) *Pseudoparacreptotrema magacetabulata,* F) *Wallinia anindoi*. Source: G. Pérez-Ponce de León, D. I. Hernández-Mena, and B. Solórzano-García. License: CC BY-NC-SA 4.0.

from North America, South America, Europe, Russia, China, Japan, and Africa. Even though the genus is recognized as Palearctic, 2 species have been reported from South America in non-cyprinid hosts (Ostrowski de Nuñez et al., 2017), and 7 from Africa, with most records in cyprinids and some in characiforms and siluriforms (Kudlai et al., 2018).

Auriculostoma **Scholz et al., 2004**

Auriculostoma astyanace **Scholz et al., 2004; type host; Characidae (*Astyanax* sp.); Nicaragua**

The genus contains 9 species; 5 of these are found in South America, including *Auriculostoma macrorchis* from perciforms and siluriforms in Argentina, *A. platense* from

siluriforms and gymnotiforms in Argentina and Brazil, *A. stenopteri* from Characidae in Uruguay, *A. diagonale* and *A. foliaceum* from Characidae in Peru, *A. astyanace* from Characidae in Nicaragua, *A. totonacapanensis* and *A. lobata* from Characidae in Mexico, and *A. tica* from Gymnotidae in Costa Rica (Scholz et al., 2004; Kohn et al., 2007; Curran et al., 2011; Razo-Mendivil et al., 2014; Hernández-Mena et al., 2016; 2019; Ostrowski de Nuñez et al., 2017).

***Bunodera* Railliet, 1896**

***Bunodera luciopercae* (Müller, 1776) Lühe, 1909; type host; Percidae; Holarctic**

Seven species comprise the genus *Bunodera*: *B. acerinae* in percids in Russia, *B. eucaliae* and *B. inconstans* in freshwater sticklebacks in the United States and Canada, *B. luciopercae* in percids in the Holarctic, *B. mediovitellata* in the threespine stickleback and *Gasterosteus aculeatus* in the Holarctic, *B. sacculata*, an endemic North American species found in percids, and *B. vytautasi* in gasterosteids (*Pungitius pungitius*) in northeast Asia (McDonald and Margolis, 1995; Hoffman, 1999; Petkevičiūtė et al., 2010; Atopkin et al., 2018).

***Bunoderella* Schell, 1964**

***Bunoderella metteri* (Schell, 1964); type species; tailed frog *Ascaphus truei*; United States**

The genus is monotypic. The only known species is *Bunoderella metteri*, from tailed frogs in Idaho and Washington, United States. The species is characterized by having 2 anterodorsal and 2 ventrolateral muscular oral lobes. No additional records have been published after the original description.

***Caudouterina* Martin, 1966**

***Caudouterina rhyacotritoni* Martin, 1966; type species; Olympic salamander *Rhyacotriton olympicus*; United States**

The genus is monotypic. The only described species is *Caudouterina rhyacotritoni* from the Olympic salamander in Oregon, United States. The species lacks muscular oral lobes and possesses a uterus that reaches the posterior end of the body. No additional records have been published after the original description.

***Crepidostomum* Braun, 1900**

***Crepidostomum metoecus* (Braun, 1900); type species; Salmonidae (brown trout) and occasionally in other fish families; Palearctic**

The genus *Crepidostomum* has a rather complex taxonomic history. According to Atopkin and Shedko (2014), the genus contains 40 nominal and 24 valid species. However, many species have been synonymized and there is not a current revision of the species composition in the genus (see Hoffman, 1999). Figure 6 corroborates that the genus is not monophyletic and requires detailed taxonomic revision based on phylogenetic analysis. In this chapter, 16 species are recognized, some of which have molecular data available, namely, *Crepidostomum affine* from the mooneye, *Hiodon tergisus* in the United States; *C. auritum* from the freshwater drum, *Aplodinotus grunniens* in the United States; *C. auriculatum* from sturgeons, *Accipenser schrenki* and *Huso dauricus* in the Holarctic; *C. bailcalense* from several fish families in Eurasia; *C. brevivitellatum* from *Anguilla rostrata* in Canada; *C. chaenogobii* from gobiids and cottids in Japan and the Russian Far East; *C. cooperi* from centrarchiids in North America; *C. cornutum* from centrarchiids in North America; *C. farionis* from various fish families in the Holarctic; *C. illinoiense* from hiodontids in the United States; *C. isostomum* from several fish families in the United States; *C. latum*, in several fish families in Europe; *C. metoecus* from salmonids in the Palearctic; *C. opeongoensis* from *Hiodon* spp. in Canada; *C. oshmarini* in Balitoridae and Cottidae in Europe; *C. percopsisi* from the trout perch, *Percopsis omiscomaycusi* in Canada; *C. wikgreni* in several fish families in Europe (Nelson et al., 1997; Hoffman, 1999; Choudhury and Nelson, 2000; Moravec, 2002; Tkach et al., 2013; Atopkin and Shedko, 2014; Shimazu, 2016b; Petkevičiūtė et al., 2018).

***Creptotrema* Travassos et al. 1928**

***Creptotrema creptotrema* Travassos et al. 1928; Characiformes and Siluriformes; Brazil, Argentina**

The genus contains 8 nominal species, 6 of them distributed in South American characiforms or siluriforms, that is, *Creptotrema creptotrema, C. lynchi, C. paranaensis, C. pati, C. sucumbiosa,* and *C. lamothei*; and in Central America, in mountain mullets, *C. agonostomi,* and 1 in fundulids of the United States, *C. funduli* (Kohn et al., 2007; Curran, 2008; Curran et al., 2012). Another species (yet undescribed) was recorded in pimelodid catfishes in Panama (Choudhury et al., 2017). Furthermore, another study demonstrates that the genus *Creptotrema*, as currently defined, is not monophyletic. *Creptotrema agonostomi,* and 3 genetic lineages corresponding to independent species, all from the Central American mountain mullets, *Dajaus monticola*, belong to the genus *Pseudoparacreptotrema* (see Figure 6). A new genus will be required to accommodate *C. funduli*.

***Creptotrematina* Yamaguti, 1954** (Figure 7A)

***Creptotrematina dissimilis* (Freitas, 1941) Yamaguti, 1954; type species; Characidae; Brazil, Argentina**

The species in this genus were originally placed in *Creptotrema*, but Yamaguti (1954) erected the genus *Creptotrematina.* It currently contains only 3 species: *C. dispar* and *C. dissimilis* in Characidae from Argentina and Brazil, and *C. aguirrepequenoi* from *Astyanax* spp. in Central America (Kohn et al., 2007; Curran et al., 2011; Razo-Mendivil et al., 2014; Ostrowski de Nuñez et al., 2017).

***Margotrema* Lamothe-Argumedo, 1970** (Figure 7B)

***Margotrema bravoae* Lamothe, 1970; type species; Goodeidae; central Mexico**

The genus belongs to Allocreadiidae (Pérez-Ponce de León et al., 2007), and contains only 2 species, the type species and *Margotrema resolanae* from the leopard splitfin, *Xenotaenia resolanae* (Goodeidae), in west-central Mexico (Pérez-Ponce de León et al., 2013).

***Paracreptotrema* Choudhury et al., 2006** (Figure 7C)

***Paracreptotrema mendezi* (Sogandares-Bernal, 1955) Choudhury et al., 2006; type species; Poeciliidae; Panama**

The genus *Paracreptotrema* includes 3 species, all of them found in Central American poeciliids: *P. blancoi* from *Priapichthys annectens* in Costa Rica, *Paracreptotrema mendezi* from *Brachyrhaphis episcopi* in Panama, and *P. rosenthali* from *Xiphophorus malinche* and *Pseudoxiphophorus jonesii* in Mexico (Choudhury et al., 2006; Bautista-Hernández et al., 2015; Pérez-Ponce de León et al., 2016).

***Paracreptotrematoides* Pérez-Ponce de León et al., 2016** (Figure 7D)

***Paracreptotrematoides heterandriae* (Salgado-Maldonado et al., 2012) Pérez-Ponce de León et al., 2016; type species; *Pseudoxiphophorus bimaculatus* (Poeciliidae); Mexico**

The genus is monotypic. The only species described in the genus is *Paracreptotrematoides heterandriae*, from the twospot livebearer *Pseudoxiphophorus maculatus* in Veracruz, Mexico. No additional records have been published after the original description.

***Pseudoparacreptotrema* Pérez-Ponce de León et al., 2016** (Figure 7E)

***Pseudoparacreptotrema profundulusi* (Salgado-Maldonado et al., 2012)**

The genus includes 2 nominal species, *Pseudoparacreptotrema macroacetabulata* from the killifish *Profundulus candalarius* in Chiapas, Mexico, and *Pseudoparacreptotrema profundulusi* from the killifish *Profundulus punctatus, Pr. balsanus*, and *Pr. oaxacae* from Oaxaca and Guerrero, Mexico (Pérez-Ponce de León et al., 2016). Newly gathered molecular data allowed the authors to determine that *"Creptotrema" agonostomi* from the mountain mullet, *Dajaus monticola* in Mexico actually belongs in *Pseudoparacreptotrema*. The same dataset led to the determination that 3 additional species of the genus had to be described as parasites of mountain mullets in localities of Central America (Pérez-Ponce de León et al., 2020).

***Wallinia* Pearse, 1920** (Figure 7F)

***Wallinia valenciae* Pearse, 1920; type species; *Geophyrocharax valenciae* (Characidae); Venezuela**

The genus *Wallinia* has 5 valid species: The type species from characids in Venezuela; *W. chavarriae* from *Bryconamericanus scleroparius* and *Astyanax aeneus* (Characidae) in Costa Rica, *W. mexicana* from *Astyanax mexicanus* in Mexico, *W. brasiliensis* from 2 species of *Astyanax* in Brazil, and *W. anindoi* from *A. aeneus* in Mexico (Choudhury et al., 2002; Pérez-Ponce de León et al., 2015; Dias et al., 2018; Hernández-Mena et al., 2019).

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Digenea, Plagiorchiida

Xiphidiata

Haematoloechidae Odening, 1964 (Family)

Virginia León-Règagnon

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Family Haematoloechidae

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 44

Haematoloechidae Odening, 1964 (Family)

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Introduction

Members of the family Haematoloechidae are parasites of the lungs of amphibians and they are found on every continent except Antarctica. Their life cycle includes a snail and an aquatic arthropod as first and second intermediate hosts, respectively, and a frog or salamander as the definitive host.

Main Characters

Their body is elongate, the forebody is usually tapered, and the posterior body is oval; the tegument may be spined or not, but spines are easily lost. The oral sucker is well developed and is located near the anterior end of the body. The pharynx is well developed, the esophagus is short, and the digestive ceca extend to near the posterior extremity (Figure 1). The ventral sucker is located anterior to the midbody; in most species it is well developed, but in a few of them it is reduced and difficult to observe (Figure 2). The male reproductive system is composed of 2 oval or lobed testes in the posterior part of the body that are usually diagonally arranged (rarely symmetrical or in tandem); they are intracecal, with the exception of 1 species, *Haematoloechus exoterorchis,* in which they are extraceacally arranged (Figure 3). Species in this family have a cirrus sac that is cylindrical, narrow, and long, winding between the 2 suckers. The genital pore is ventral, median, and located at the level of the pharynx or esophagus. The female reproductive system comprises the ovary, which is located between the ventral sucker and the testes, and can be oval or lobed. The seminal receptacle is a large structure located laterally and dorsal to the ovary. Mehlis' gland is dorsal to the seminal receptacle and ovary. The vitelline follicles are arranged in clusters, sometimes overlapping each other; they are distributed laterally along the ceca for most of their length; in some species they are distributed only in the pretesticular region. The uterine loops fill the entire hindbody, passing

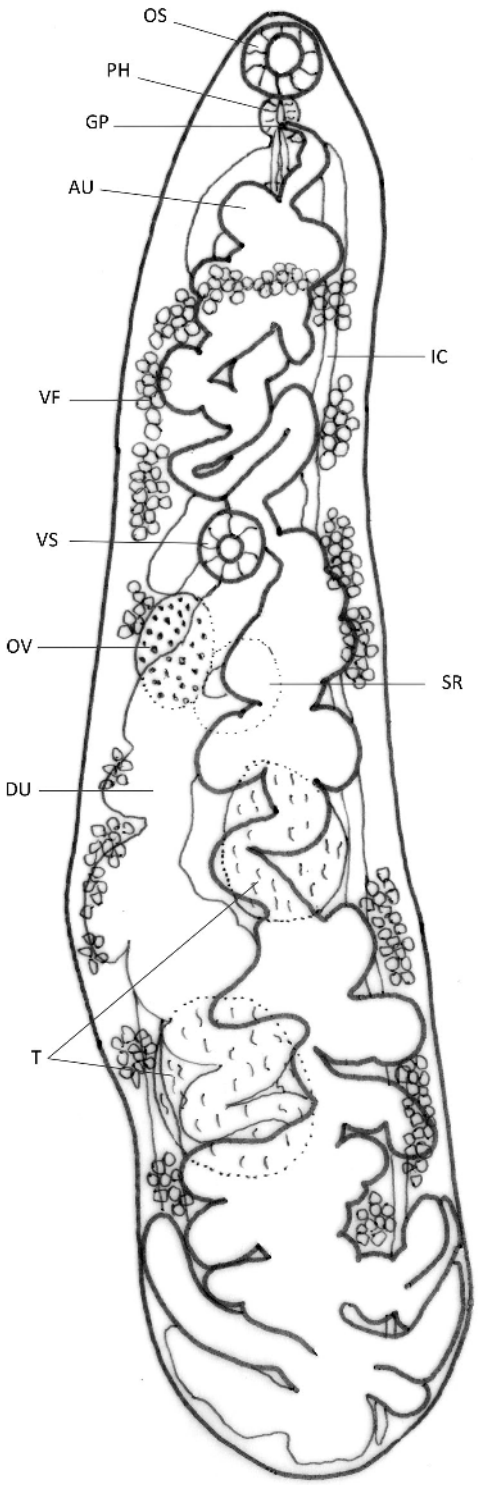

Figure 1. *Haematoloechus caballeroi*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

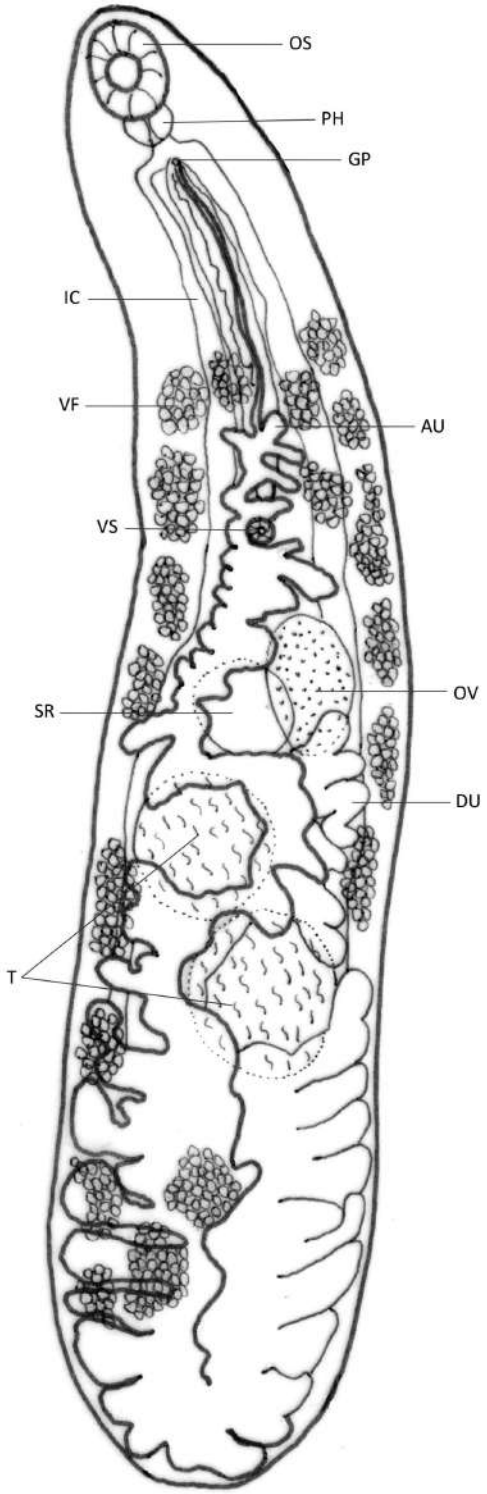

Figure 2. *Haematoloechus meridionalis*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

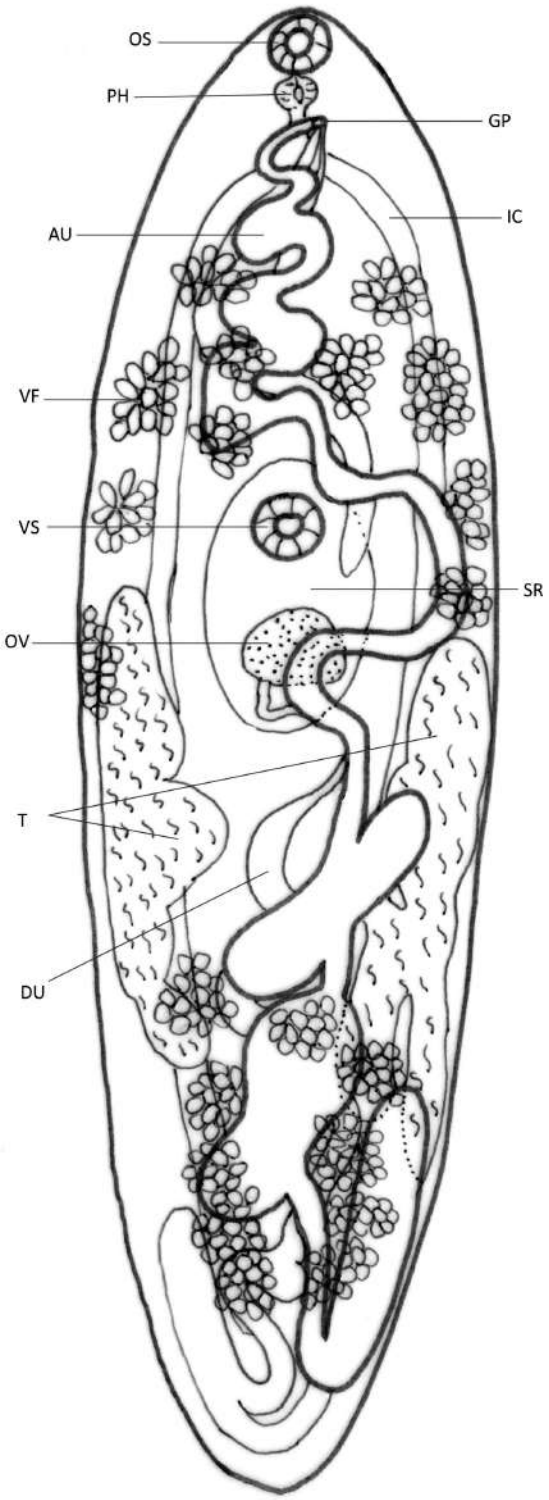

Figure 3. *Haematoloechus exoterorchis*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

Figure 4. *Haematoloechus caballeroi*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

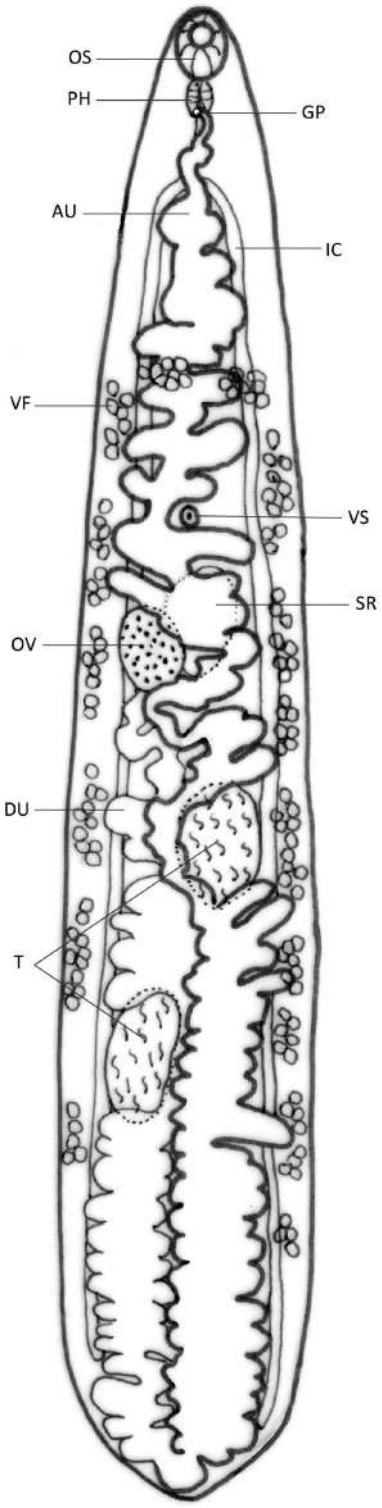

Figure 5. *Haematoloechus medioplexus*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

among the testes, sometimes forming extracecal loops that can extend forward at different levels; the ascending uterus forms several loops in the forebody. The eggs are tiny and numerous; in the distal uterus they are heavily pigmented and can obscure other structures (Figure 4).

Taxonomy and Phylogenetics

The taxonomic history of this family has been complicated. The first member of this family was formally described in the early 19th century. That species was originally named *Distomum variegatum* Rudolphi, 1819, and later was transferred to the newly erected genus *Haematoloechus* by Looss (1899). A few years later, the type genus of this family was renamed as *Pneumonoeces* Looss, 1902 because a hemipteran genus had previously been named *Haematoloecha* Stal (Looss, 1902). Although Harwood (1932) and Ingles (1932) independently reinstated *Haematoloechus* based on the existing International Code of Zoological Nomenclature (ICZN, 1895), some other authors continued to use *Pneumonoeces* (Mehra, 1937; Skrjabin and Antipin, 1962).

The first time this taxonomic group received a formal name was as the subfamily Pneumonoecinae (Mehra, 1937), then renamed Haematoloechinae (Freitas and Lent, 1939) within the Plagiorchiidae. It was not until 1964 that this group was recognized as an independent family within the Plagiorchioidea, the Haematoloechidae (Odening, 1960), for which the monophyly is currently supported by phylogenetic analyses based on ribosomal and mitochondrial DNA sequences (Tkach et al., 2000; 2001; León-Règagnon and Topan, 2018).

Several genera have been proposed to include species of haematoloechids based on the varying arrangement of the uterine loops or the distribution of the vitelline follicles. *Ostiolum* Pratt, 1903 was proposed for species lacking extracecal longitudinal uterine loops (Pratt, 1903) as in *Haematoloechus medioplexus* Stafford, 1902 (Figure 5) or *H. complexus* Seely, 1906. *Pneumobites* Ward, 1917, was proposed for those with longitudinal uterine loops extending to the pre-acetabular region of the body, with *H. longiplexus* as its type species (Ward, 1917) (Figure 6). *Skrjabinoeces* Sudarikov, 1950 was proposed for species with vitelline follicles in clusters limited to the pre-testicular region, with *H. similis* as its type species (Sudarikov, 1950) (Figure 7). Odening (1958) recognized the genera *Ostiolum* and *Haematoloechus,* this later with 3 subgenera based on the arrangement of the vitelline follicles: *Skrjabinoeces* (as described above), *Anomolecithus* (vitelline follicles not in clusters, extending to the post-testicular region, like in *H. asper* (Figure 8)) and *Haematoloechus* (vitelline follicles in clusters, extending to the post-testicular region, as in *H. longiplexus* (Figure 6) and most other members of Haematoloechidae). The genus *Neohaematoloechus* Odening, 1960 was erected for those species described as lacking a ventral sucker, with *H. neivai* (Travassos and Artigas, 1927) as its type species (Odening, 1960) (Figure 9).

The genus *Ostioloides* Odening, 1960 was proposed to include *Haematoloechus rappiae,* which was first described as *Haplometroides rappiae* Szidat 1932, then transferred to *Haematoloechus* by Yamaguti (1958), and finally used as the type species to erect *Ostioloides* by Odening (1960). The intestinal ceca extending only two-thirds of the body and the post-bifurcal position of the genital pore in *O. rappiae* supports the validity of the genus *Ostioloides* and indicates that it does not belong to the family Haematoloechidae, but to the Plagiorchiidae (Tkach, 2008).

In his extensive revision of the Trematoda, Yamaguti (1971) recognized the genera *Haematoloechus, Neohaematoloechus*, *Ostioloides*, and erected a new genus, *Metahaematoloechus* Yamaguti, 1971*,* for species with extracecal testes, with *H. exoterorchis* Rees, 1964 as the type species.

More recent research, using molecular data, has aided the identification of morphological characters that are useful for the differentiation of species, and revealed that most genera previously included in Haematoloechidae are not monophyletic (León-Règagnon et al., 1999; 2001; Snyder and Tkach, 2001; León-Règagnon and Paredes-Calderón, 2002; León-Règagnon and Brooks, 2003; León-Règagnon, 2010; Zamparo et al., 2011; León-Règagnon and Topan, 2018). Characters such as the ratio of the suckers, the oral sucker/pharynx ratio, the shape of the ovary and testes, the arrangement of the uterine loops, and the distribution of the vitellaria are valuable characters to differentiate species. Nevertheless, none of them appears to reflect the evolutionary history of the group (see León-Règagnon and Topan, 2018). Mapping the morphological traits that led to previous taxonomic arrangements into their phylogenetic tree, these authors found that they do not support the monophyly of previously proposed groups, consequently synonymizing *Ostiolum, Pneumobites, Anomolecithus, Neohaematoloechus*, and *Metahaematoloechus* with *Haematoloechus* (León-Règagnon and Topan, 2018). There is no molecular evidence for species previously assigned to the subgenus *Skrjabinoeces*; it is necessary to include these species in a phylogenetic framework to test the validity of this genus. Tkach (2008) considered it to be valid based on the morphology of the cercariae. According to León-Règagnon and Topan (2018), the only valid genus in the family is *Haematoloechus,* containing 70 valid species.

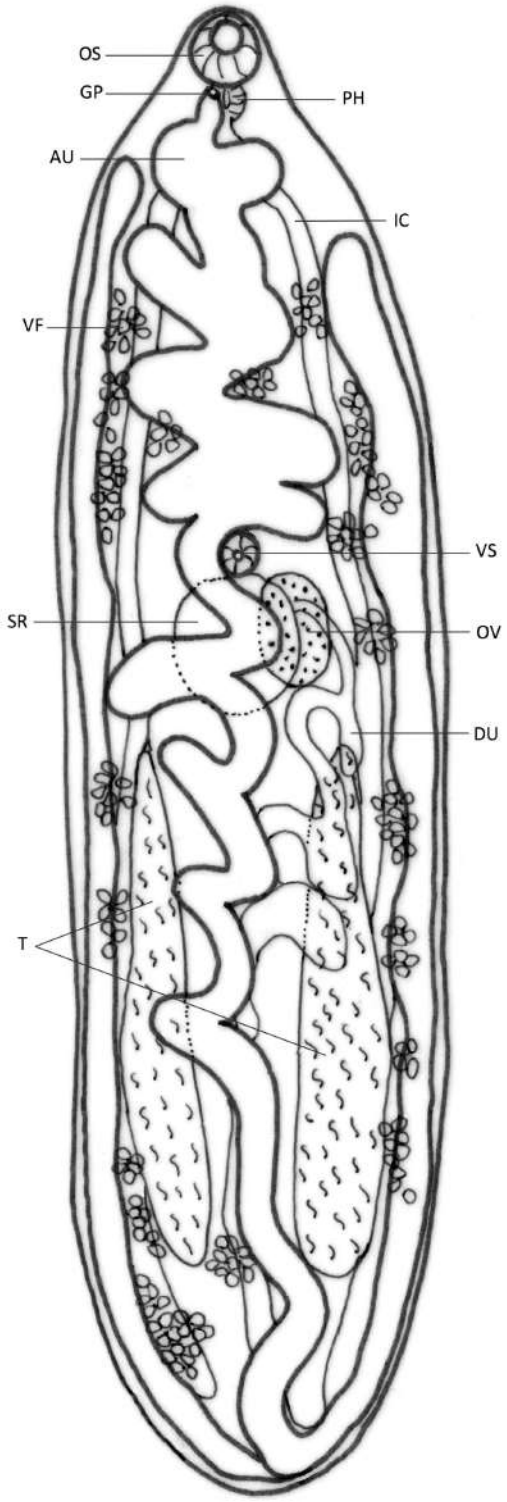

Figure 6. *Haematoloechus longiplexus*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

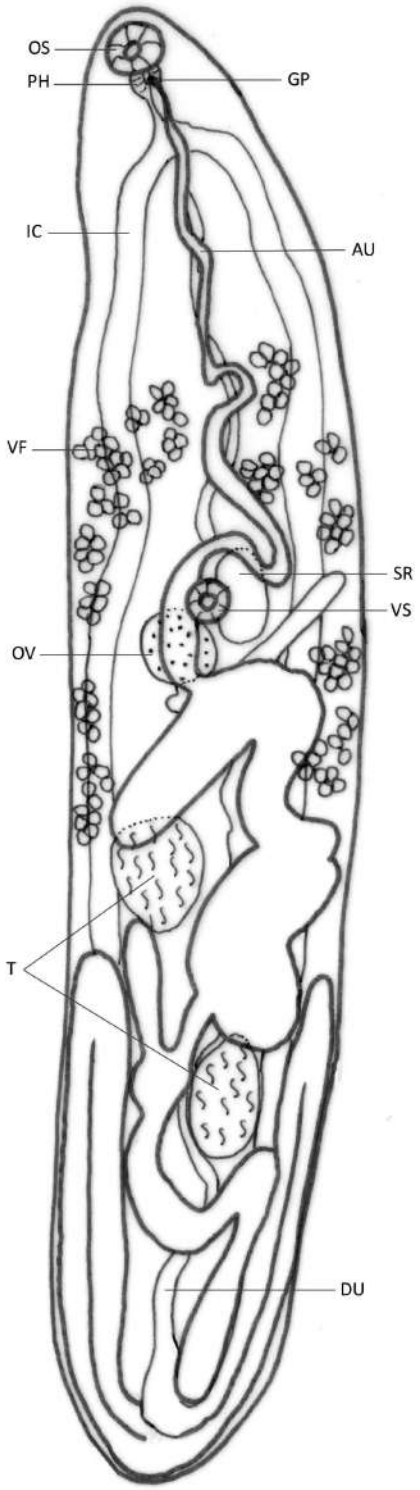

Figure 7. *Haematoloechus similis*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

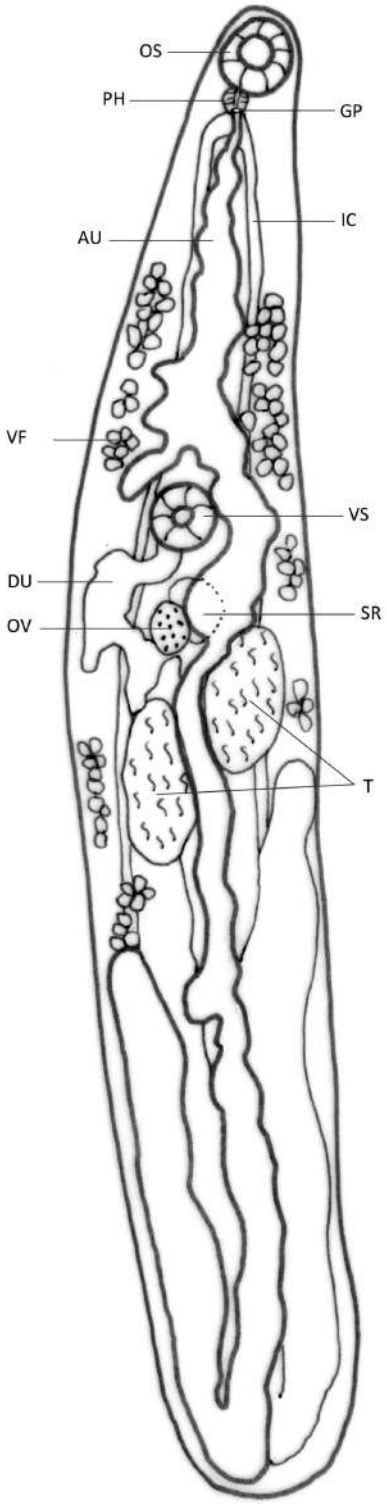

Figure 8. *Haematoloechus asper*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

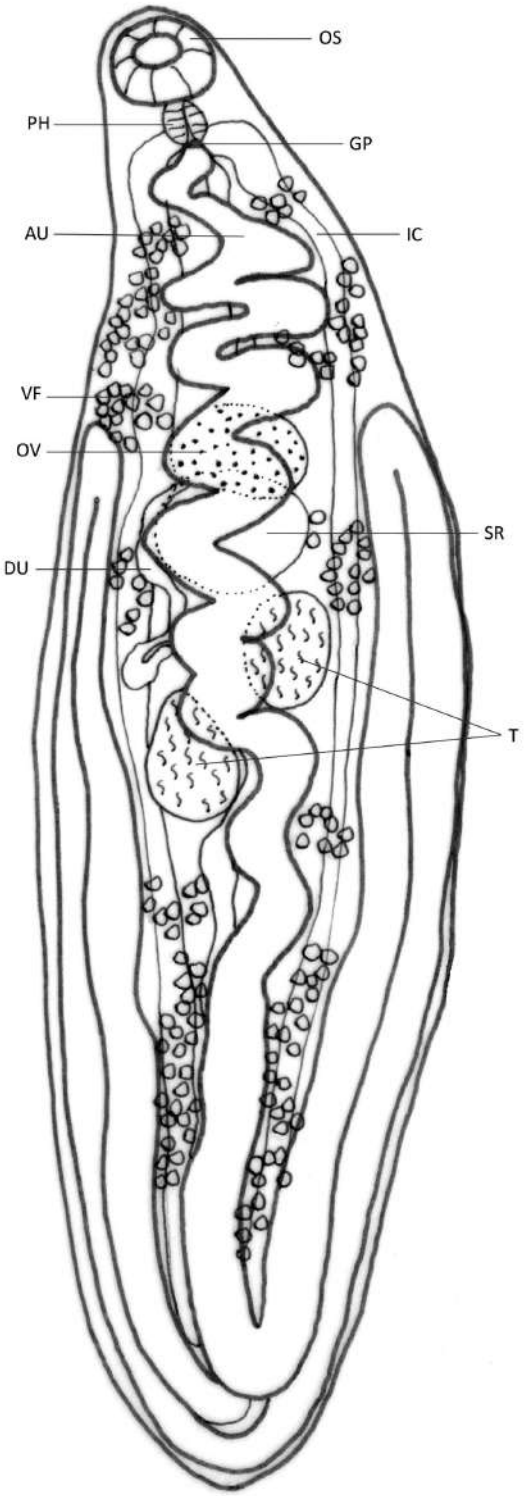

Figure 9. *Haematoloechus neivai*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

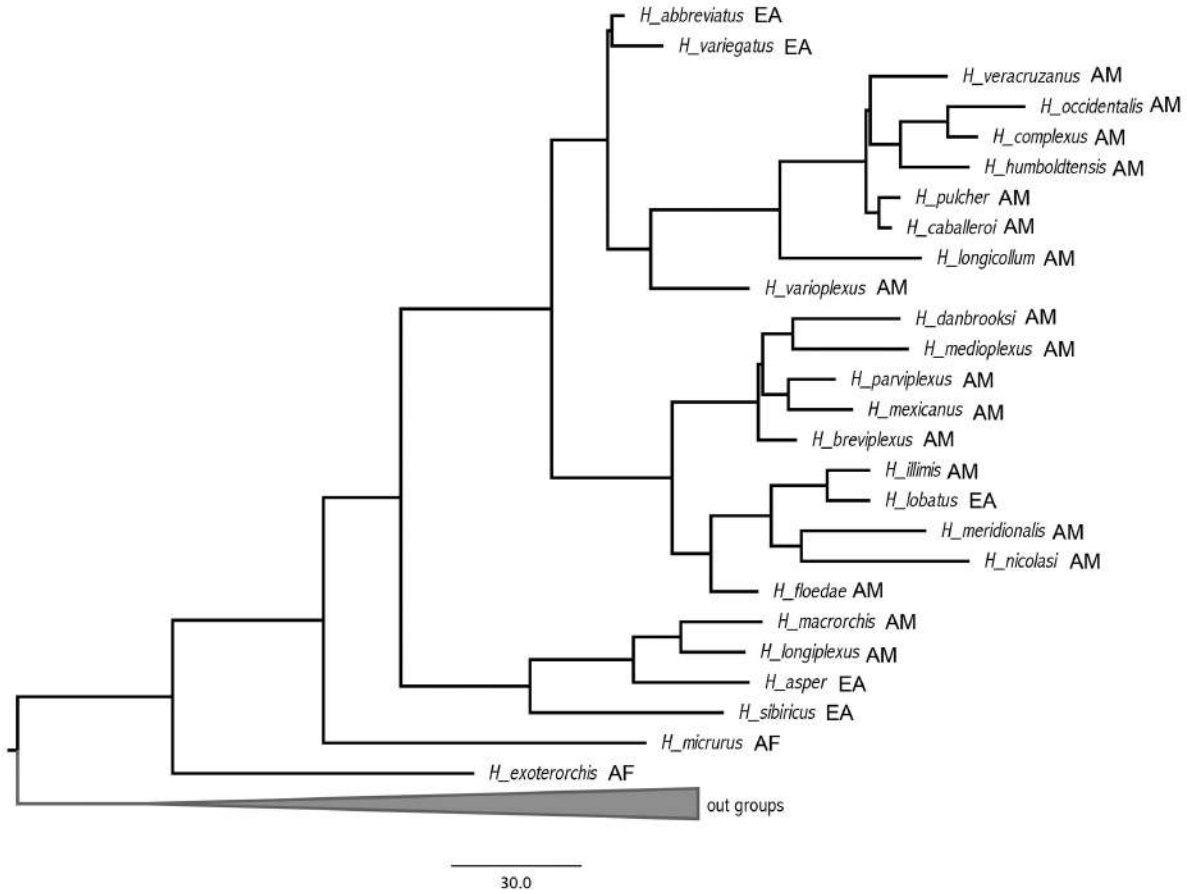

Figure 10. Phylogenetic hypothesis of *Haematoloechus* spp. based on mitochondrial and ribosomal DNA. AF: Africa, AM: America, EA: Eurasia, Out groups: *Brachycoelium salamandrae*, *Glypthelmins brownorumae*, *Opisthioglyphe ranae*, and *Plagiorchis koreanus*. Source: Adapted from León-Règagnon and Topan, 2018. License: CC BY-NC-SA 4.0.

Host Range

Most species of Haematoloechidae prefer amphibians of the family Ranidae (sensu Bossuyt et al., 2006) as their definitive hosts, although accidental infections (a few isolated records of species that are common in ranids) have been recorded in members of Bufonidae or Salamandridae, and some species have colonized members of other families, such as Ambystomatidae, Bombinatoridae, Leptodactylidae, Telmatobiidae, and others (León-Règagnon and Topan, 2018).

Snyder and Janovy (1994; 1996) examined the second intermediate host specificity of 4 North American species of *Haematoloechus*, including, *H. complexus, H. longiplexus, H. medioplexus,* and *H. varioplexus*. They found that cercariae of *H. complexus* act in a generalist way and are able to penetrate the intersegmental membranes of all 9 species of arthropods used in their experiments. *Haematoloechus longiplexus* penetrated only the base of the caudal gills of anisopteran (damselfly) and zygopteran (dragonfly) odonate naiads, while cercariae of *H. medioplexus* and *H. varioplexus* do not actively penetrate intermediate hosts, but are drawn into the branchial basket respiratory apparatus of the anisopteran naiads. They rarely attach to and never penetrate experimental hosts, suggesting that the evolution of disparate patterns of behavior among the cercariae of these 4 congeners directly affects subsequent patterns of transmission to the definitive host (Snyder and Janovy, 1994; 1996). Snyder and Tkach (2001) suggested that, based on the phylogenetic analysis of rDNA of 8 species of *Haematoloechus* and available data on life cycles of those species, intermediate host specificity reflected the evolutionary history of the group. However, this trait has been studied in only a few species of the genus and information in many more species is needed in order to corroborate this hypothesis.

Historically, host specificity (now referred to as host range) has been associated with the inability of the parasite to colonize a different host species, that is, the parasite has specialized in 1 (or a few) host species and is not capable of parasitizing other host species (for an extensive review, see Brooks and McLennan, 2002). Nevertheless, if the parasite has specialized in a resource, not in the host species, and if

this resource is phylogenetically conserved among many host species, the parasite will be able to colonize other host species that carry that same resource if the opportunity presents itself. As noted elsewhere in this book, this phenomenon is called ecological fitting (Janzen, 1985). Even when host specificity is observed during a moment of time and in a particular space, it does not mean that host switching is not possible if the conditions change. For example, if new hosts are introduced in the area, or the original host is introduced in a new area, considering climate change and globalization can affect situations that are very common nowadays (Brooks and Hoberg, 2013; Brooks et al., 2014; 2019).

Haematoloechus floedae is a species native to the southeastern United States where it lives in the lungs of the bullfrog, *Lithobates catesbeianus*. When bullfrogs were introduced to the southwestern United States, the parasite went with them, and is now found in bullfrogs in that part of the country. Interestingly, the lung fluke was recently reported in other groups of frogs in the Yucatán Peninsula, Mexico (*L. brownorum*—leopard frog—and *L. vaillanti*—palmipes group) and in 2 leopard frogs, *Rana taylori* and *R.* cf. *forreri*, from the Área de Conservación Guanacaste, Costa Rica, where bullfrogs were introduced (León-Règagnon et al., 2005).

Haematoloechus floedae, despite having a supposedly complex, specialized life cycle, has become established in a number of endemic species in localities where bullfrogs were introduced, and even when in some of those localities bullfrogs have been extirpated, the parasite persists. These host switching events are clear examples of ecological fitting (Brooks et al., 2006).

Biogeography

According to León-Règagnon and Topan (2018), the association between *Haematoloechus* spp. and their hosts predates the ranid diversification in the Cretaceous Period (Bossuyt et al., 2006). Several African species of *Haematoloechus* (for example, *H. aubriae*, *H. combesi*, *H. darcheni*, *H. dollfusinum*, and *H. lobogonadus*) parasitize members of the Conrauinae, Ptychadeninae, and Pyxicephalinae groups that originated early in the radiation of the Ranidae in Africa (Bossuyt et al., 2006). When ancestral ranids colonized Europe (*Rana* and *Pelophylax*) and the New World (*Lithobates*) in the Oligocene or Miocene Period (Bossuyt et al. 2006), they must have been already associated with *Haematoloechus*, which is clearly reflected in the phylogenetic hypothesis presented by León-Règagnon and Topan (2018), as the African species *H. exoterorchis* and *H. micrurus* appear to have diverged early in the evolution of the group, and European and American species are present in the 3 larger clades of the tree (Figure 10).

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45

Digenea, Plagiorchiida

Xiphidiata

Lecithodendriidae Lühe, 1901 (Family)

Jeffrey M. Lotz

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Family Lecithodendriidae

doi:10.32873/unl.dc.ciap045
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Chapter 45

Lecithodendriidae Lühe, 1901 (Family)

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Introduction

The Lecithodendriidae Lühe, 1901 is a family of cosmopolitan digeneans in the suborder Xiphidiata. Adult lecithodendriids inhabit the intestinal tract of insectivorous bats (and occasionally birds). They are of mostly minor consequence in human and veterinary health but have been more important for parasite ecology. They use an aquatic snail as first intermediate host, an insect as second intermediate host, and the bat as definitive host. Humans and other mammals can become infected when ingesting infected insects. For veterinary science, the Lecithodendriidae have been found to be reservoirs for the causative agent of Potomac horse fever. They are important for studies of parasite ecology because they comprise a substantial component of the infracommunities of bats. Several species of Lecithodendriidae are often found in chiropteran infracommunities providing communities of several closely related members. Studies of those communities have contributed to better understanding of the assembly, structure, and dynamics of parasite communities.

Identifying Lecithodendriidae

Adults of the Lecithodendriidae (Figure 1) are typically less than 1 mm in length, possess an acetabulum, oral sucker, pharynx, and short cecae. They are monoecious with a single ovary, restricted follicular vitellaria (found in fore-, mid-, or hindbody), and a uterus containing tanned eggs most of which are found in the hindbody. From the ovary the uterus expands into a seminal receptacle and the Laurer's canal empties on the dorsal surface. The uterus empties into the genital atrium near the acetabulum (most often anterior). The male reproductive system comprises 2 testes, with vasa efferentia that meet to form the vas deferens. The vas deferens expands into a seminal vesicle which then narrows and is surrounded by the pars prostatica (Figure 2). The seminal vesicle is contained in a thin membranous sac (pseudocirrus sac) in members of the subfamily Lecithodendriinae; however, it lies free in the parenchyma of members of the subfamily Ophiosacculinae (genera *Ophiosacculus* and *Castroia*–Figure 1D and 1G). A true cirrus (eversible terminal male reproductive tract—vas deferens) is lacking and, therefore, a cirrus sac is lacking; however, the pseudocirrus sac is likely homologous with the cirrus sac of other digeneans. The male and female systems empty into a common genital atrium before exiting the body. The genital atrium of lecithodendriids is most commonly a modest expansion that receives contents from the vas deferens and the metraterm and exits through the genital pore (Figure 2A). However, variations exist in the terminal genitalia among many genera. For example, in *Glyptoporus* (Figure 1A) and *Caprimulgorchis* (Figure 2D) the genital atrium is protrusible and may resemble a cirrus. In other species the atrium is not eversible but is expanded and armed as in *Acanthatrium* (Figure 1E, Figure 2C) or contains a papilla as in *Papillatrium* (Figure 2B). Typically, the excretory bladder is V-shaped and the flame cell pattern is 2 ((2 + 2 + 2) + (2 + 2 + 2)).

Systematics and Taxonomy

The Lecithodendriidae belongs to the superfamily Microphalloidea. The morphological characteristics that hold the Microphalloidea together are few and the best evidence for their relationship is molecular (Olson et al., 2003; Tkach et al., 2003; Bray, 2008). The application of molecular systematics to the Microphalloidea and Lecithodendriidae began in 2000 (Tkach et al., 2000) and continues to help clarify the relationships among the families. Those relationships as well as the content of the families are regularly being revised and undoubtedly will continue to be so for the foreseeable future. Further, more needs to be known of the life cycles and larval characteristics of the lecithodendriids both for possible systematic importance and for understanding the evolution of the group. The microphalloid families for which at least 1 life cycle is known are the Zoogonidae, Pleurogenidae, Prosthogonimidae, Leyogonimidae, Collyriclidae, Phaneropsolidae, and Microphallidae. The families of the Microphalloidea for which no life cycles are known are the Faustulidae, Anenterotrematidae, Eumegacetidae, Exoditdendriidae, and Stomylotrematidae.

An interesting character found only among the Microphalloidea is the virgula organ of the cercaria (Figure 3). The virgula is a mucin reservoir contained in the oral sucker of most members. Lotz and Font (2008) included the Lecithodendriidae among a group of digeneans the members of which possess a virgula in the cercaria. At the time they suggested that the virgula might form a synapomorphy for that group of digeneans. However, based on the phylogeny proposed by

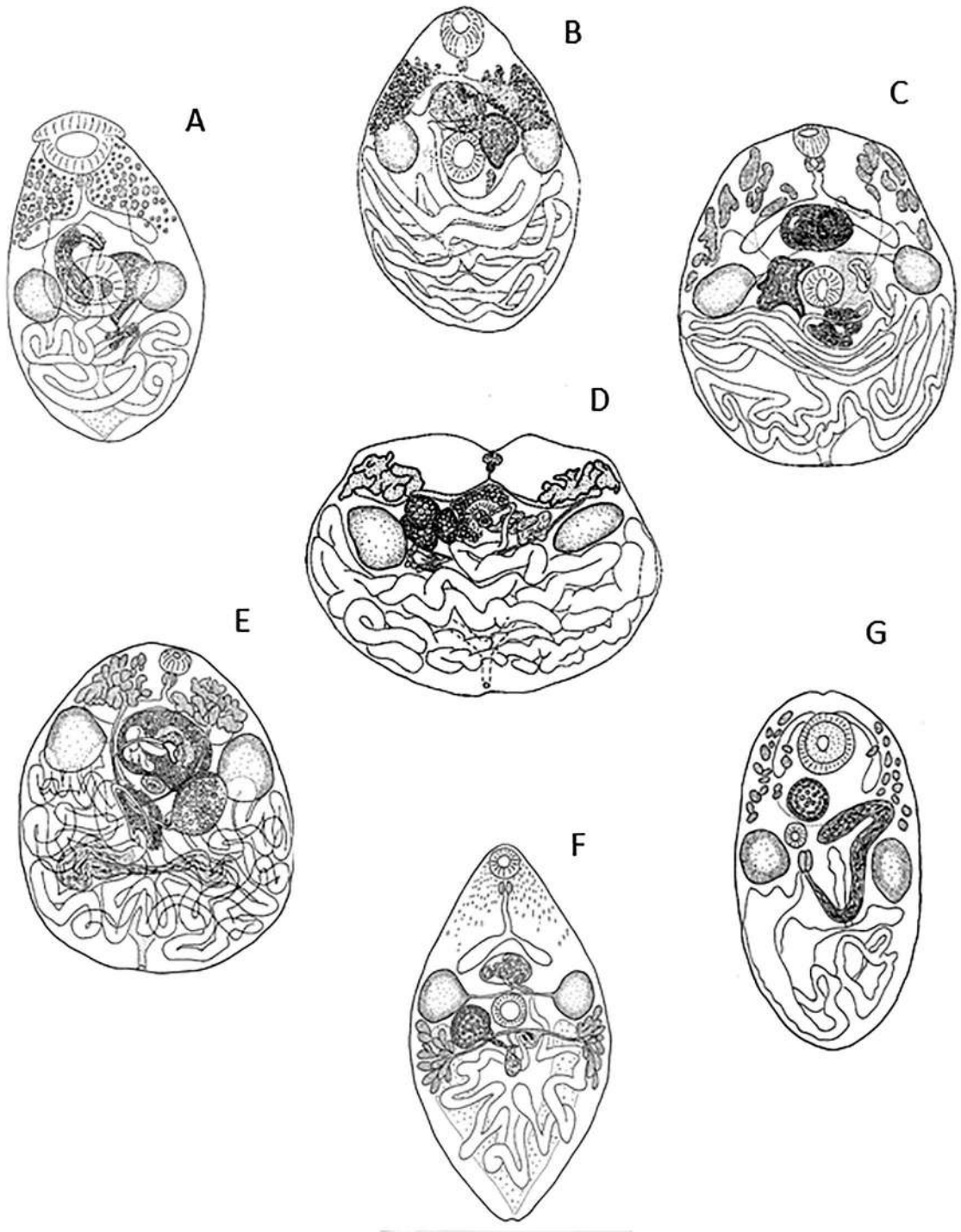

Figure 1. A) *Glyptoporus noctophilus*. B) *Paralecithodendrium swansoni* Macy, 1936. C) *Ochoterenatrema labda*. D) *Castroia silvai* Travassos, 1928. E) *Acanthatrium nycteridis* Faust, 1919. F) *Lecithodendrium linstowi*, Dollfus, 1931. G) *Ophiosacculus mehelyi* (Modlinger, 1930) Macy, 1935. Source: Lotz and Font, 2007. License: CC BY-NC-SA 4.0.

Olson and colleagues (2003) it appears that the virgula has either arisen more than once, has been lost in various clades, or a combination of the two. At least 2 species of Lecithodendriidae (*Paralecithdendrium chilostomum* and *Lecithodendrium linstowi*) (Kudlai et al., 2015; Enabulele et al., 2018) have been shown by molecular matching to lack a virgula in the cercaria. Further, Enabulele and colleagues (2018) found the first intermediate host to be a pulmonate rather than a prosobranch snail (the most common for microphalloids generally). Among the families of Microphalloidea whose life cycles are

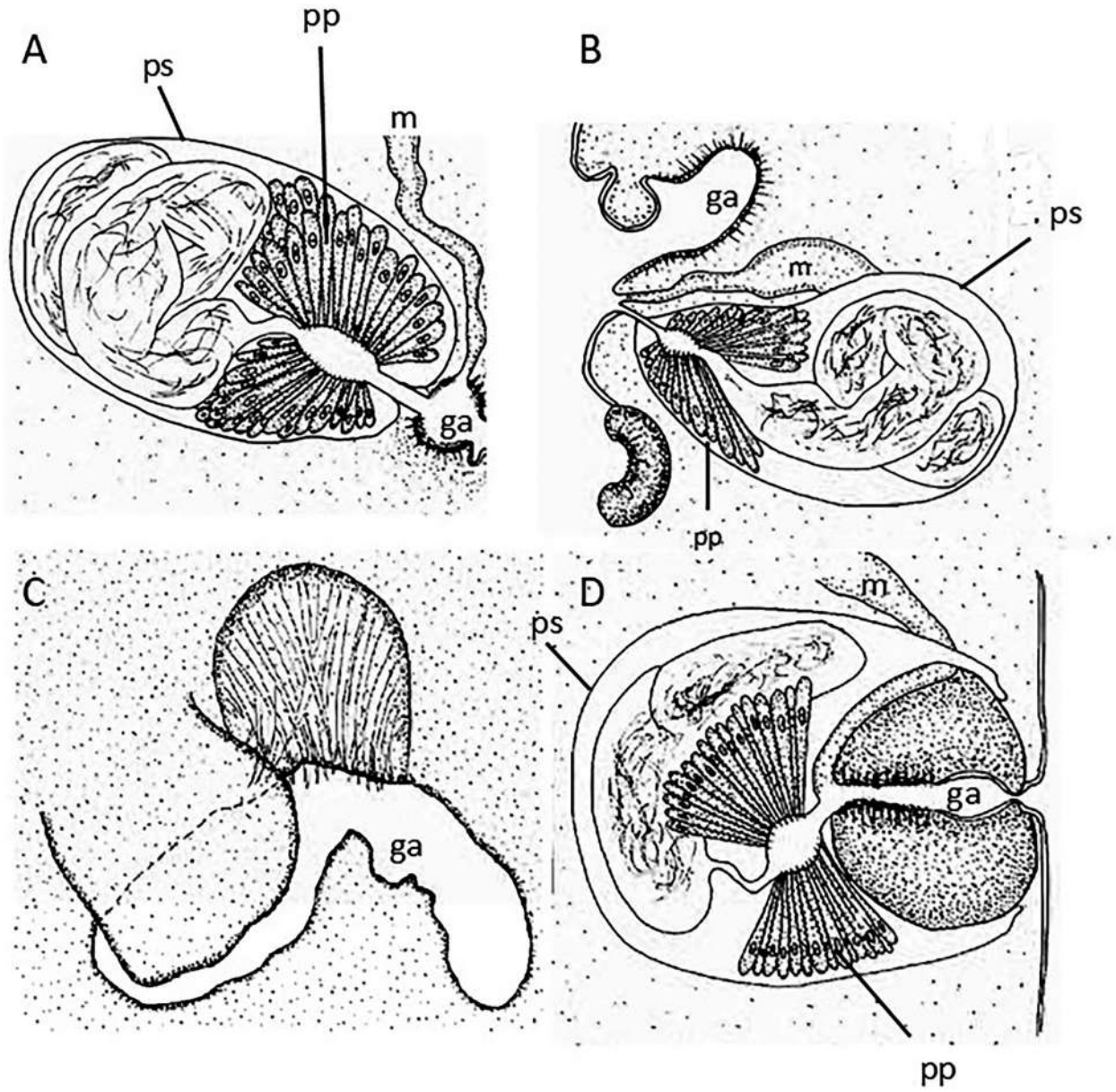

Figure 2. A) *Paralecithodendrium ovimagnosum* (Bhalero, 1926); B) *Papillatrium parvouterus* (Bhalero, 1926); C) *Acanthatrium eptesici* Alicata, 1932; D) *Caprimulgorchis molenkampi* (Lie Kian Joe, 1951). A, B, and D are sagittal sections. C is a ventral view of terminal genitalia of whole mount. Ps: Pseudocirrus sac; m: Metraterm; sv: Seminal receptacle; ga: Genital atrium (hermaphroditic duct if narrowed); pp: Pars prostatica. Sources: A, B, D) Lotz and Palmieri, 1985; C) Lotz and Font, 1983. License: CC BY-NC-SA 4.0.

known, the virgula is absent in the Zoogonidae, Microphallidae, and Prosthogonimidae but present in the Lecithodendriidae, Phaneropsolidae, Collyriclidae, and Pleurogenidae.

Life Cycles

Members of the Lecithodendriidae have a typical digenean 3-host life cycle. Operculated eggs are passed from the definitive chiropteran host. Life cycle studies have not reported whether those eggs contain miracidia at release. Therefore, embryonation of eggs must be determined from examination of eggs from adults. However, it is very difficult to observe the development of the miracidium in the eggs of lecithodendriids because the eggs are small and numerous. The only explicit mention in the literature of egg embryonation in adults was made by Etges (1960) noting that eggs were unembryonated in adult *Acanthatrium anaplocami*. On the other hand, a number of authors have reported intrauterine embryonated eggs in allied families. Hall (1959) reported them in *Mosesia chordeilesia* (a putative phaneropsolid). For pleurogenids they have been reported by Vaucher (1968) in *Paraleyogonimus baeri*,

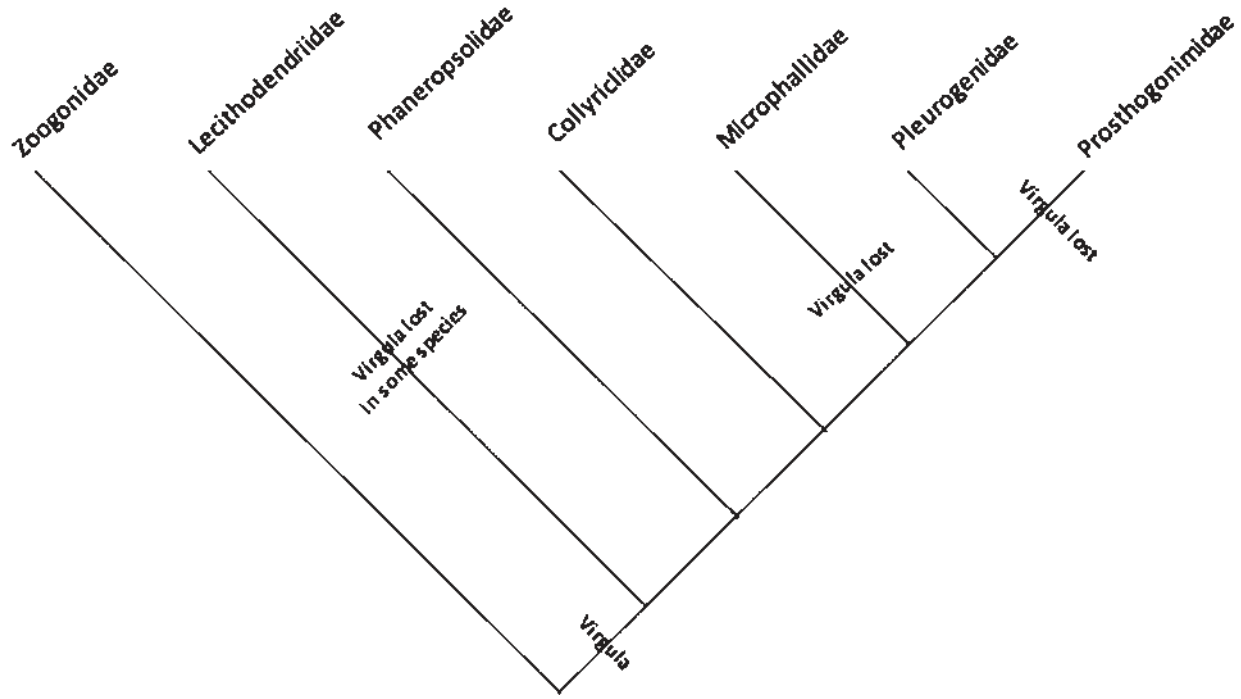

Figure 3. Distribution of virgulate cercariae among families of the Microphalloidea for which cercariae are known. Cladogram of the Microphalloidea. Sources: Adapted from Olson et al., 2013; Kanarek et al., 2014. License: CC BY-NC-SA 4.0.

Madhavi et al. (1987) in *Pleurgenoides orientalis*, Janardanan and Prasadan (1991) in *Pleurgenoides ovatus*, and Świderski et al. (2014) in *Brandesia turgida*.

Studies of lecithodendriid life cycles have rarely addressed infection of snails from eggs or miracidia, therefore, it is rarely known whether an egg hatches a free-living miracidium which penetrates the snail or the egg is ingested before hatching. Although this information does not exist for the Lecithodendriidae, it does for a few allied families. In pleurogenids the eggs hatch only upon ingestion by the snail (Madhavi et al., 1987; Janardanan and Prasadan, 1991; Retnakumari et al., 1991). Further, the egg is ingested for reported life cycles of the Prosthogonimidae and Microphallidae. The first intermediate host of lecithodendriids is primarily a prosobranch snail, although pulmonates have been reported (Enabulele et al., 2018).

Within the first intermediate host the egg hatches and presumably the miracidium penetrates the intestinal wall and becomes a mother sporocyst. It is not known how many generations of daughter sporocysts are produced; however, snails typically harbor numerous daughter sporocysts, suggesting more than 1 generation of daughter sporocysts. Of significance, the sporocysts hold relatively few cercariae. Burns (1961) found 4–20 cercariae in the sporocycts of 5 virgulate digeneans. However, Etges (1960) reported sporocysts with up to 150 developing cercariae for *Acanthatrium anaplocami*.

Following intramolluscan development, cercariae leave the snail host, then seek out and penetrate the second intermediate host. The second intermediate host is the aquatic larva or naiad of an insect. For lecithodendriids second intermediate hosts have been reported from members of the insect orders Diptera, Trichoptera, and Plecoptera (see Brown, 1933; Etges, 1960; Burns, 1961; and El-Naffar et al., 1979). Second intermediate hosts for pleurogenids additionally include Megaloptera, Ephemeroptera, Odonata, Hemiptera, and Coleoptera. Subsequent to metamorphosis of the insect larva or naiad the adult infected host conveys the metacercaria to the definitive host.

The cercaria (Figure 3) is armed and the oral sucker of most members contains a unique mucin reservoir, the virgula. Investigations of the development and function of the virgula have been done most extensively by Kruidenier (1951). The virgula is embedded in the oral sucker ventral to the buccal cavity (Kruidenier, 1951). It is formed quickly in developing cercariae from swelling of the distal ends of pre-virgula mucoid glands during development of the cercariae in the sporocysts (Kruidenier, 1951). The virgula stores mucins that are released from those glands. According to Kruidenier (1951) the virgula contents are used both before and after penetration of the arthropod second intermediate host. However, most of the contents are used after penetration. Presumably the mucins released from the virgula aid in cercarial migration within the second intermediate host but appear not to

aid in penetration per se as the virgula does not diminish in size as the cercaria penetrates into the arthropod second intermediate host.

Burns (1961) noted that when a cercaria of *Acanthatrium oregonense* finds a suitable host it enters through thin portions of the cuticle, such as the gills. Further, he noted that upon contacting the gill, cercariae release mucous threads resulting in a capsule or cyst forming over the larval stage (Figure 4).

This has also been observed for other lecithodendriids and pleurogenids. Burns (1961) observed the cercaria of *Gyrbascus* (= *Allassogonoporus*) *vespertilionis* penetrate its second intermediate host. In this case no external cyst was formed but a mucous layer was secreted that covered the cercaria and appeared to enhance their chance of sticking to the gills of caddisfly larvae. Hall and Groves (1963) confirmed external cyst formation during penetration in several virgulate cercaria at the time of penetration but those cercaria have not been matched to adult worms; presumably they are lecithodendriids or pleurogenids.

Upon penetration, the cercaria of *Acanthatrium oregonense* does not encyst immediately but migrates through the insect's body and may not encyst until after metamorphosis (Brown, 1961). Brown (1961) reported that 31 days after exposure of caddisfly larvae to cercariae only insects that had metamorphosed into adults harbored encysted metacercariae. Those that were still in the larval stage or had developed into pupae harbored motile metacercariae. Etges (1960) found only unencysted metacercariae after exposure of mayfly naiads to cercariae of *A. anaplocami.* Although he never examined adult mayflies he assumed that metacercariae would encyst after metamorphosis. Brown (1933), although never observing the cercaria or performing laboratory studies, examined wild-caught caddisflies and found only unencysted metacercariae of *Paralecithodendrium chilostomum* in caddisfly larvae. However, he found encysted metacercariae in pupal and adult mayflies. On the other hand, El-Naffar and colleagues (1979) found encysted metacercariae in dipteran larvae after exposure to the cercariae of *Lecithodendrium granulosum*; however, only metacercariae from adult mosquitos were infectious to the definitive host.

The life cycles of the virgulate pleurogenids do not appear to have delayed metacercarial encystment. Brown (1961) found *Gyrbascus* (= *Allassogonoporus*) *vespertilionis* to encyst shortly after entry into caddisfly larvae. Macy (1964) reported that metacercariae of *Pleurgenoides tener* encysted in odonate naiads at 5 days and that 5-day-old metacercariae from naiads were infectious to the lizard definitive host. Extended unencysted periods for other pleurogenid metacercariae have not been reported (for example, Grabda-Kazubska, 1971; Brinesh and Janardanan, 2014).

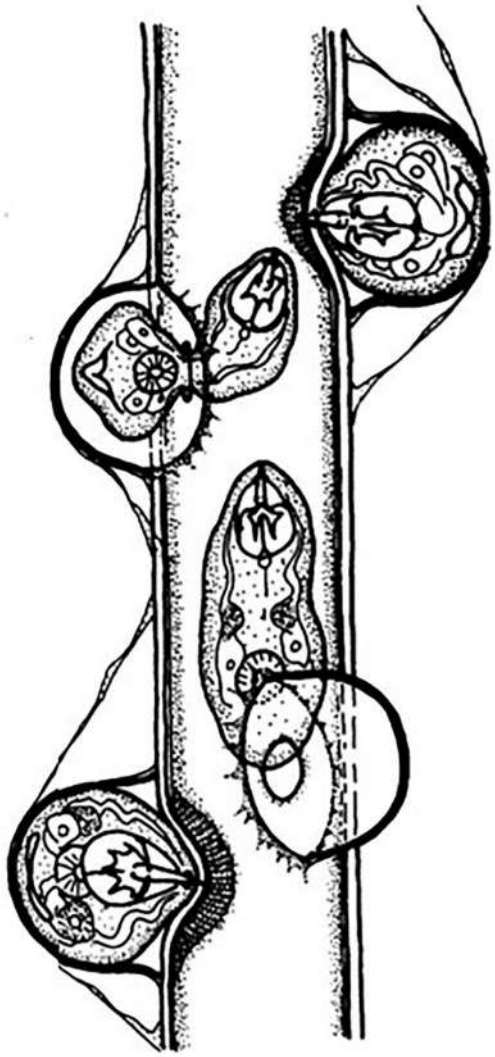

Figure 4. Cercaria of *Acanthatrium oregonense* encysting on and penetrating the gill of a larval caddisfly Source: Adapted from Burns, 1961b. License: CC BY-NC-SA 4.0.

Human Significance

Caprimnulgorchis molenkampi (Lie Kian Joe, 1951) Lotz and Palmieri, 1985 was first described by Lie Kian Joe (1951) from 2 human necropsies in Indonesia. Manning and colleagues (1971) recovered this fluke from 14 human necropsies in Thailand. *Caprimnulgorchis molenkampi* is not considered pathogenic, although high intensities may cause some symptoms. The prevalence of *C. molenkampi* is usually obtained coincidently with surveys for the more pathogenic bile duct and gallbladder inhabitant, *Opisthorchis viverrini*. Chai and colleagues (2009) reported human infections with prevalences of 3.4–24.5% from Laos. They recovered worms after treating stool-sample-positive individuals with an anthelminthic, specifically, Paziquantel.

Manning and Lertprasert (1973) working in Thailand investigated part of the life cycle of *Caprimnulgorchis molenkampi*. They found the rodent *Rattus rattus* and 2 species of bats (*Scotophilus kuhlii* and *Taphosous melanopogon*) to be naturally infected. Lotz and Palmieri (1985) found *T. melanopogon* infected with *C. molenkampi* in Malyasia. Manning and Lertprasert (1973) discovered metacercariae in naiads and adult dragon- and damselflies in Thailand. It is likely that human infections occur throughout southeast Asia, particularly where consumption of odonates is practiced. Manning and Lertprasert (1973) estimated that over a million people in Thailand and Laos may be infected.

Veterinary Significance

The Lecithodendriidae have been implicated in the transmission of Potomac horse fever (PHF), an acute inflammation of the digestive tract producing fever and diarrhea in horses of all ages, as well as abortion in pregnant mares. The causative agent is *Neorickettsia risticii* (order Rickettsiales, family Anaplasmataceae). The intracellular bacterium infects cells, particularly monocytes, of the small and large intestine. The infection results in acute colitis, which is one of the principal clinical signs of PHF (Madigan, 2010).

PHF occurs when horses ingest the reservoir host, a digenetic trematode, as is the case for other species of *Neorickettsia*. As early as 1924 insects (mayflies) were implicated in transmission of PHF, then called horse cholera (Baird and Arroyo, 2013). However, confirmation and the role of digeneans in the disease epidemiology would take some time to work out. Barlough and colleagues (1998) reported that the prosobranch snail, *Juga* spp., was positive for *N. risticii* but did not look for any trematode infections in those snails. However, they did suggest that the rickettsia might actually infect a trematode parasite of the snail, including *Acanthatrium oregonense*. Pusterla and colleagues (2000) successfully transmitted PHF to horses by feeding sporocycsts and cercariae of an unidentified digenean species isolated from naturally infected snails, *Juga yrekaensis*, and re-isolating the bacterium from them. Kanter and colleagues (2000) reported *N. risticii* from an unidentified virgulate cercaria and their sporocysts parasitizing the prosobranch, *Elimia livescens*. Chae and colleagues (2000) detected *N. risticii* (= *Ehrlichia risticii*) in metacercariae in the juveniles and adults of caddisflies, mayflies, damselflies, dragonflies, and stoneflies. Although it is likely that horses acquire infection from ingestion of insects, it is also possible infection could occur by ingestion of infected snail or even free-swimming infected cercariae.

Bats are important in the epidemiology of *Neorickettsia risticii*. Pusterla and colleagues (2003) found *N. risticii* in the lecithodendriids *Acanthatrium* spp. and *Lecithodendrium* spp. inhabiting the intestine of the bat *Myotis yumanensis* collected in northern California, United States. Maintenance of *N. risticii* in the wild is likely enhanced by vertical transmission. Gibson and colleagues (2005) revealed that *N. risticii* is present in the eggs of *A. oregonense* infecting bats providing evidence that it is vertically transmitted in the trematode which contributes to the maintenance of *N. risticii*. Greiman and colleagues (2016) demonstrated that presence of *N. risticii* occurs in all stages of the life cycle of digeneans (*Plagiorchis elegans*) providing further evidence that transmission of the infection may occur from the adult to larvae through the egg and horizontal transmission is not required. Greiman et al. (2017) reported that *N. risticii* was likely worldwide in distribution and consisted of a number of recognizable genotypes.

Ecology

Species of Lecithodendriidae are important components of many parasite community ecology studies. Bats have elevated metabolism and require high caloric intake. As such they are voracious aerial insect feeders and may consume 25–100% of their body weight daily, most coming from insects (Tuttle, 2005; Kunz et al., 2011). The high rate of insect consumption consequently results in high recruitment rates of helminth species that are transmitted by insects to bats, such as Lecithodendriidae and related digeneans. This may result in high diversity and high worm burden in bat helminth infracommunities with up to 11 species in some infracommunities (Coggins et al., 1982; Lotz and Font, 1983; 1991; Pistole, 1988; Estaban et al., 2001; Lord et al., 2012; Warburton et al., 2016a).

A basic question in parasite community ecology is, "What processes structure infracommunity assemblages?" The Lecithodendriidae as components of bat helminth infracommunities have been used to attempt to answer that question. Lotz and Font (1983; 1991; 1994) concluded that infracommunities were most likely the result of random recruitment and within-host interactions were of little importance. The majority of pairs of co-occurring species exhibited no associations; however, of the pairs that did, they found that pairs were more likely to be positively associated rather than negatively associated. Lotz and colleagues (1995) suggested that the structure of helminth infracommunities might be best explained by co-transmission of intermediate stages.

Warburton et al. (2016a; 2016b) examined external factors that might influence infracommunity differences and found that environmental variables, especially amount of land used for human development, explained most differences within a set of helminth component communities. The component communities reflect the pool of helminth species available to

form infracommunities within a geographical site. At the infracommunity level they found that host body condition and host immune response significantly affected total worm burden and, likely, community structure.

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Digenea, Plagiorchiida

Xiphidiata

Opecoelidae Ozaki, 1925 (Family): The Richest Trematode Family

Storm B. Martin

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Family Opecoelidae

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Chapter 46

Opecoelidae Ozaki, 1925 (Family): The Richest Trematode Family

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Introduction

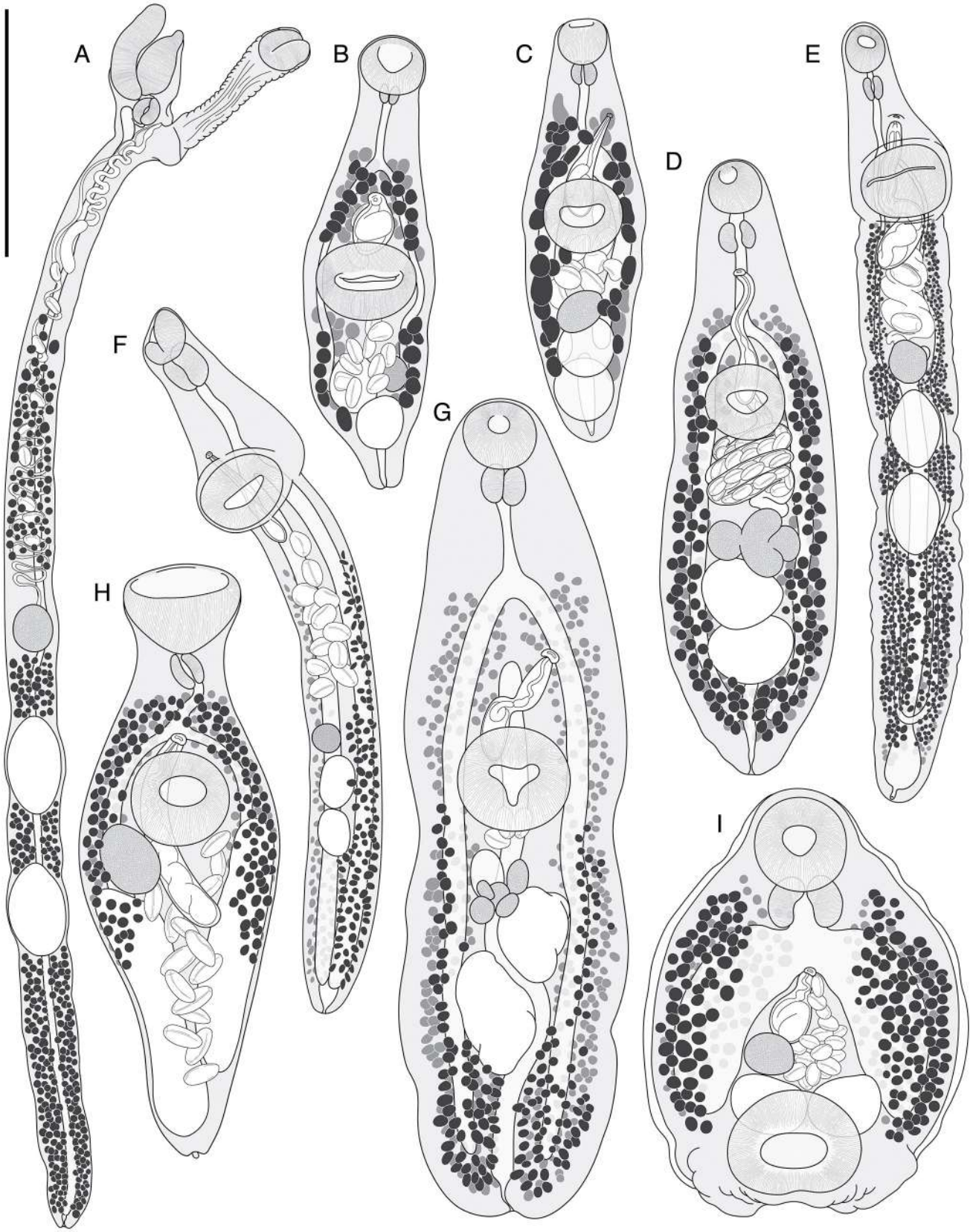
The Opecoelidae Ozaki, 1925 is the richest of all trematode families. It comprises over 1,000 described species presently arranged into about 100 genera. Adult opecoelids are benign endoparasites, typically residing in the intestines, pyloric ceca, or rectum of phylogenetically and ecologically diverse teleost fishes worldwide. They exploit both marine and freshwater fishes and are among the best represented trematode lineages known from polar and deep sea fishes (Bray, 2004; Faltýnková et al., 2017; Martin et al., 2018d). Therefore, although no opecoelids are known to have any economic importance, they are often among the lineages of trematodes most frequently encountered by ichthyoparasitologists in the field.

Identifying the Opecoelidae

Although opecoelids are a hugely speciose and evolutionarily derived group, they are neither diverse nor specialized in their morphology relative to other trematode lineages. Nevertheless, opecoelids are usually readily recognizable, even under stereomicroscope in the field, by the combination of some general characters together with the absence of certain specialized characters that are seen in other groups (select opecoelids depicted in Figure 1). Most species are 1–5 mm in length, although a few apparently never exceed 1 mm, for example, *Choerodonicola arothrokoros* Martin et al., 2018 (Martin et al., 2018a), species of *Fairfaxia* Cribb, 1989 (Cribb, 1989; Hassanine and Gibson, 2005), and some species of *Plagioporus* Stafford, 1904 (Fayton et al., 2017; 2018), and specimens of some species may exceed 5 mm, for example, species of *Macrourimegatrema* Blend et al., 2004 (Blend et al., 2017) and some species of *Hamacreadium* Linton, 1910 (see Bray and Justine, 2016; Martin et al., 2017b). Most opecoelids are dorsoventrally flattened and elongate-oval, oval, or linguiform. The body may also be elongate and subcylindrical or squat and robust to almost round. The tegument is never spinous, although species of *Poracanthium* Dollfus, 1948 (subfamily Opecoelinae) possess specialized spines surrounding the genital pore (Cribb, 2005a). In some other species, especially those of the subfamily Opistholebetinae, the tegument may be thick, wrinkled, or rugose, and in *Scorpidotrema longistipes* Aken'Ova & Cribb, 2003 and species of *Holsworthotrema* Martin et al., 2018 (subfamily Stenakrinae), it is covered by small, fine projections (Martin et al., 2018d).

Opecoelids have 2 usually-large suckers. The oral sucker is always anteriorly terminal or subterminal and the ventral sucker is usually larger and typically situated in the anterior half of the body. In species of several unrelated genera, the ventral sucker is supported by a long peduncle, while in others it may protrude prominently from the ventral body surface, be surrounded by fleshy tegumental folds or obvious muscle fibers, or be provided with papillae (Cribb, 2005a). In species of some genera belonging to the subfamily Opistholebetinae, the ventral sucker is situated in the posterior half of the body (such as *Heterolebes* Ozaki, 1935 and *Pseudoheterolebes* Yamaguti, 1959) or even near the posterior extremity, such as *Opistholebes* Nicoll, 1915 and *Parallelolebes* Martin et al., 2018 (see Martin et al., 2018e). Opecoelids always have a well-developed pharynx and a bifurcated intestine. The ceca usually reach near to the posterior extremity and may be blind, open into separate ani, or may unite, in which case they may form a cyclocoel, open into a common anus, or open into the excretory vesicle to form a uroproct (Cribb, 2005a). The excretory vesicle is probably always tubular, although in many opecoelids it has not been described and in others it has been reported as Y-shaped. *Pacificreadium serrani* (Nagaty & Abdel Aal, 1962) is exceptional in that its excretory vesicle is diverticulate anteriorly. The length of the excretory vesicle is important for distinguishing some genera; in most opecoelids it terminates anteriorly at about the level of the ovary, but in some species, especially freshwater taxa belonging to the subfamily Plagioporinae, it is shorter, and in others it enters the forebody. The excretory pore is usually terminal posteriorly, sometimes subterminal.

Opecoelids are simultaneous hermaphrodites. The male reproductive system consists of the testes connected via the vas deferens to the terminal genitalia. The testes are always situated in the posterior half of the body and are usually 2, although species belonging to 2 probably unrelated genera, *Decemtestes* Yamaguti, 1934 and *Helicometrina* Linton, 1910, have approximately 10. The male terminal genitalia include the seminal vesicle, a sperm storage organ, and the cirrus, an eversible copulatory organ, all of which may (or may not) be entirely or partially enclosed in a muscular, or sometimes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membranous, cirrus sac (Cribb, 2005a). The ejaculatory duct runs through the cirrus and opens, together with the uterus, into a common genital atrium. The genital pore is always ventral and in the forebody (that is, anterior to the ventral sucker). The single ovary may be smooth to deeply lobed and round to irregular. It is usually situated anterior to the testes, although in species of *Hysterogonia* Hanson, 1955, *Pseudoplagioporus* Yamaguti, 1938, *Sphaerostoma* Rudolphi, 1809, and *Urorchis* Ozaki, 1927 it is between the testes and in *Orthodena tropica* Durio & Manter, 1968 it lies beside the testes. The ovarian complex may or may not include a canalicular seminal receptacle, a specialized envagination of the Laurer's canal. The vitellarium includes fields of typically numerous, dense follicles. These are usually extensively distributed and the precise distribution is frequently important for distinguishing genera and species. The uterus is usually restricted in distribution to the intercecal zone between the gonads and cirrus sac, although in species of some genera it may extend beyond the ceca laterally or between or beyond the testes posteriorly. Eggs are tanned, operculate, and unembryonated. Exceptionally small eggs, < 30 µm-long, are diagnostic for 2 genera, *Choerodonicola* and *Diplobulbus* Yamaguti, 1934 (see Cribb, 2005a). Likewise, filamented eggs are a defining characteristic of the subfamily Helicometrinae, but bifilamented eggs also occur in species *Diplobulbus*.

In the field, it is often necessary to distinguish opecoelids from taxa belonging to the Fellodistomidae Nicoll, 1909, Lecithasteridae Odhner, 1905, Lepocreadiidae Odhner, 1905, Monorchiidae Odhner, 1911 and Zoogonidae Odhner, 1902, other rich and frequently encountered groups exploiting the intestine of teleost fishes. Lepocreadiids, monorchiids, and zoogonids have a spinous tegument. The ventral sucker in lepocreadiids and monorchiids is also usually much smaller relative to that of most opecoelids and remnant eye spot pigment is often visible in the forebody. The fellodistomids and lecithasterids have a smooth tegument and usually a large ventral sucker similar to that of opecoelids. Distinguishing these groups from opecoelids requires assessment of some internal characters and can therefore be more difficult. Typically, compared with most opecoelids, the distribution of the vitelline follicles in both fellodistomids and lecithasterids is highly restricted and the distribution of the uterus is much more extensive. This distinction also usually applies to the Monorchiidae and Zoogonidae.

Smaller groups exploiting fishes which may potentially be confused with opecoelids are the Acanthocolpidae Lühe, 1906, Allocreadiidae Looss, 1902, Apocreadiidae Skrjabin, 1942 and Enenteridae Yamaguti, 1958. All acanthocolpids and many apocreadiids have a spinous tegument. However, in some acanthocolpids, specifically of the genus *Acanthocolpus* Lühe, 1906, the spines are easily lost during handling and fixation of the specimens and may be mistakenly identified or described as opecoelids (see Bray and Gibson, 1991; Martin et al., 2018c). Some enenterids are highly similar to some opecoelids, but they are a small group specializing mainly in 1 small family of herbivorous fishes, the drummers (Perciformes: Kyphosidae) (Bray and Cribb, 2001). Likewise, the allocreadiids are now recognized as a relatively small group restricted to freshwater fishes (Cribb, 2005b). Many allocreadiids can be distinguished from opecoelids by the presence of a remnant eye spot pigment, a papillate oral sucker, or an extensive uterus, but others are less distinctive and more closely resemble generalized opecoelids.

The unspecialized morphology of opecoelids is perhaps best exemplified by comparison to species of *Biospeedotrema* Bray et al., 2014 and *Zdzitoweickitrema incognitum* Sokolov et al., 2018. These taxa are known only from deep sea fishes and are morphologically indiscernible from the opecoelids (Bray et al., 2014; Sokolov et al., 2019). However, phylogenetic analyses suggest closer affinity with the Gorgoderoidea (Sokolov et al., 2019). Thus, these enigmatic taxa are presently without a suitable family designation.

Figure 1. Select representative taxa belonging to the Opecoelidae: A) *Pseudopecoeloides tenuis* Yamaguti, 1940 (subfamily Opecoelinae), original ex. *Priacanthus macracanthus* Cuvier, the spotted bigeye, collected in Moreton Bay, Australia; B) *Fairfaxia lethrini* Cribb, 1989 (subfamily uncertain), original ex. *Lethrinus nebulosus* (Forsskål), the spangle emperor, collected off Lizard Island, Australia; C) *Plagioporus ictaluri* Fayton et al., 2018 (subfamily Plagioporinae); D) *Helicometra* sp. cf. *H. fasciata* (Rudolphi, 1819) Odhner, 1902 (subfamily Helicometrinae), original ex. *Thalassoma lunare* (Linnaeus), the moon wrasse, collected off Heron Island, Australia; E) *Bathycreadium brayi* Pérez-del-Olmo et al., 2014; F) *Polypipapiliotrema citerovarium* Martin et al., 2018 (subfamily Polypipapiliotrematinae), original ex. *Chaetodon quardimaculatus* Gray, the fourspot butterflyfish, collected off Ra'ivāvae, Austral Archipelago, French Polynesia; G) *Hamacreadium* sp. cf. *H. mutabile* Linton, 1910 (subfamily uncertain) (original ex. *Lutjanus carponotatus* (Richardson), the Spanish flag snapper, collected off Heron Island, Australia; H) *Hexagrammia longitestis* Schell, 1973 (subfamily Stenakrinae); I) *Opistholebes amplicoelus* Nicoll, 1915 (subfamily Opistholebetinae), original ex. *Tetractenos hamiltoni* (Richardson), the common toadfish, collected in Moreton Bay, Australia. Scale bar: 0.5 mm. Sources: A, B, D, F, G, I) S. B. Martin; C) Adapted from Fayton et al., 2018; E) Adapted from Pérez-del-Olmo et al., 2014; H) Adapted from Schell, 1973.

Systematics and Taxonomy

The morphological similarity between allocreadiids and opecoelids is reflected in the confused taxonomic history of these groups. Many opecoelid genera were originally proposed in the Allocreadiidae and, until recently, most authors considered the Opecoelidae and Allocreadiidae to be closely related, belonging to the superfamily Allocreadioidea Looss, 1902, together with the Acanthocolpidae and Brachycladiidae Odhner, 1905 (see Cribb, 2005a; 2005b). Although phylogenetic relationships among families within the Xiphidiata are not yet entirely resolved, combined evidence from recent analyses (Olson et al., 2003; Bray et al., 2005; 2009; Curran et al., 2006; Littlewood et al., 2015) demonstrate that true allocreadiids are not especially closely related to opecoelids. Instead, they resolve as sister to the Gorgoderidae Looss, 1899 and, thus, the Allocreadioidea is best considered synonymous with the Gorgoderoidea Looss, 1899 (see Littlewood et al., 2015). The Acanthocolpidae and Brachycladiidae are closely related and are now combined into the superfamily Brachycladioidea Odhner, 1905. The opecoelids appear to be closer to this group than to the gorgoderoids (Olson et al., 2003), but are sufficiently distinctive such that they were recognized by Littlewood and colleagues (2015) in a separate superfamily, the Opecoeloidea Ozaki, 1925. However, the establishment of Opecoeloidea is not a new concept; the separation between the Opecoelidae and Allocreadiidae was appreciated much earlier by some taxonomists, specifically Cable (1956) and Dollfus (1959).

Life Cycles

Opecoelid life cycles, where known, usually involve 3 hosts (Figure 2). Eggs are passed with feces of the definitive host, which is always a teleost fish, and miracidia hatch from the eggs and seek and penetrate the first intermediate host, which is always a gastropod (Cribb, 2005a). Within the gastropod, the miracidium develops into a mother sporocyst which produces more sporocysts. These may be mother sporocysts themselves or may be daughter sporocysts, which produce cercariae (Cribb, 1985).

Opecoelid cercariae lack eye spots, possess a penetration stylet, and, usually, have a stumpy, cup-shaped tail. These cercariae do not swim, but crawl in a leech-like manner (Cribb, 2005a). However, one cercaria, that of *Helicometra gibsoni* Meenakshi et al., 1993, has a very long tail (Meenakshi et al., 1993). It belongs to what is potentially the most basal opecoelid lineage, the subfamily Helicometrinae, leading to intriguing speculation as to the original tail condition in the earliest opecoelids.

Opecoelid cercariae penetrate and encyst as metacercariae in a wide variety of second intermediate hosts, including crustaceans, aquatic insects, oligochaetes, echinoids, gastropods, scleractinian anthozoans, and fishes (McCoy, 1930; Meenakshi et al., 1993; Aeby, 1998; Jousson et al., 1999; Cribb, 2005a; Yoshida and Urabe, 2005; Yano and Urabe, 2017; Martin et al., 2018b). The metacercariae reach the definitive host via trophic transmission. This transmission is usually passive, however, in the case of *Polypipapiliotrema stenometra* (Pritchard, 1966) (subfamily Polypipapiliotrematinae Martin et al., 2018), infection with metacercariae increases the chance of the second intermediate host, reef-building corals of the genus *Porites*, being preyed upon by the definitive hosts, corallivorous butterflyfishes (Chaetodontidae) (Aeby, 1998; 2002). Infection of the coral polyp by the metacercaria triggers a growth response (possibly an immune response) which causes pink discoloration and abnormal growth such that the polyp may be unable to retract into its calyx (Cheng and Wong, 1974; Aeby, 1998). Thus, infected polyps are both more vulnerable and more nutritious, and this change in condition is prominently advertised to the butterflyfishes, which preferentially prey on the infected polyps (Aeby, 2002).

Facultatively progenetic life cycles, where eggs are produced and released by precocial metacercariea within the second intermediate host, have been documented for at least 9 opecoelid species belonging to 6 genera (Lefebvre and Poulin, 2005). In these species, the definitive host may be skipped. Exceptionally, in the life cycle of *Plagioporus sinitsini* Mueller, 1934, the second intermediate host, or even both the second intermediate and definitive host, may be facultatively skipped (Barger and Esch, 2000). In this species, cercariae may develop into metacercariae within the daughter sporocyst which emerges from the gastropod and is consumed directly by the definitive teleost host. However, the metacercariae may also develop into adult worms which produce eggs within the daughter sporocyst; the sporocyst emerges from the gastropod and releases miracidia ready to infect the next gastropod (Barger and Esch, 2000).

The nature of the opecoelid life cycle means that definitive teleost hosts are overwhelmingly predators or omnivores, but several opecoelid species belonging to genera in the Opecoelinae and Helicometrinae subfamilies have been reported from herbivorous perciform fishes, namely Acanthuridae (surgeonfishes), Blenniidae (blennies), Girellidae (luderick), Scaridae (parrotfishes), and Siganidae (rabbitfishes). Some opecoelids even appear to specialize in such fishes. Two species of *Choerodonicola* and 3 species of *Diplobulbus* are known only from fishes of the family Scaridae (Yamaguti, 1934; 1942; 1952; Martin et al., 2018a), and species of *Holsworthotrema* and *Scorpidotrema* (monotypic) (subfamily Stenakrinae) are known only from species of Kyphosidae (drummers) and a species of Scorpidiidae (sweep),

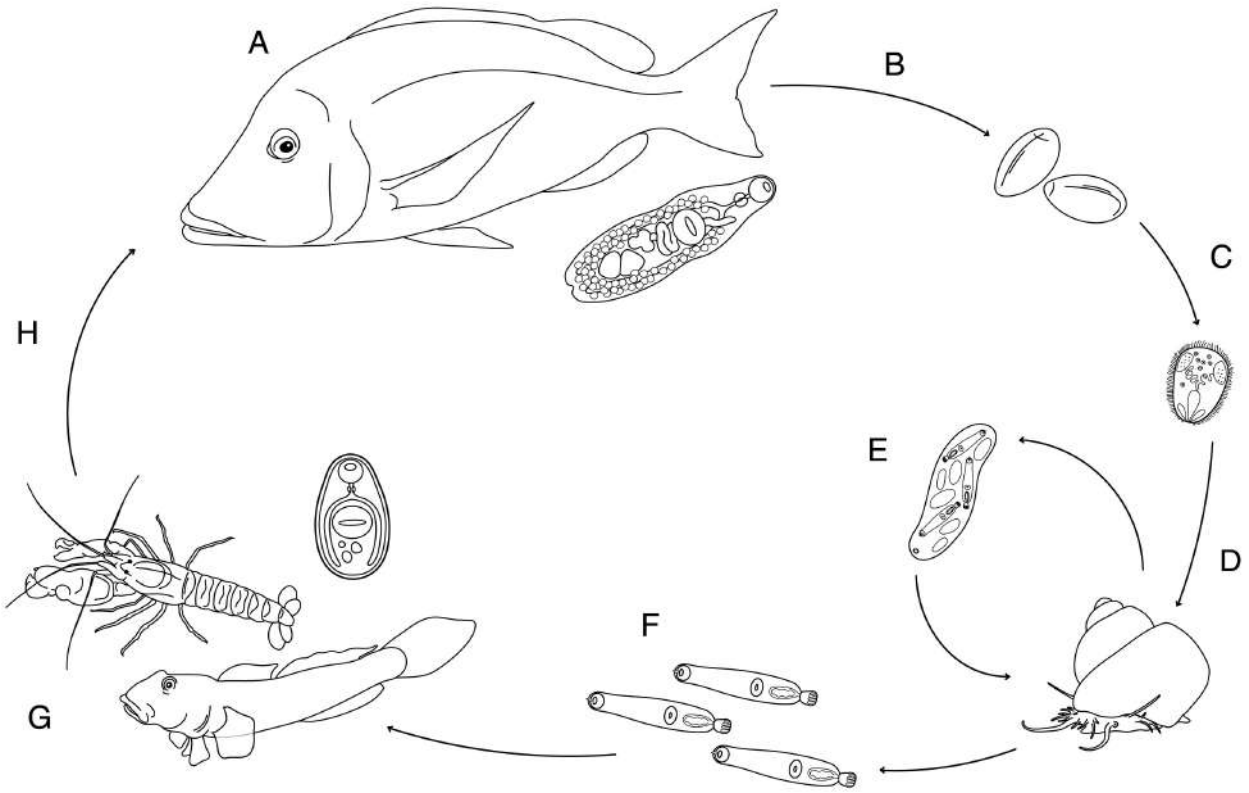

Figure 2. Generalized opecoelid life cycle. A) Adult opecoelids reside within the intestines of teleost fishes; B) Opecoelid eggs are passed to the environment in definitive host feces; C) Eggs hatch in the environment, giving rise to the ciliated miracidia larvae; D) Miracidia seek and penetrate the first intermediate host, which is always a gastropod; E) Within the gastropod, the miracidia sporocyst. Asexual reproduction produces generations of sporocysts, some of which produce cercariae; F) Cercariae emerge from the first intermediate host and seek and penetrate the second intermediate host using a stylet. Most opecoelid cercariae have a stumpy tail and do not swim but crawl; G) Various groups of invertebrates, and sometimes vertebrates, are exploited as intermediate hosts. The cercaria encysts within host tissue and develops into the metacercaria; H) Metacercariae are transmitted to the definitive host when they prey on an infected second intermediate host. If this fish is an appropriate (physiologically compatible) definitive host, the metacercariae may mature into reproductive adults. Source: S. B. Martin. License: CC BY-NC-SA 4.0.

respectively (Martin et al., 2018d). It is not known how these trematodes reach their definitive hosts. Presumably they penetrate invertebrates living among algae which are incidentally ingested by the definitive fish hosts, but it is also possible that they have secondarily adopted a 2-host life cycle with direct encystment of metacercariae onto algae or the substrate. This strategy is known for other trematode families specializing in herbivorous fishes such as the Atractotrematidae (Huston et al., 2018), Gorgocephalidae (Huston et al., 2016), Gyliauchenidae (Al-Jahdali and Hassanine, 2012), Haplosplanchnidae (see the Haplosplanchnata chapter for more information), and Microscaphidiidae (Hassanine et al., 2016). Intriguingly, the cercaria has been described for one of the species of *Choerodonicola* and, unlike most other opecoelid cercariae, it lacks a stylet, the specialized organ used to penetrate the second intermediate host (Martin et al., 2018a).

Host Range

The host range of opecoelids across various species of fishes is variable and a complete understanding of patterns within the family, as in many other trematode groups, is hampered by a multitude of dubious or unsubstantiated records, poor descriptions, and the persistence of many polyphyletic genera (these are genera in which species are lumped by researchers even though there is no phylogenetic/evolutionary ancestor descendant relationships among the species). However, it seems that for the most part, host range is very low, especially for species from tropical marine systems. For example, on the Great Barrier Reef, Australia, only 2 opecoelids, *Helicometra fasciata* (Rudolphi, 1819) and *Trilobovarium parvvatis* Martin et al., 2017, are reliably known from fishes belonging to more than a single family (Miller et al., 2011; Martin et al., 2017a). Compelling cases for lower

specificity are more frequent among taxa exploiting freshwater, deep sea or polar fishes.

Phylogenetics

Determination of phylogenetic affinities among opecoelid genera and the identification of major lineages within the family is an area of active study for the Opecoelidae. Since the early 1980s the organization of the Opecoelidae has been dominated by a 4-subfamily classification hypothesis established by Gibson and Bray (1982; 1984). This hypothesis is based principally on the presence versus absence of 2 features of the adult worm, a well-developed cirrus sac and a canalicular seminal receptacle. However, with increasing availability of phylogenetically informative rDNA sequence data, recent analyses have demonstrated that this classification does not adequately reflect the evolutionary history of the group (Bray et al., 2016; Fayton and Andres, 2016). Consequently, the classification of the Opecoelidae is rapidly being revised; it presently comprises 9 subfamilies (Bathycreadiinae, Helicometrinae, Opecoelinae, Opecoelininae, Opistholebetinae, Plagioporinae, Podocotylinae, Polypipapiliotrematinae, and Stenakrinae), although analyses of currently available sequence data, both published and unpublished, suggest that at about 14 subfamilies might be required.

These analyses do not suggest that the morphological characters used by Gibson and Bray (1982; 1984) are not informative at the subfamily level, but rather that these characters, as well as others of adult worms, together with consideration for the ecological and phylogenetic groups of hosts exploited must all be considered (Martin et al., 2018d). In particular, it appears that radiation of some major lineages within the family occurred following switches of the second intermediate hosts exploited. Thus, the Opecoelinae, Helicometrinae, and Podocotylinae appear to exploit only crustacean second intermediate hosts, whereas the Plagioporinae, a freshwater group, use aquatic insects and annelids, the Opistholebetinae are only known to use hard-bodied invertebrates, namely gastropods (snails) and echinoids (urchins), the Polypipapiliotrematinae are the only known trematodes to exploit scleractinian anthozoans (corals), and species of an as yet unnamed clade appear to specialize in using small fishes (Martin et al., 2018b; 2018d; 2018f). This diversity in second intermediate host groups exploited has almost certainly been an important driver for the huge richness and success of the family. However, it must be appreciated that these patterns are based on few known life cycles, especially when considered against the enormous richness of the family. Therefore, the elucidation of further life cycles will most likely prove crucial for understanding the phylogenetic organization of the Opecoelidae and interpreting the evolutionary history of lineages within the group.

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Digenea

Summary of the Digenea (Subclass): Insights and Lessons from a Prominent Parasitologist

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Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

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Chapter 47

Summary of the Digenea (Subclass): Insights and Lessons from a Prominent Parasitologist

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Introduction

Digeneans serve as marvelous parasites to study because they are so very diverse. Part of this diversity exists because they infect both intermediate and final hosts. Intermediate hosts include the first intermediate host, a mollusc, except for a few that use polychaetes. Second intermediate hosts include many groups of invertebrates, as well as many vertebrates. Final hosts include fishes, amphibians, reptiles, birds, and mammals.

Considerable knowledge about digeneans has resulted from United States federal funding directed toward schistosomiasis. This has helped understanding of the group, but species of the genus *Schistosoma* are somewhat unusual for digeneans in that they have separate male and female individuals, rather than each individual being hermaphrodites, and that they live in blood vessels where direct competition among other helminths is limited or nonexistent for the most part.

Several higher level taxa will be discussed in this chapter, primarily following the classification of Littlewood and colleagues (2015), and will be discussed with some addenda toward the end. When reading through the chapter, note that there will often be exceptions for any statement.

Morphology

Very little is actually known about most morphological structures of digeneans. In some cases, the structures are very similar to those found in other taxa of Platyhelminthes but offer opportunities for intriguing studies for students to compare features of specific digeneans with those of the other digeneans or other Platyhelminthes. And in other cases, the function of the various structures offers wonderful opportunities for investigation.

To learn more than what is covered here, names and features of morphological structures of adult digeneans occur in many other textbooks, albeit those for the same or similar structures may differ and for different structures may be referenced as the same (for example, Ginetsinskaya, 1988; Noble et al., 1989; Roberts and Janovy, 2013). Names and morphological features may also be found in reference books (for example, the CAB International 3-volume series Keys to the Trematoda, edited by Gibson et al., 2002; Jones et al., 2005, and Bray et al., 2008, as well as important treatments by Yamaguti, 1971; 1975) and articles (for example, Manter, 1970; Gibson and Bray, 1979; and Bullard and Overstreet, 2008).

Following are descriptions of some tegumental features, including spines (and other attachment structures), the alimentary tract, and the reproductive system, including eggs.

Tegumental Features

The tegument of a trematode is the outer body structure that interfaces with the host, providing both protection and some structural integrity to the trematode. The tegument (integument) is a complex structure. Over 100 years ago, Pratt (1909) described the cuticle and subcuticule of trematodes and cestodes. He, however, showed that rather than being cuticles, the outer layer of tissue in both served as teguments formed from parenchymal cells or their secretions, with the subtegument (as subcuticle) actually consisting of parenchymal cells.

Long after Bils and Martin (1966) set a groundwork for study of the ultrastructure, Świderski and colleagues (2013) described the tegument of the microphallid *Maritrema feliui* as consisting of a 2-layered syncytial epithelium. The outer layer consists of an external anucleate cytoplasmic region connected to an inner layer of nucleate perikarya (cytons) deeply embedded in the cortical parenchyma. The plasma membrane of the surface contains deep invaginations in which pinocytosis occurs and also 2 types of tegumental spines. The inner layer produces disc-shaped granules that are passed on to the surface layer. Those authors remind us of the publication by Schulte and colleagues (2013), who point out that the most efficacious schistosome vaccines thus far developed are directed against tegumental structures. Published research on the tegument of spineless paramphistomes suggests there exist 4 layers, with the innermost layer resting on and coupling with a thick basal lamina (Anuracpreeda et al., 2014). It may be that the abundance of negative

Box 1. Notes on the Study of Digenean Biology

Nollen conducted a variety of relatively simple elegant studies providing valuable results on the biology of digeneans. Nollen (1983) also reviewed patterns of sexual reproduction among the digeneans, monogeneans, and cestodes as well as isotopic labeling techniques useful to evaluate spermatogenesis, oogenesis, and mating in a variety of different species. Fried also conducted simple elegant studies, usually collaborating with students (for example, Fried and Rosa-Brunet, 1991). For example, they describe a simple way of cultivating echinostome metacercariae into relatively large, ovigerous adults, albeit with eggs not necessarily producing developed embryos. The method involved excysting metacercariae on a chick chorioallantoic membrane maintained at 38.5 °C and a relative humidity of 60 to 65%. They noted worm length increased from 0.5 mm at 2 days to about 3.0 mm at 6 days post-inoculation. When they transferred the immature adult to a second membrane, it reached 6 mm and was producing over 100 eggs in 17 days. These worms can be used for a variety of studies.

Nollen extracted adult philophthalmids, also relatively large worms related to the echinostomes, from under the nictitating membrane of chickens, conducted initial phase experiments on them, and then replaced the individuals adjacent to the eye.

Overstreet found that he could obtain adult microphallids of some species to study by placing the encysted progenetic metacercariae in saline in a glass stender dish into a 40 °C waterbath for a few hours to days. If the metacercariae did not hatch on their own, he added a small amount of trypsin or trypsin with ox bile salts, which rapidly digest the outer host portion of the cyst wall; once hatched, the metacercariae have to be maintained in fresh, warm saline or a cell culture solution like medium 199 and allowed to produce eggs over a 1–5-day period. There are lots of tricks enabling one to obtain healthy, living worms for study. Since it is difficult to rear most trematodes, Overstreet found that placing 1 or more individuals into dialysis tubing; tying off both ends; surgically implanting the tube into the body cavity of an appropriate host such as a chicken, rat, or large fish; and then later, surgically removing the tube with the developed worms protected from the host cellular response provides a useful method.

charges on the surface could protect paramphistomes from immune attacks by the host. This research as well as that of a related species also show the variety of papillae and tegumental folds (Panyarachun et al., 2010). These structures can be compared with those of the spined tegument of *Depropristis inflata* (Depropristidae) from the eel *Anguilla anguilla* (see Filippi et al., 2013).

Spines (and other attachment structures)

As mentioned above, spines occur in the tegument of many digeneans. Different digeneans have different shaped body spines that presumably have different functions, including moving, attaching, and feeding. Usually in the adult there exists an embedded basal portion of the spines, but some have completely embedded spines. Radev and colleagues (1998) described 4 different types of spines in the eye fluke *Philophthalmus hegeneri*: 1) Circular and scale-like; 2) scale-like with distal points; 3) oval with a distal spine; and 4) spine-like with 1–6 segments. Some spines are covered and do not protrude above the tegumental surface while others have the basal portion only embedded. Overstreet and Heard (1995) show scanning electron microscope images (SEMs) of differences of scale-like spines at 3 levels of an individual of *Megalophallus reamesi* (Microphallidae). *Maritrema madrynensis* also has a variety of scales and spines (Diaz and Cremonte, 2010). The spines of *Cryptocotyle lingua* cercariae are shed shortly after penetration and encystment in the fish host (Køie, 1977). Some body spines in adults do not occur near the posterior end, near the ventral sucker, or on the dorsal surface; these placements are often important taxonomic features in closely related species, and in some species they occasionally occur inconsistently. See Table 1 for an example of a comparison of body spines in members of one genus.

Not all trematodes have external spines, especially those like hemiuroids, most of which inhabit the high-acid stomachs of their fish hosts. Species of other families have an enlarged ring of oral tegumental spines such as some echinostomes, heterophyids, cryptogonimids, and others. Monorchiids have various tegumental spines as well as various shaped spines in the terminal genitalia, including beautiful rose-thorn-shaped spines used in copulation (for example, Overstreet and Brown, 1970). The shape, size, and

Table 1. Examples of body spines in members of the genus *Homalometron* (see Overstreet, 1969; Curran et al., 2013a; Fayton et al., 2016).

Species name	Description of tegumental spines
Homalometron foliatum	Cover the entire immature specimen but just near the testicular level
H. cryptum	Entirely absent in the thick tegument
H. robisoni	Limited to anterior 13%
H. palmeri (phenotypically similar to *H. pallidum*)	Measure 12–17 µm long, broad, and scale-like over the entire body
H. pallidum (phenotypically similar to *H. palmeri*)	Measure 6–9 µm long, delicate, and mostly devoid in the posterior half

distribution of spines on the cirrus of *Maritrema madrynensis* are the primary means of distinguishing it from a closely related species (Diaz and Cremonte, 2010). Students of parasitology interested in structure and importance of terminal genitalia (including spines on the cirrus) should also read the literature dealing with these features in other groups (for example, Doe and Smith, 2016).

Some trematodes attach to various places in the host by means of adhesive organs. For example, Erasmus and Öhman (1965) conducted ultrastructural studies of the gland cells and host-parasite interface of the adhesive organ of the diplostomoid *Cyathocotyle bushiensis* in its bird host. The pear-shaped gland cells and their ducts within the parenchyma of the adhesive organ produce a complex secretion comprising densely granular bodies, finely granular material, and mitochondria. When the retracted organ everted to attach to its host, the microvillus external surface attached to the host mucosal tissue and served to discharge the secretions into the host and perhaps also allow absorption of nutrient materials from the host.

In some individuals of the apocreadiid *Crassicutis archosargi* (Apocreadiidae) that attach to the intestine of its only-known definitive host, *Archosargus probatocephalus* (Sparidae, the sheepshead fish), the permanently attached region of the trematode consists of a modification of the tegument on either the dorsal or ventral side and extends among the host's intestinal villi. This modified tegument can be studied easily as it stains distinctively by the PAS (periodic acid-Schiff) method digested with diastase, as well as by other methods (Overstreet, 1976b).

Papillae

Different types of externally protruding papillae occur in different locations in the tegument on most digeneans and, in many cases when relatively consistent, can be used as a diagnostic characteristic (if the specimens are heat-killed and clean), meaning that identification of the species can be determined based on observation of this character.

Several researchers have studied the ultrastructure of sensory receptors in both adult and cercariae of different species. For example, Torimi and colleagues (1989) described 4 types of sensory structures in adults of *Echinostoma hortense* using SEM and transmission electron microscopy (TEM). Two contained ciliated papillae and 2 contained papillae without cilia; each occurred near either the oral sucker or ventral sucker. Although electrophysiological studies on this or other worms have not been conducted by cited researchers, most of them speculated that the morphological features and the distribution of these papillae indicate that they function as contact or stretch receptors during attachment and feeding of the worms. Many studies are waiting to be conducted!

Neurophysiological aspects

Because digeneans do not have a true circulatory system nor true endocrine organs, an understanding of the numerous neuropeptides becomes important for determining current anthelmintic and other drug-target selection. Adequate nerve and muscle function for many key behavioral determinates involves sensory perception/host location, invasion, locomotion/orientation, attachment, feeding, and reproduction (McVeigh et al., 2012). Some trematodes possess a type of lymphatic or osmoregulatory system that is metabolically active, suggesting a circulatory or excretory role (Sharma, 1978; Fried and Haseeb, 1991); in some cases, the structure and overall shape of this system serve as a taxonomic character. McVeigh and colleagues (2012) reviewed neuropeptide signaling in the Nemata and Platyhelminthes, including digeneans, highlighting a suite of 19 protein families that affect phenotypes in helminth reverse genetic screens. The types and organization of neurosecretory cells, nerve fibers, and perikarya have been reported from several digeneans, including light microscopy, ultrastructure, histochemistry, and immunochemistry (for example, Sharma and Sharma, 1981; Riddell et al., 1991; Mohammed and Al-Attar, 2000).

Even though dealing with a gill monogenean, Maule and colleagues (1990) tackled a well-reviewed

Box 2. Digeneans, Morphology: Alimentary Tract — Study It

There are a variety of ways for a student to investigate the alimentary tract of digeneans. Nollen (1968b) approached this with a rather simple system. He used species of *Philophthalmus* that as adults resided in the ocular sac of birds. That made it possible for him to remove the worms for experimental treatment and then, when necessary, return them to the host without surgery. In the cited paper, he exposed *Philophthalmus megalurus* to tritiated compounds later processed for autoradiography, using freeze-dried specimens embedded and sectioned in epoxy resin, with standard histological techniques for paraffin sections as controls, to detect the incorporated compounds (glucose, tyrosine, leucine, and thymidine). Absorbed glucose through the tegument became distributed widely within 1 min; then within 15 min, glucose converted to glycogen. The limitation of alkaline phosphatase within the excretory system showed the unimportance of that enzyme to glucose absorption. Tyrosine and leucine entered the worm, mostly through the gut within 5 min, and became distributed throughout the body within 15 min. Those 2 amino acids became incorporated in the vitellarium within 10 min. In contrast, it took 8 hr for tyrosine injected into infected birds to become incorporated by the vitelline cells, which in turn formed egg-shell material. It took 10 days for the eggs to be laid by the worm. However, within 30 min, the thymidine became incorporated within the reproductive system. Rapid entrance and incorporation of thymidine, glucose, tyrosine, and leucine into developing miracidia within the eggs demonstrated that the uterus was much more than a passive conduit for eggs.

immunocytochemical study using confocal scanning laser microscopy that would provide a splendid background for a digenean study. Moczoń and Świderski (1983) took an opposite approach. They infected specimens of a male South African pouched mouse (*Saccostomus campestris*) with 200 cercariae of the Tanzanian strain of *Schistosoma haematobium*. After 3 months, they treated the mice with a series of doses of niridazole, and then examined the region of the schistosomes posterior to the ovary for the presence of any ultrastructural and histochemical pathological changes from untreated specimens.

Alimentary Tract

Unlike cestodes, which only acquire nutrients through their tegument, most trematodes acquire nutrients through both the tegument and an alimentary tract.

The cellular tissue that lines the cecum is called the cecal epithelium, or gastrodermis, and it differs among different digenean species and also among different groups of Platyhelminthes. For example, the monogenean *Calicotyle kroyeri*, from rays, ingests epidermal tissues and associated mucus from the skin of its fish host. Halton and Stranock (1976) studied the common columnar cell in *C. kroyeri* that is filled with heterogeneous vacuoles from the viewpoint of both histochemistry and ultrastructure. This cell in the monogenean has an apical endocytotic complex comprising cell surface lamellae, apical vesicles, and numerous tubular invaginations of the plasmalemma. The luminal surface bears a highly organized array of peg-like structures that take up particulate food material from the gut lumen for transfer by means of other vesicles to the vacuoles in the columnar cells for digestion. The digestive elements of the cell are histologically reactive for protein, mucus, and carboxylic esterases. Indigestible residues and lipid droplets accumulate in the large apical vacuole and are periodically released into the lumen by exocytosis. The resulting distinction of the lumen of this worm involves an outflow of digestive secretions from the gastrodermis cells. These enzymes are secreted into an environment that is slightly acidic and important in several ways for regulating digestive processes, such as those involving cysteine and aspartic proteases.

Molecular and cellular studies of proteases (which are best characterized as a series of cathepsins) were instrumental in the discovery that they are vital in nutrient uptake from the host by degrading blood tissue proteins and other tissues into free amino acids (Dalton et al., 2005). Proteases involved in tegumental turnover, parasite excystment, egg hatching, and host penetration have played a pivotal role in the development of parasitism.

Since trematodes may produce peptidases that are specific for certain species, these enzymes may be targeted by researchers to develop practical applications in diagnostics, chemotherapy, and perhaps vaccination. For example, cysteine proteases such as cathepsin B1 serve as a primary target for small-molecule cysteine protease inhibitors. For example,

Box 3: Studying Digeneans: Anomalies — Study It

Numerous anomalous conditions occur in trematodes, but relatively few are reported. When a researcher finds an anomaly, they usually just toss the affected worms away or do not use them for descriptive purposes. In some researchers' experience, most anomalies appear to be found in unfertilized specimens, resulting in a poorly formed vitellarium, Mehlis' gland, or other reproductive structure. For example, Stunkard and Nigrelli (1930) noted that 1 of many specimens of *Lintonium vibex* contained 1 rather than 2 testes, and the worm appeared otherwise normal. Sometimes, these abnormalities may create an informative genetic situation like the number of testes in the common opecoelid *Helicometrina execta* from wrasses in Florida (Overstreet, 1969). Collections of 55 mature specimens contained individuals with from none to 5 testes, and some additional specimens from a total of 8 species of wrasses and non-wrasses contained 4, 7, 8, and the predominate 9 testes.

Out of about 1,500 specimens of *Philophthalmus megalurus*, a single 6-day old experimentally produced specimen had a portion of the anterior testis containing typically appearing ovarian tissue (Nollen, 1970). In a different case, an individual of a digenean presently known as the apocreadiid *Homalometron cryptum* in Florida without evidence of injury or degeneration exhibited 1 anomalous cecum interrupted to form a short branch joining the normal cecum and another portion with 2 blind ends extending the length of the vitelline follicles and lacking the well-developed epithelium of the normal cecum (Overstreet, 1969).

administering this protease to human test subjects infected with *Schistosomes* has been shown to decrease worm burdens of *Schistosoma mansoni*. Other cathepsins may serve as targets for other trematodes. Nevertheless, several trematodes other than blood flukes obtain their nutrition from blood and parasitologists have long questioned the source of hemoglobin in adults and metacercariae of digeneans (Cain, 1969a; 1969b; Vandergon et al., 1988).

Reproductive System

With the exception of schistosomes from mammals and a few didymozoids from fishes, digeneans are hermaphroditic. Species having a longevity of months to years continuously produce ova and sperm. In fact, some hemiuroids and didymozoids possess a Juel's organ, a modified appendage to the Laurer's canal and an organ that serves as a disposal unit, which recycles excess or unused reproductive material (Gibson and Bray, 1979). The Laurer's canal, which in most other digeneans links the oviduct dorsally with the exterior or to a seminal receptacle, seems to serve as a drainage conduit for excess or spent seminal and vitelline material.

In trematodes with a short lifespan, such as some microphallids (Digenea: Microphallidae) that occur in migratory birds, some species known to use a crustacean intermediate host are progenetic (meaning that they mature very quickly) and develop quickly and are able to rapidly develop and produce eggs in the feces of the bird before the bird acquires a good meal and leaves the area, continuing it migration. The area is often inhabited by endemic snails or crustaceans hosts of the microphallids and perpetuation of the worm's life cycle requires the use of a similar flyway (geographic area of migration) each season for the bird to maintain the microphallid species.

As mentioned elsewhere in this chapter, some microphallids as well as hemiurids and other digeneans in their intermediate host are progenetic and exhibit precocious development. Jackson and colleagues (1997) considered the condition in *Hemiurus levinseni* in mysidaceans from cold bays in Nova Scotia to result from accelerated gamete production or a shortened life cycle where the usual obligate vertebrate host is no longer required.

The relative position and size of gonads provide important taxonomic features. Sperm ultrastructure of various digeneans also provides informative taxonomic and phylogenetic features. There are several characters that provide useful features, and these should be compared among close species and other taxa (for example, Ternengo et al., 2009; Quilichini et al., 2016).

Some researchers have found the Mehlis' gland, a cluster of gland-cells, to be an important taxonomic structure as well as one producing materials necessary for lubricating the uterus, forming and protecting the egg and eggshell, and probably activating the spermatozoa. Depending on the species, up to 5 different types of cells may be observed in the 'gland' that appear morphologically different and can be differentiated when stained with neutral red.

Box 4. Digeneans, Morphology: Reproductive System — Study It

The ultrastructure of the oviduct of the lung fluke *Paragonimus ohirai* was studied in detail by Orido (1990), showing there are 5 principal regions with cilia confined to 2 separate areas. His investigation (1991) also included the ultrastructure of the Mehlis' gland from which 2 types of secretory products were produced.

Smyth and Halton (1983) assumed the secretion played an important role in eggshell formation, and a variety of studies involving different trematodes have suggested the presence of a complex polysaccharide.

Moczoń and Świderski (2000) determined using ultrastructure that the secretions produced by the gland as well as by the wall of the distal ootype in *Schistosoma mansoni* included neutral glycoproteins.

Several researchers have used histochemistry and light microscopy to study the Mehlis' gland and its secretions in a variety of trematodes (for example, Del Conte, 1970; Sharma et al., 1981).

Those interested in this field of study should also investigate the similar structures, including the vitellarium, in cestodes (for example, Smyth and Halton, 1983; Świderski et al., 2011b), monogeneans (for example, El-Naggar et al., 1990), turbellarians (for example, Chandler et al., 1992), and other trematodes (for example, Świderski et al., 2011a).

Table 2. Selected general keys to genera, some accompanied by lists of digenean species.

First-listed author(s)	Year	Description of resource
Yamaguti	1971	Vertebrates: List of species, descriptions of genera
Yamaguti	1975	Life cycles
Schell	1985	North America north of Mexico
Gibson et al.	2002	Generic and higher level keys – Keys to the Trematoda, Volume 1
Jones et al.	2005	Generic and higher level keys – Keys to the Trematoda, Volume 2
Bray et al.	2008	Generic and higher level keys – Keys to the Trematoda, Volume 3

Eggs

Little is known about the ultrastructure of digenean eggs, especially when compared with those of cestodes, which are much more diverse. Conn and colleagues (2018) described some features from small operculated eggs from microphalloids. Those consist of an embryo surrounded by eggshell, with the shell material derived from vitellocyte secretions similar to that process found in the eggs of bothriocephalidean and caryophyllidean cestodes. Fried and Haseeb (1991) described eggs and miracidia of several trematodes. Nollen (1971) reported an early study on the quinone tanning system in eggshells. Future studies assessing eggs and miracidia of those trematode groups with large eggs without an operculum should provide biologically and taxonomically useful results.

As a general rule, trematodes that have large operculated eggs have a diagnostic miracidium that hatches from the egg and uses chemoreception to locate the molluscan first intermediate host. Stimulation for hatching usually differs among species and depends on various environmental conditions. See the text box (Box 6) for examples of the variations that have been observed.

Trematodes with tiny eggs such as hemiurids often contain many thousands of them and deposit them in the feces of their host, which in the case of hemiurids are fishes. The eggs, which usually spread out over a wide geographic area, have the statistical chance for the first intermediate host to feed on them. In the case of what was reported as *Hirudinella ventricosa* from a mackerel in India, the eggs were released from the worm in strings, with each string containing active spermatozoa and numerous oval, thick-shelled, translucent eggs containing fully-developed miracidia (Murugesh and Madhavi, 1990).

Note for Tables 2, 3a, 3b, and 3c: Be aware that in many cases the taxa names are not acceptable to recent authorities, so be sure to examine online sources like WoRMS for marine species or recent literature for accepted names.

Box 5. Digeneans, Morphology: Reproductive System, Eggs — Study It

Eggs of *Echinostoma caproni* under either light or dark conditions at 27 °C from an experimental mouse infection developed fully in 9 days, compared with 10 days when from a hamster (Behrens and Nollen, 1993). When exposed to light as a trigger for hatching, with incandescent light providing more consistent stimulation than florescent light, miracidia from eggs from mice took 13 days to hatch, whereas those from hamsters took 11 days. Both lots hatched between 11:00 and 16:00 hr, indicating a diurnal circadian rhythm, and, when stored in the dark for over 56 days, the miracidia from those eggs displayed abnormal swimming behavior; those eggs stored for 70 days did not hatch when exposed to light.

When developed eggs of the same echinostome species were experimentally exposed by Fried and Reddy (1999) to snail-conditioned (*Biomphalaria glabrata*) water, a greater number of eggs hatched than when maintained in artificial springwater.

Ford and colleagues (1998) studied the effects of salinity, pH, and temperature on the half-life and longevity of the same species of miracidia and found they were unable to tolerate much salt and lived longer at lower temperatures but never longer than 15 hr. Unlike eggs of echinostomes that require time to embryonate in an aquatic environment, those from schistosomes released from the minimally salty gut into a freshwater environment are already developed and hatched immediately.

When the chemosensitivity of miracidia of 2 species of *Philophthalmus*, which are positively phototactic and geotactic, were compared by Nollen (1990a), they showed an opposite response to some of the tested chemicals. The miracidium of the blood fluke *Schistosoma mansoni* also exhibited a positive phototactic response but was negatively geotactic in contrast to that of *Schistosoma haematobium*, which responded oppositely for both sensitivities (Shiff, 1974).

Biology

General

Numerous species of digeneans exist, and, with proper attention, students can find infections in most vertebrate hosts although infection depends on habitats and general prevalence. There are about 150 families, and identification at the superfamily, family, subfamily, and genus levels of members can be best accomplished by using the 3 volume keys entitled Keys to the Trematoda (Gibson et al., 2002; Jones et al., 2005; Bray et al., 2008) or updated articles on specific genera or groups. Details of many of these families are included in other chapters in this online textbook. Nevertheless, mention of a few examples will occur elsewhere in this chapter.

Digeneans are especially powerful for students to study because they come in all sizes and shapes; they exhibit a variety in feeding, reproducing, moving, and surviving; some can be harmful to their hosts, and, consequently, many have economical or medical importance. The taxon provides laboratory features that can utilize a variety of tools to investigate them. What a wonderful experience to have the opportunity to spend hours extracting an adult specimen of *Nematobibothrioides histoidii* over 6–12 m-long and in tangled masses extending from 1 side to the other just under the skin of a moribund *Mola mola* (ocean sunfish) (Noble and Noble, 1964; Bullard and Overstreet, 2008, personal experience).

A clear understanding of the biology of most digeneans remains poorly understood because of their lack of medical importance; little actual work has been done on the wildlife infecting species that are of little medical or veterinary importance. For example, most emphasis for intense study of trematodes has been focused on blood flukes, however, blood flukes and are not the normal run of the mill trematodes that comprise 95% of the species that exist. The blood flukes are truly exceptional because some groups even have separate sexes. However, understanding of trematodes without great medical importance can be had as shown with the following example: Many years ago, Nollen (1968a; 1968b; 1978) used radioactive ^{3}H-thymidine, as mentioned elsewhere, to determine exciting aspects such as development of stages within eggs still in the uterus, mating of different sized individuals, and other examples. Most of his studies were conducted on worms that matured under the nictitating membrane in the eyes of baby chickens (chicks); he could remove

Table 3a. Selected literature sources regarding digeneans in marine fishes.

First-listed author(s)	Year(s)	Location(s)
Linton	1910	Dry Tortugas
Manter	1947	Dry Tortugas
Yamaguti	1934, 1954, 1958, 1971, 1975	Worldwide
Sogandares-Bernal	1959	Gulf of Panama and Bimini
Siddiqi and Cable	1960	Puerto Rico
Nahhas and Cable	1964	Curaçao and Jamaica
Overstreet	1969	Biscayne Bay, Florida
Williams and Bunkley-Williams	1996	Puerto Rico lists
Yamaguti	1970	Hawaii
Palm and Bray	2014	Hawaii
Madhavi and Bray	2018	India

Table 3b. Selected literature sources regarding digeneans in freshwater fishes.

First-listed author(s)	Year	Location
Bunkley-Williams and Williams	1994	Puerto Rico
Hoffman	1999	North American (keys)
Thatcher	2006	Amazon fishes
Kohn et al.	2007	South American

Table 3c. Selected literature sources regarding digeneans of other animals.

First-listed author(s)	Year	Location and/or type of source and/or subtopic(s)
Travassos et al.	1969	Brazil, vertebrates
McDonald	1969a, 1969b	Bibliography and catalog, anatid birds
McDonald	1981	Keys, waterfowl
Prudhoe and Bray	1982	Amphibians
Forrester and Spalding	2003	Florida, wild birds
Jacobson	2007	Reptiles
Atkinson et al.	2008	Wild birds
Samuel et al.	2008	Wild mammals
Overstreet et al.	2009	Gulf of Mexico, lists of adult digeneans from all marine vertebrates
Fernandes et al.	2015	South America, birds and mammals
De Baets et al.	2015	Fossil evidence
Overstreet and Hawkins	2017	Gulf of Mexico, diseases of fishes and other animals prior to 2010

the individuals, treat them, replace them to their original sites, remove them again, and analyze them. With modern tools, students can now investigate many more aspects of the biology of digeneans.

After a 6-hour exposure to ^{3}H-thymidine, isolated adult specimens of *Philophthalmus gralli* were transplanted to chicks and labeled oogonia became primary oocytes within 4 days and then enclosed in newly formed eggs by day 12. In adults labeled in vitro and transplanted singly to chicks, only 2 of 28 self-inseminated. Labeled adults transplanted with unlabeled ones never self-inseminated but cross-inseminated with approximately 40% of the available individuals. Transplanted adults localized in 3 micro-habitats within the chicks' orbit. In only 1 of 21 attempts, did a labeled worm inseminate an un-labeled one outside of the micro-habitat where it was found (Nollen, 1978). Nollen (1984) also conducted mating studies with

Box 6. *Hirudinella ventricosa* — Learn More

These relatively large marine worms when constricted are about the size of a human thumb and are commonly known as walnut worms.

There are probably several species presently known as *Hirudinella ventricosa*, but they occur primarily in offshore predatory fishes.Ribosomal DNA from specimens from 3 different pelagic fishes in the Gulf of Mexico shows that there are at least 4 species in the Gulf of Mexico (Calhoun et al., 2013), including what tentatively is supported by morphological differences (specimens from type localities were not sequenced) to be *Hirudinella ventricosa* from the wahoo (*Acanthocybium solandri*), *Hirudinella ahi* from the yellowfin tuna (*Thunnus albacares*), and 2 different unidentified species of *Hirudinella* sp. from the blue marlin (*Makaira nigricans*) with 1 of those also infecting the benthic yellow goatfish (*Mulloidichthys martinicus*).

One would probably win a bet with a fisherman who just caught a wahoo shorter than 160 cm by wagering that exactly 2 individuals of *Hirudinella ventricosa* will infect the stomach of their catch (Overstreet, 1978).

P. gralli and *P. megalurus* and determined when single, transplanted, labeled *P. megalurus* were transplanted into chicks with unlabeled *P. gralli*, interspecies mating occurred, but there was no evidence of hybrids. Opposite studies with labeled *P. gralli* differed because interspecies mating did not occur. When Nollen (1999) recovered young adults of *Echinostoma trivolvis* and *E. paraensei*, he labeled the sperm and transplanted those individuals singly to uninfected hamsters that contained several unlabeled worms of the same or opposite species or both species. After 5 days, when no recipient worm of the same species was present, only 1 interspecies mating occurred out of 113 possible recipients. When single donor worms had a choice of either species of recipient worm, no interspecies mating took place, but self-insemination occurred.

Each species has its own biological eccentricities, and it is up to the readers to see how to untangle those of the species presently under the objective lenses of their microscopes. For example, *Cyclocoelum oculeum* occurs in the nasal sinuses of coots. McLaughlin and Marcogliese (1983) studied the migration, growth, and development of the species in *Fulica americana.* They orally intubated 40 encysted metacercariae to each of 1 group and artificially excysted another batch and injected 40 intraperitoneally to that group. Those injected into the body cavity migrated through the air sacs and air passages to the sinuses and migrated faster and grew larger than the others in an asynchronous manner. In fact, no infection resulted from orally fed worms after 6 weeks! Infection with the related *C. mutabile* involved an invasion of the liver after penetrating the intestine, and it remained there about 2 weeks before migrating to the air sacs where specimens matured synchronously, with that species exhibiting a more complex cycle.

A Note on Preparation Methods

Morphological characters used to identify worms to the level of genus or species can be modified by methods of fixation and preservation. For example, workers fixed digeneans decades ago in alcohol, formalin, and acetic acid (AFA) and killed them under coverslip pressure. The acetic acid in AFA, along with ethanol and formalin, eroded spines, especially in specimens left in the fixative for a long period. Also, some workers relax trematodes in distilled or tap water and killed them with a cold fixative. That may be acceptable for acanthocephalans that will not be sequenced or used for ultrastructure. However, structures in digeneans may degenerate or otherwise result in shifted features or otherwise altered structures.

For example, a study by Curran and colleagues (2001) involved digeneans from colubrid snakes in Vietnam. When the specimens of *Singhiatrema vietnamensis* were bathed in fresh water and then cold-killed, they were wider, the pharynx and esophagus were distorted, the ceca were shorter, and the cirrus sac was oriented differently. These alterations could have resulted in a misidentification to species. However, when treated similarly, the vitellarium of *Szidatia taiwanensis* exhibited distorted, confluent follicles rather than separate ones, a feature of *Gogatea* rather than of *Szidatia*. Before you go to collect parasites be sure to use the most current and up to date methods for collecting and preserving parasites and their hosts. Some sources include Gardner and Jiménez-Ruiz (2009), Gardner and colleagues (2012), and Galbreath and colleagues. (2019).

Phylogeny and Classification

Olson et al. (2003) combined their sequences from various digeneans to develop species-level phylograms based

Box 7: Personal Note from the Author, Robin M. Overstreet (from 2018)

Readers should be aware that the late Ray Cable was probably the most "forceful international inspiration for contemporary cercarial studies," with a long list of cercarial studies (for example, Overstreet, 1997b) and my copy of several (for example, Cable, 1956; 1965) stand well worn. He was also an interesting parasitologist!

Another contemporary authority on cercariae and life cycles is Marianne Køie (for example, 1985).

And I am embarrassed to confess that Richard Heard and I have amassed one of the largest collections of unpublished digenean experimental life cycles, which we hope to publish before either of us expire.

on Bayesian inference of combined data, ssrDNA + lsrDNA, and a revised classification based on the phylograms showing relationships among the different higher level taxa. Since that time, information gaps have been filled and much more is known about the relationships among families, genera, and species. A few general updates on methods and relationships followed (Nolan and Cribb, 2005; Olson and Tkach, 2005) and many have added to and sorted out the relationships and are cited where the corresponding taxa are treated below.

Before the availability of marvelous molecular tools, various researchers used morphological and developmental means to show those relationships. Some were highly inaccurate, though a relationship tree developed by Cable (1974) was unexpectedly close. Cable's accomplishment is truly amazing when one finds that worms that appear very similar morphologically are not phylogenetically related. Cable also showed convergent evolution of distantly related Microphallidae, Heterophyidae, and Fellodistomatiidae. On the other hand, worms that appear distinctly different may be shown to be closely related, as established by molecular means. Clearly, improvements in molecular tools, including entire genomes, will open new doors. They will also allow researchers to much better understand the biology and history of the digeneans.

Life cycles also have been used to assess the evolution of digeneans. A discussion of the importance of morphological features of adults and cercariae in understanding the phylogeny of digeneans occurs elsewhere in this chapter. Sinitsin (1931) and others, who considered that digeneans originated as gastropod parasites, were challenged by Heyneman (1960) who considered flatworms evolved from dalyelloid rhabdocoels. Cribb and colleagues (2001b, 2003) critically examined the nature and evolution of digeneans, looked at Diplostomida and Plagiorchiida separately, and still could not be definitive about how the complex cycle arose and how variation within the group evolved. Is a gastropod or vertebrate the primary original host? There is still lots of good reading such as that by Pearson (1972; 1988; 1992), who presumed that digeneans evolved from free-living rhabdocoels with a mollusc first origin, Cable (1965; 1974; 1982) and Gibson (1987) to compare with the molecular data that places the major helminth taxa (Trematoda, Monogenea, and Cestoda) and minor ones (Gyrocotylidea and Amphilinidea) together as the monophyletic Neodermata (Littlewood et al., 1999a; 1999b). That monophyly allowed the use of parsimony but has not definitively settled the origin of digeneans.

LaRue (1957) established a grouping based on embryological aspects of the excretory system of cercariae, which, with modifications, is similar to the accepted scheme used today. His suborders Anepitheliocystidia and Epitheliocystidia are no longer accepted because the cellular structure of the excretory vesicle as assumed by light microscopy for some members of Epitheliocystidia was shown to be a syncytium with transmission electron microscopical evaluation, the development of the excretory system in the tail was not clear cut, and most important, these features do not fit an acceptable phylogeny.

Classification and phylogeny evolved into using morphological adult characters and characteristics, and characteristics of all life stages combined to produce cladograms from cladistic analysis (for example, Brooks et al., 1985; 1989; Brooks and McLennan, 1993). Classification developed further by utilizing phylogenetic relationships determined from genetic sequences of specific genetic fragments (Tkach et al., 2000; Olson et al., 2003). The 3 publications by Brooks and colleagues pointed out the ambiguous data for the unresolved Plagiorchiata and tried to better establish members of the clade. Tkach and colleagues (2000) considered those works valuable and an important basis for other investigations. In fact, those molecular works supported some of the conclusions and straightened out other relationships.

Molecular analyses during the next 2 decades have clarified the higher level digenean taxa. Presumably, this classification will be perfected even more by using entire genomic sequences in the near future. Nevertheless, many articles on specific groups have added to or corrected the earlier phylogram of Olson and colleagues (2003). For example, Overstreet and Curran (2005a; 2005b) classified the haploporoids, all known to infect fishes only, based on morphological features. But once they collected and analyzed molecular sequences, they straightened out several aspects of the early classification (see the classification of Haploporoidea elsewhere in this chapter).

Classification

Littlewood and colleagues (2015) updated Olson's work from the point of view of diversity, showing that at that time there were 24 major groups (superfamilies) of Digenea, with 150 families, 1,777 described genera, and 12,012 described species. Not all families include any sequenced individual, and, in most groups, fewer than 5% or 10% have been sequenced. Looking at the numbers from a different point of view, Bullard and Overstreet (2008) estimate that they amass the largest group of monozoic plathyhelminths, perhaps about 18,000 nominal species, with fishes hosting an astonishing number of digeneans. Considering there exist about 27,977 extant fish species, accounting for just over half of all living vertebrates, and considering the number of new digeneans named yearly, with approximately half the species being named and examined for digeneans, the number of digeneans infecting fishes will soon probably exceed the number of fish species. Moreover, most sequenced species from fishes represent fewer than 5% of the known members in their representative families (Littlewood et al., 2015).

Subclasses Diplostomida and Plagiorchiida

As indicated above, the Digenea contains the subclasses Diplostomida and Plagiorchiida, with the former containing 19 families in 3 superfamilies, with most attention directed toward the Schistosomatoidea. Orélis-Riberio and colleagues (2014) provided a helpful tree illustrating phylogenetic relationships among all 3 blood fluke families using 83 blood fluke partial D1–D2 domains of 28S sequences.

Family Schistosomatidae

Members of the Schistosomatidae infect birds and mammals, those of the Spirorchiidae infect turtles, and those of the Aporocotylidae infect fish. The family for those in fishes has been considered both Sanguinicolidae and Aporocotylidae, but Bullard and colleagues (2009) determined it should be Aporocotylidae. Bullard and colleagues (2008) and Orélis-Ribeiro and colleagues (2014) showed that plesiomorphic members of the Aporocotylidae and maybe other blood flukes are some of the only digeneans that can show an association with some primitive hosts.

Family Diplostomatidae

Barcodes using cytochrome *c* oxidase 1 were analyzed by Locke and colleagues (2015) on 52 species of Diplostomatidae based on larval forms from fishes with more success than using the barcode on other digenean groups. That study was useful for detecting 23 of 40 unidentified species supported by at least 1 additional line of evidence.

Superfamily Brachylaimoidea

The superfamily Brachylaimoidea, according to Littlewood et al. (2015) is the sister group of Schistosomatoidea and Diplostomoidea, even though Heneberg and colleagues (2016; 2018) considered it in the Plagiorchiida. Those latter authors, however, provided trees with several brachylaimoids and showed that they really belonged in the Diplostomida. Locke and colleagues (2012) provided molecular and morphological information on the Holarctic distribution of *Urogonimus macrostomus*, confirming that several 'prior' species and individuals showing a wide degree of biological and geographical variation did indeed belong to this leucochloridiid brachylaimoid. The brachylaimoids occur in 7 families, 29 genera, and 227 species. Some leucochloridiids have furcocercariae, and some have colorful branched sporocysts that are visible within their land snail host, attracting their definitive hosts. The cercaria is often a cercarium (without a developed tail), and the metacercaria, usually encysted in the intermediate host, has a well-developed reproductive system. The definitive hosts for members of this superfamily are amphibians, reptiles, birds, and mammals.

Superfamilies Bivesiculoidea and Transversotrematoidea

The subclass Plagiorchiida contains 21 superfamilies of which the Bivesiculoidea and Transversotrematoidea each contain a single-family, each with 5 or fewer genera, and the 2 superfamilies not being significantly related to each other. These constitute the most primitive plagiorhiids. The bivesiculids are atypical in they have a single testis and completely lack ventral and oral suckers, assuming one accepts the anteriorly located muscular structure as a pharynx; those with a known life cycle have a furcocystocercous cercaria that swims and is eaten directly by the definitive marine or freshwater fish host, resulting in a 2-host cycle. The

presence of *Bivesicula claviformis* in large groupers presented a challenge. Cribb and colleagues (1998) found immature specimens in a wrasse that compared with adults from the grouper, but not with specimens of 2 other species, using both molecular sequencing and morphological structures. They suggest that the immature specimens constituted a true metacercaria and an obligate stage in a 3- rather than 2-host cycle. The family was originally proposed by Yamaguti (1934) as a subfamily of the Monorchiidae, which, of course, it is not.

The transversotrematids are transversely elongate or pyriform digeneans with a cyclocoel gut (posterior portions join, making a cyclocoel), and those with a known life cycle have a furcocercous cercaria with distinctive arm processes at its bases that allow them to attach directly on the skin of their marine or freshwater fish host, allowing them in turn to mature into an adult under the host scales without passing through a metacercarial form. The family is unique.

Superfamily Azygioidea

One of the next 5 related plagiorchiid superfamilies also has members with a forked-tailed cercaria, even though the tails of some of the 5 differ considerably. Azygioidea is confined to 1 family, Azygioidae, with 4 genera and 40 species that mature in the stomach or body cavity of elasmobranchs and in the stomach of freshwater teleost and holosteans. The cercaria of some is an active, large, colorful, usually yellowish or orangish, and appears as an insect to a hungry fish. The superfamily is a sister superfamily with that of the related Hemiuroidea.

Superfamily Hemiuroidea

The Hemiuroidea contains 13 families, 212 genera, and 1,334 species. They infect the gut, especially the stomach, of marine teleosts, but they are also common in freshwater teleosts and less common in elasmobranchs, amphibians, and reptiles as well as progenetic in invertebrates. For example, Overstreet and Hochberg (1975) reported adults of the fish hemiuroid *Derogenes varicus* in the cuttlefish, *Sepia officinalis,* and included a reference for egg-bearing specimens in an arrowworm (Chaetognatha); when Køie (1979) described the life cycle of *D. varicus*, she found natural infections of immature metacercariae in the arrowworm *Sagitta elegans*. Along the same vein, Overstreet (1969) found a 3.2% prevalence of progenetic metacercariae of a different hemiuroid in the coelom of 250 specimens of *Sagitta hispida*. When 2 of 3 of the arrowworms were maintained in separate beakers for 29 days, the egg-bearing digenean migrated into the host's uterus. A hemiuroid's body surface is usually smooth (without spines) but can be rugate or plicate. As indicated elsewhere, some hirudinellids are quite large and occur in the stomach of large, carnivorous, marine teleosts (Overstreet, 1978; Bullard and Overstreet, 2008). Other hemiuroids occur in the esophagus of frogs and are well known because they have 3 intermediate hosts (for example, Yamaguti, 1975).

Family Didymozoidae

Members of another related family, Didymozoidae, are atypical because some are not hermaphroditic and most occur in tissues, embedded on gills, or in body cavities of oceanic pelagic fishes (for example, Yamaguti, 1971; Bullard and Overstreet, 2008). A relatively early comparison of phylogenies of genomes versus morphology of hemiuroids was conducted by Blair and colleagues (1998).

Family Heronimoidea

Heronimoidea is a single family with 1 accepted species, *Heronimus mollis*. Rather than a relic of an ancestral form, the species appears to be an aberrant form with a secondarily reduced life cycle and not related with the paramphistomoids. It is found in the lungs and trachea of freshwater turtles, and its eggs are retained in the adult and hatch when the adult migrates to the mouth of the turtle host and escapes into the water; the hatched miracidia may already contain cercarial embryos in the mother sporocyst. The cercaria does not encyst (Jones, 2005).

Superfamily Bucephaloidea

The next recognized superfamily, Bucephaloidea, has 2 reported families, 29 genera, and 416 species. Curran and Overstreet (personal communications) find that the 28S sequences of several genera do not match the morphological findings (Overstreet and Curran, 2002); perhaps extensive genomic sequences will clarify the phylogeny of this group. Known cercariae possess oxbow-shaped tails unlike an atypical forked-tail cercaria. Overstreet and Curran (2002) provide descriptions of the genera; tentatively do not accept but describe and discuss the second family, Nuitrematidae; a reader should note that bivalves serve as the first intermediate host, fishes as the second intermediate hosts, and teleosts as definitive hosts. One species is known from a salamander, and I suspect adult worms that we encountered in elasmobranchs off Mississippi (Overstreet et al., 2009) as being acquired from sharks eating teleosts containing adults of those species. This family deserves extensive study, although life cycle investigations and other studies would make good student projects.

Superfamily Gymnophalloidea

The superfamily Gymnophalloidea with a forked tail is quite complicated and deserves extensive study. Bray (2002) listed 5 families rather than the 4 by Littlewood and colleagues (2015), but all should be investigated molecularly. The family Gymnophallidae contains relatively small worms in birds and mammals; molecular studies are needed to distinguish several of those species. The rest of the families occur in fishes, and most of the species in those have been placed in the Fellodistomidae, a family that has been considered a 'catch-basket group.' Many of its members require molecular attention and investigation of their life cycles.

Superfamilies Paramphistomoidea and Pronocephaloidea

The next 2 superfamilies have gymnocephalus cercariae that attach on vegetation or some other substratum. The first, Paramphistomoidea, contains 11 families, 135 genera, and 431 species, and most have their ventral sucker located at or near the posterior end. In some species there is a modified attachment organ. Many are quite large and occur in the rectum of their vertebrate hosts. The second, Pronocephaloidea, fits as a sister-group of above superfamily and members are commonly referred to as monostomes because they lack a ventral sucker or a typical pharynx; many have a head-collar or longitudinal rows of papillae on the ventral surface. Many occur in the digestive tract, but many others also occur in the respiratory tract, oviduct, urinary and gallbladders, pancreas, liver, and tissue sites of their teleost, reptiles (turtles and iguanid lizards), birds, and mammals. There are 6 families, 49 genera, and 293 species.

Superfamilies Haplosplanchnoidea and Echinostomatoidea

The above 2 superfamilies are sister groups to the Haplosplanchnoidea and Echinostomatoidea. The Haplosplanchnoidea has but a single family, and it was once thought closely related to Haploporoidea, members of which are also found in the gut of fishes only. It has 9 genera and 50 species that have a smooth tegument, and nearly all have a single cecum.

The Echinostomatoidea is a much better known superfamily because it is larger, and members infect primarily birds, even though some occur in reptiles and mammals in addition to fishes. Tkach and colleagues (2016) updated the list of Littlewood and colleagues (2015) with its 1,098 described species included in 9 families and 105 genera. It is the last of the related superfamilies with gymnocephalus cercariae; however, the cercariae also occur in haploporoids as discussed below. Tkach and colleagues (2016), using partial genetic sequences for 80 species, representing 8 families and 40 genera, elevated 2 subfamilies to families, created a new family, and abolished 2 families and 3 subfamilies as well as refined the generic boundaries within 3 abundant families. In addition to illustrating the phylogenetic relationships among the taxa, they also provide a schematic representation of that tree including intermediate and final hosts, making it one of the best known superfamilies.

Superfamily Opisthorchioidea

The superfamily Opisthorchioidea contains numerous species (839), and, which by itself, has most of its members with pleurolophocercus cercariae that infect fish as second intermediate hosts. The superfamily Opisthorchioidea in this study comprises the Heterophyidae, Opisthorchiidae, and Cryptogonimidae. Several of the species of the first 2 families infect humans and other mammals as well as birds and reptiles, and many have been sequenced (for example, Dao et al., 2017). Recently, several cryptogonimids, a taxon with members infecting freshwater and marine fishes as well as crocodilians (for example, Brooks and Holcman, 1993), the odd snake (Tkach and Bush, 2010), and amphibians (Miller and Cribb, 2008) have been sequenced and studied. Fishes provide a variety of different model systems such as lutjanids (for example, Miller and Cribb, 2007b), haemulids (Miller and Cribb, 2007a), and Mexican cichlids (for example, Razo-Mendivil et al., 2008). Martínez-Aquino and colleagues (2017) provided a nice phylogenetic tree from Bayesian inference analysis of the concatenated data involving larval forms of crocodilian species from Mexico as well as an acanthostomine from a Southeast Asian snake along with numerous fish cryptogonimids and species reported as members of Heterophyidae/Opisthorchiidae. There are many species and genera just being seen for the first time and many more to be seen in the future. Some are cryptic species, some have a wide distribution, and some make exceptional indicators—more areas and hosts should be studied (Miller and Cribb, 2007b; Overstreet, personal observations). Several species in both families infect humans.

Superfamily Apocreadioidea

The Apocreadioidea represents a superfamily infecting primarily fishes with a few reptiles. Pulis and colleagues (2014) were the first to investigate the phylogenetic position of the Megaperidae. In doing so, they changed the rank of the latter family to a subfamily within the Apocreadiidae. Blend and colleagues (2017) then reorganized the

Schistorchiinae and considered Megaperidae as a synonym of Apocreadiidae, a decision not accepted by Gibson (2017 in the WoRMS database because it was non-compliant with Article 35.5 of the International Code of Zoological Nomenclature (ICZN, 2012)). In any event, Apocreadiidae is the sole family in the superfamily and contains many cryptic species that had been misidentified for many years. For example, the genus *Homolometron* contains 34 accepted species, with 6 similar species described since 2010 (Parker et al., 2010; Curran et al., 2013a; 2013b; Barger and Wellenstein, 2015; Fayton et al., 2016). One of those, *Homolometron palmeri*, described in 2013 (Curran et al., 2013b) had been reported by a few workers as *Homolometron pallidum* since 1958, and it infects at least 7 fish hosts. Species of other apocreadiid genera also have been sequenced (for example, Scholz et al., 2004; Curran et al., 2013a; Tkach et al., 2013), and the genera are now better understood than when reported earlier based on morphological characteristics (for example, Caira, 1989).

Superfamily Lepocreadioidea

The Lepocreadioidea is sister of the above and others; members infect marine fishes and coastal birds. Bray and colleagues (2009) assessed partial *lsr*DNA and *nad1* sequences of 55 species and found the group, with the exception of 2 species of the putative Enderidae genus *Cadenatella*, formed a monophyletic polytomy of 5 clades. There occurred some odd findings: a significant proportion of *nad1* did not necessarily evolve under positive selection, all deep sea species were not related, different life cycles existed and perhaps a representative lepocreadioid cycle included a bivalve, encystment of vegetation, and a herbivorous fish host. Morphological features did not indicate strong value when relating higher level relationships, but many similarly appearing species infected related hosts.

Superfamily Monorchioidea

The Monorchioidea consists of 2 families, both of which infect bony fishes. The Lissorchidae, the most primitive, infects freshwater fishes, and the Monorchiidae infects both marine and freshwater fishes. The superfamily is sister to several others, all of which have cercariae with a stylet (xiphidiocercaria) except for the Haploporoidea. There are several paraphyletic species/taxa published and unpublished (for example, Wee et al., 2018; Cribb et al., 2018), and students under Thomas H. Cribb (Nicholas Q.-X. Wee) off the coast of Australia and Robin M. Overstreet (Apryle Panyi) in and off the coast of the Southeast United States have recently studied various species, and the combination of the findings should provide a much better understanding of the Monorchiidae. Bray and colleagues (2005) determined that *Cableia pudica* was a basal monorchiid rather than an acanthocolpid. A variety of life cycles are known.

Superfamily Haploporoidea

As indicated above, the superfamily Haploporoidea has a gymnocephalus type cercaria that differs from the remaining 5 superfamilies in the subclass Plagiorchiida. All members infect fishes, both marine and freshwater, and most of those members contain a single testis like most monorchiids and lissorchids. Overstreet and Curran (2005a; 2005b) presented keys for the Haploporidae and Atractotrematidae before any of the tentatively allotted species had been sequenced. We erected a new subfamily to make 4 in the Haploporidae. Since then, our group has collected many species throughout the world, sequenced many of them, described new species and genera, and published on most but not all of them. We were correct in accepting Atractotrematidae and including the apparently dissimilar genera in it (Andres et al., 2016a; Andres et al., 2018). Seven subfamilies are now accepted in the Haploporidae. Additional species of *Cadenatella* to those removed from the Lepocreadioidea by Bray and colleagues (2009) were sequenced, and the monophyletic group was a clear haploporid. *Hapladena*, which was originally in the Magasoleninae belongs in a separate subfamily. Blasco-Costa and colleagues (2009), who sequenced many haploporids from the Mediterranean Sea, erected Forticiculcitinae without the type species, but we accept the taxon, and Andres and colleagues (2015) included new species from Argentina and freshwater in Florida as well as a newly erected genus (*Xiha*) for *Dicrogaster fastigatus* from the northern Gulf of Mexico; that species had previously been assumed to be a haploporine associated with members from the Mediterranean Sea. Several haploporid species when sequenced (Pulis and Overstreet, 2013; Andres et al., 2014a; Andres et al., 2018), including *D. fastigatus*, turned out to be grouped into subfamilies different from those in which they were originally placed by Overstreet and Curran (2005b). As more haploporids are sequenced, additional changes in classification may be made in the freshwater chalcinotrematines (Pulis et al., 2013; Curran et al., 2018), 'megasolines,' and other subfamilies, but present data support a common marine ancestor with 2 testes, shifting from a primarily marine life history with eupercarian hosts to a more euryhaline one with diadromous, mostly mullet, hosts as originally suggested by Manter (1957).

Superfamily Gorgoderoidea

The Gorgoderoidea contains 12 families, but members of most from the variety of hosts (Elasmobranchii, Chondrostei, Teleostei, Amphibia, Reptilia, Aves, and Mammalia) have not been sequenced. Cutmore and colleagues (2013) examined members of 3 subfamilies of Gorgoderidae from teleosts, elasmobranchs, and deep sea teleosts and combined specimens from frogs and detected a variety of clades, including 4 in Gorgoderinae. These will provide a good baseline for additional specimens from different host groups for a future dissertation. The true allocreadiids, according to Curran and colleagues (2006) belong in this superfamily as sister to the Gorgoderidae. The 'Allocredioidea' indicated by Olson and colleagues (2003) was split into Brachycladioidea and Opecoeloidea, which will be discussed below (Littlewood et al., 2015).

Superfamily Opecoeloidea

The Opecoeloidea includes 1 family split into several subfamilies, including the Opistholebetinae, and infects freshwater, marine, and deep sea fishes (Cribb, 2005; Bray et al., 2016). The family, Opecoelidae, is the largest digenean family and contains over 90 genera with nearly 900 described species. It had been suggested by Curran and colleagues (2006) to be considered a member in the Brachycladioidea, but, to avoid confusion, I am accepting Opecoeloidea. Recent articles based on genetic sequences demonstrate the complex nature of the various clades in the family (Andres and Overstreet, 2013; Andres et al., 2014b; 2014c; Bray et al., 2014; Bray et al., 2016). The cercariae of many opecoelids contain a short, suckered tail that allows them to move in a leech-like manner to infect their typically crustacean hosts. Køie (1981) described the ultrastructure of 2 related species exhibiting differences in their tegument and a few other features.

Superfamily Brachycladioidea

Bray and colleagues (2005) sequenced several species of Brachycladioidea in *Stephanostomum* and related genera (Acanthocolpidae) from fishes and 1 species in Brachycladiidae from marine mammals and showed the relationship among them; also, they used those plus other data on sister taxa from Olson and colleagues (2003) to suggest that the brachycladiids from fish-eating marine mammals were derived from piscivorous marine fish parasites. As an example of recent uncertainty, Fernandez and colleagues (1998) pointed out that the marine mammal campulids (so-called) had historically been associated with Fasciolidae or Acanthocolpidae (the most speciose genus being *Stephanostomum*) on the basis of morphology. Orecchia and colleagues (2006, now unavailable online) considered them as Diplostomoidea. As indicated above and in WoRMS, the several genera are now separated into the subfamilies Brachycladiinae and Nasitrematinae of the Brachycladiidae (Superfamily Allocreadioidea) with 7 prior subfamilies considered as synonyms. Additional data should further clarify the relationships.

Superfamily Plagiorchioidea

The Plagiorchioidea includes 26 families infecting freshwater fishes and amphibians as well as terrestrial reptiles, birds, and mammals. However, a few members of Macroderoididae occur in brackish and marine environments in addition to fresh water. Tkach and Kinsella (2011) reported 4 species of *Macroderoides* plus 1 of the closely related *Paramacroderoides* in the same individual of the Florida gar. Three of those also infected bowfin from the same locality. A closely related species in 1 of the 2 North American clades is specific to pickerel, and it is the only 1 in a North American teleost rather than a holostean host. Moreover, the single member of *Paramacroderoides* mentioned above showed fewer differences in the number of variable sites with 1 particular species of *Macroderoides* than between it and the other species (Tkach et al., 2010). Analysis of additional species should show whether *Macroderoides* is a junior synonym or an additional genus is warranted. Many studies await attention of parasitologists. The Plagiorchioidea are sister to the Microphalloidea, and because the nearly 1,000 species in freshwater and terrestrial habitats and their available hosts that could serve as a large source of parasitology projects. Tkach and colleagues (2003) provided a good phylogenetic analysis of the superfamily.

Superfamily Microphalloidea

The Microphalloidea is considered sister of the Plagiorchioidea, and both have many families, genera, and species. Of the over 1,335 species, many have had their life cycles determined. Since many microphallid metacercariae from crustacean hosts are progenetic and can be cultured, the adults from a wide range of hosts as similar for the plagiorchioids can readily be compared with their metacercariae morphologically and molecularly. Speciation is complex; that of *Micophallus pygmaeus* complex with a derived 2-host cycle in the Holarctic speciated by host switching rather than co-speciation (Galaktionov et al., 2012). Since intermediate hosts of zoogonids and faustulids include

echinoderms, cnidarians, and other invertebrates, infections with these serve as interesting indicators of feeding behavior. Good articles on analyses of sequences include those by Tkach and colleagues (2003), Bray and colleagues (2005), and Kudlai and colleagues. (2015).

Life Cycles

Sporocyst Stage

Miracidia hatch from the egg, either after the molluscan first intermediate host eats the egg or directly in the water. Several means can trigger this hatching, usually through an operculum. Most known infections result from chemosensitivity allowing attraction of the miracidium to the mollusc. Once in the mollusc, the miracidium enters tissue where germ cells undergo asexual reproduction as a mother sporocyst or redia. In fact, in a few cases, redia develop in some miracidia even when still in the definitive host. Several cited general textbooks and cited articles treat the asexual reproduction process that results from a variety of taxonomically specific ways in the production of cercariae (see other sections in this book for citations to these).

Redial Stage/Generations

Redial generations are intriguing because, unlike the more abundant sporocyst stage, this stage has an intestinal cecum, pharynx, and birth pore through which a cercaria can exit. Køie and colleagues (1977) provided an ultrastructural study on the microvillus-like and cilia-like projections on the redia of *Fasciola hepatica* as well as that of the cercaria, external cysts, metacercaria, and migratory stages. A study by Dönges (1971) using chain-transplantations of daughter rediae of an echinostomatid (*Isthmiophora melis*) from infected to uninfected snails can pass through a minimum of 42 successive generations. Similar results have been obtained using redia of at least 2 other echinostomes. Dönges thought the limiting factors for redial multiplication in the intermediate host must be the size and lifespan of the intermediate host.

Preformed redia are known to occur in miracidia of the chicken eye flukes *Philophthalmus megalurus* and *P. gralli* based on ultrastructural studies by West (1961) and Nollen (1990a). This redial stage escapes and actively moves about when the miracidium stops swimming in pond water. Nollen (1990b) found that rediae escaped much earlier when in certain culture media than when in certain salt solutions or pond water. Because some rediae can feed on sporocysts of other trematodes, creating competition among trematodes in snail hosts, the technique described by Nollen can be used to obtain rediae for such studies. Sporocysts differ from rediae by lacking the intestinal cecum, and consequently the nutrients for the developing cercariae must be absorbed through the sporocyst's syncytial tegument (Køie, 1985).

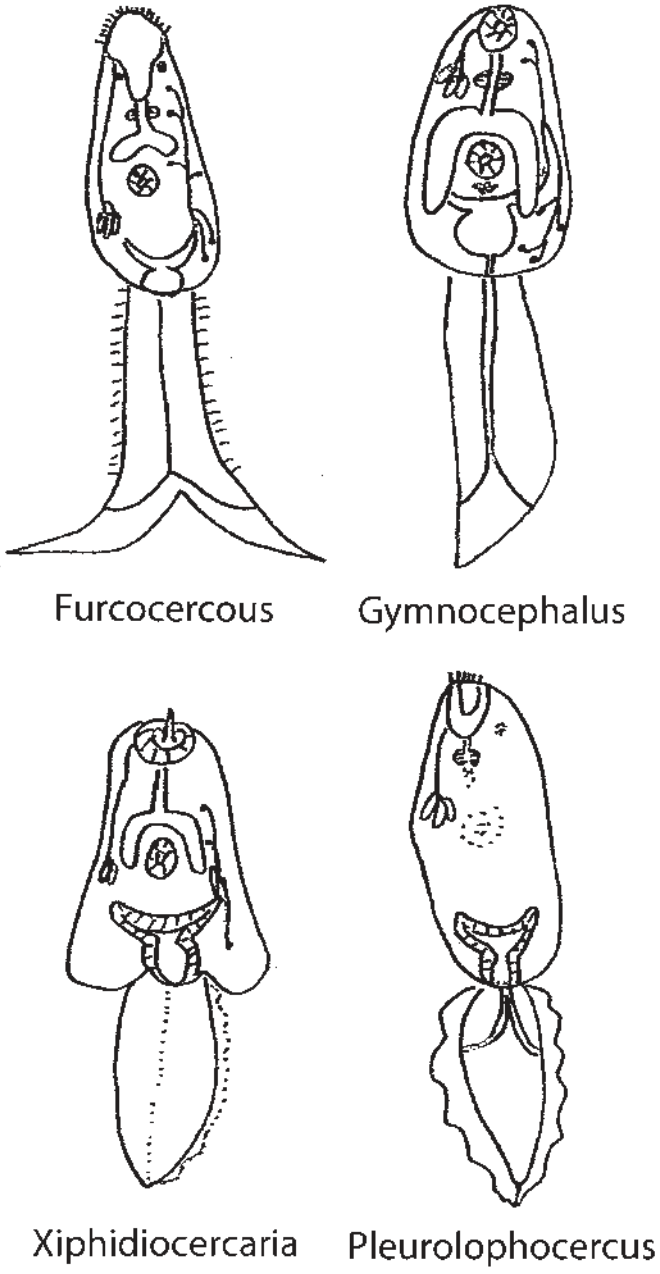

Figure 1. Furcocercous and gymnocephalus cercariae, mostly having thin-walled excretory vesicles, and xiphidiocercaria and pleurolophocercus, mostly having thick, putative epithelial excretory vesicles. The name of the furcocercous type refers to its forked tail. Source: R. M. Overstreet. License: CC BY.

Box 8. Host-Digenean Relationships — Study It

Asexual reproduction of the blood fluke *Trichobilharzia ocellata* affects reproduction of *Lymnaea stagnalis*, its snail host. Under laboratory conditions, an uninfected snail laid from 35 to 85 eggs per week and died before reaching 28 weeks, and an infected snail produced 24,500 cercariae/week, laid only 1 egg/week, grew more rapidly, had 90% survival at 28 weeks, and lost 2.9 mg of carbohydrate and 5.1 μg of protein (Bourns, 1974).

Cercarial Stage

Life histories differ among digenean families, and the cercarial type for a family plays an important role in dictating that history. Various textbooks describe or illustrate numerous types of cercariae (for example, Schell, 1985; Olson, 1974; Roberts and Janovy, 2013; Bullard and Overstreet, 2008). To avoid complexity, for purposes of this chapter, only 4 general types are covered, namely, furcocercous and gymnocephalus, mostly having thin-walled excretory vesicles, and xiphidiocercaria and pleurolophocercus, mostly having thick, putative epithelial excretory vesicles (Figure 1). Most of the 4 general types have a well-developed excretory system with flame cells that occur in specific patterns, a poorly-developed alimentary tract, oral and ventral suckers, and a tail. Remember that all sorts of exceptions exist.

The name of the furcocercous type refers to its forked tail (Figure 1). The anterior end has small spines and penetration glands with ducts that empty near the spines. The gymnocephalus type, relatively large so that it usually can be detected without a dissecting microscope, has cystogenous glands producing cysts that typically attach to or entwine on vegetation, shells of intermediate hosts, or other external substratum. A genital primordium is usually apparent. These types with relatively thin-walled excretory vesicles (bladders) differ from the next 2 with thicker walls and representing what was previously termed Epitheliocystida of LaRue (1957) rather than Anepithetheliocystida (**an** = without; Latin). Thus, a xiphidiocercaria with its 'epithelial' excretory vesicle, a feature that relates to its ability to encyst within the intermediate host, and a movable stylet anteriorly that assists in penetration into the intermediate host. There are typically 2 types of glands with ducts that exit anteriorly, 1 for penetration and 1 for encystment. The fourth—but not necessarily final—type is the pleurolophocercus; its name refers to a fin fold located on the tail which assists in both swimming and allowing the cercaria to maintain its position near the surface of the water. This type has rasping spines on the anterior end that associate with penetration glands; cells lining the excretory vesicle sometimes assist in forming material to create the inner wall of the cyst.

Most of what is known about the excretory, or paranephridial system, is known from the cercarial stage. Pearson (1986) published a good chapter on the subject that also mentions a distinct lymphatic system. Some digeneans have corpuscles and others have concretions. Both constitute important taxonomic features and both beg to be studied. Only a few trematodes, but some in different families, contain excretory concretions, and these may have different functions. Martin and Bils (1964) initiated studies by using ultrastructure of these structures in the metacercaria of *Acanthoparyphium spinulosum*. They determined that these concretions were composed chiefly of calcium carbonate and a trace of phosphate. Initially, calcium salts were delivered to the main collecting excretory vesicles in a flocculent state and ultimately deposited in concentric layers. They thought this material may be useful to fix carbon dioxide and buffer acids. Whether this is true, whether they involve lipid metabolism (Erasmus, 1967), whether they have an osmoregulatory function (Gibson, 1973), or whether these structures in different species have different functions create wonderful questions for students to ask and answer. For sure, the concretions have important biological functions and, at least for some investigators, are also important taxonomic features in adults and larval stages of some species of monorchiids, haploporids, opecoelids, hemiurids, and other digeneans. The corpuscles fill the main excretory vessels of some diplostomatids, echinostomatoids, hemiurids, and other digeneans.

Biological aspects of cercariae differ for each species and for each species under different environmental conditions. This aspect makes cercariae useful as indicators or fun to assess their ecology. Cercarial emergence of different species from their molluscan host, worms both related and not, occurs at different times related to light: dark cycling and other factors, usually related to the necessary presence of the intermediate hosts (Bell et al., 1999; Ginetsinskaya, 1988; Yamaguti, 1975).

Box 9. Metacercaria, Cysts — Study It

Weinstein and Fried (1991) used 2 species of *Echinostoma*, *Echinostoma trivolvis* and *E. caproni,* which infect the kidney of the snail second intermediate host *Biomphalaria glabrata* to experimentally infect 6–8-week-old ICR mice. The intestine of mice infected with *E. caproni* exhibited dilation, atrophied villi, a large loss of goblet cells, and retained worms compared with the expulsion of *E. trivolvis* from the mice intestine, which had an increase of goblet cells and of collagen. In another study with *E. caproni* with ICR mice, Hosier and Fried (1991) fed 25 cysts to each of 40 mice and recovered about 15 worms per mouse weekly for 20 weeks. The number of worms decreased after that with only 2 mice infected, one with 8 worms at 24 weeks and one with 1 worm at 29 weeks.

In a different researcher's study using NMRI mice, initial rejection occurred at week 12 PI, with body area per worm increasing from week 4 to week 12; the body area of worms in ICR mice was much less. In NMRI mice, the worms located in the posterior 80% of the intestine through the first 8 weeks and then occurred in the first 60 and 80% of the intestine and thereafter compared with the first 60 to 80% until week 12 and then occurred in the first 40%, demonstrating that differences in growth, body area, and distribution of the species differed between the 2 mouse strains. Once again, there are a lot of different digenean species for students to study in different hosts.

Differentiation of cercarial gland cells and the function of each will make exciting student projects!

Metacercarial Stage

The metacercariae can be free in the tissue or encysted in their second intermediate host, and the cyst wall differs among species and occasionally among families. Also, occasionally when most in a group are encysted, others are free. For example, the diplostomatid *Hysteromorpha triloba* can be situated free or encapsulated by host fibrotic tissue in tissues, usually deep within the musculature and often associated with the vertebral column, whereas *Bolbophorus damnificus* and other species in the genus and most in the family Diplostomidae are encysted.

Some metacercariae are relatively small such as that of *Paragonimus kellicotti* in the pericardial sac or soft tissues of crayfish; and these, like *Paragonimus westermani* (more common in humans in Asia), develop from a 0.4–0.7 mm metacercaria in a 0.4 mm diameter cyst into a large, 7–15 mm adult encapsulated in the lungs of their mink, feral cat, or other mammal (including human) host in the Midwest to Southeast United States. There are many species.

Some metacercariae of other families develop precociously into a stage that matures within hours. For example, some microphallids can deposit eggs in the feces of their migratory bird hosts shortly after the bird feeds on its crustacean intermediate host prey. This allows that gastropod first intermediate host to get infected. In some cases, both the snail and crustacean hosts have restricted geographical ranges, but they permit infection of the bird, which will migrate thousands of kilometers within a few days or weeks.

Cysts

Cyst walls are often similar in related species, but that is not always true. For example, in *Parorchis acanthus,* the wall is bilayered with 5 sublayers detectable with light microscopy and histochemistry. However, ultrastructural examination shows 3 layers without detectable sublayers. Perhaps some of the sublayers may constitute interfaces at surfaces and between layers. The innermost layer is laminated and formed by secretions from bâtonnet glands (Cable and Schutte, 1973) which have spiraled layers that unroll, and these layers also occur in cysts of *Fasciola hepatica.* The cyst wall of the related *Philophthalmus megalurus* has 2 layers, missing the 1 formed by bâtonnet glands. Secretions from glands in the encysting cercaria are excreted to form the outer layers. These cysts, like most from gymnocephalus-type cercariae, encyst on vegetation or external to host tissue.

Cable and Schutte (1973) considered 1 set of glands that originated in the parenchyma to be involved with encystment of the cercaria, but Haas and Fried (1974) considered those to function during post-metacercarial development. Some echinostomatids (*Echinostoma* spp.) that form cysts within the kidneys of ranid frogs, second intermediate hosts, some restricted to Bowman's capsule if in ranid frogs (Bowman, 2014), but all had a fibrous capsule of host origin. Some of those cysts with thicker encapsulations turned brownish, and the metacercariae in these often died (Martin and Conn, 1990).

Cysts formed by xiphidiocercariae in crustacean intermediate hosts also show a variety of types of cyst walls. Strong

and Cable (1972) described the ultrastructure of a 4-layered wall in *Microphallus opacus* carried from the crayfish intermediate host gill where the cercaria penetrated into the digestive gland where the cyst embedded. Heard and Overstreet (1983) studied the cercariae of *M. basodactylophallus* infecting the blue crab and *M. turgidus* from several species of *Palaemonetes.* Cercariae of these 2 species both deposit what we called penetration cysts on a gill lamella to allow leverage in penetrating through the gills after shedding their tails. Both have 2 pairs of cystogenous glands. The larger anterior ones stain dark red with neutral red until the penetration cysts (pseudocysts by Prévot [1974]) form. The smaller glands located near the midbody also have ducts emptying near the stylet similarly to where the anterior gland ducts exit, take on a lighter stain with neutral red, and do not change color until the beginning of encystment.

Cyst walls of most trematodes exhibit resistance to host inflammatory cells. Howell (1973) demonstrated the resistance of cysts of *Stictodora lari* (Heterophyidae) to encapsulation by cells of the fish host. When he implanted glass beads into the abdominal cavity of the western mosquitofish, he noted encapsulation in 3 days as opposed to 21–23 days for encapsulation of the trematode in the same site, presumably because the outer layer of the cyst made up of host material acts as 'self.' The rigid cyst wall of the related *Cryptocotyle concavum* has 4 layers of parasitic origin surrounded by a host-derived capsule (El-Mayas and Kearn, 1995). Some species in a variety of digenean families in some specific hosts produce chromatophores in the outer host encapsulations (for example, Overstreet and Heard, 1995). Whether in fishes or crustaceans, these black spots can be involved in attraction of predators or in degeneration of the metacercariae.

Entering the Definitive Host

The digenean usually enters the final, or definitive, host as a free or encysted metacercaria when that final host feeds on the second intermediate host. Numerous exceptions exist such as some haploporoid and bivesiculid cercaria whose bodies withdraw into the base of their tails and are eaten directly unencysted. Also, the blood fluke cercaria penetrates the final host. In the case of some blood flukes, a stage known as a schistosomulum undergoes development in some site different than from where it resides as an adult. This name is sometimes reserved for the stage of mammalian members of Schistosomatidae; however, when parasitologists carefully examine infected fishes, they find immature specimens developing in a site different from where the adult resides. They should be considered a schistosomulum, a counterpart of metacercariae.

In the final host, the typical adult digenean usually occurs in the alimentary tract, but they can occur in almost any tissue, depending on the species. Examples are muscle tissues, lungs, bile duct, body cavity, gills, and others (for example, Yamaguti, 1971; Bullard and Overstreet, 2008).

Types of Digenean Life Cycles

There are many different digenean life cycles, such as a 4-host cycle (for the hemiurids *Halipagus* spp.: *Physa*-like snail, cyclops-like copepod, dragonfly, and frog). Hundreds of such cycles exist (Yamaguti, 1975) and more than that have yet to be discovered and offer wonderful opportunities for students such as those reading this chapter. Molecular sequencing has certainly been able to piece different stages of a cycle. For example, it clearly demonstrated that the cercaria, known as *Cercaria sevillana*, was the metacercaria of the microphallid *Gynaecotyla longiintestinata* as reported by Pina and colleagues (2007).

Host-Digenean Relationships

Host-digenean relationships typically result in little pathological alteration, but when a cycle necessitates the intermediate host to be attractive to the definitive host, pathological alterations can be involved. In numerous cases, a definitive host may respond heavily to a parasite, but it usually relates to an abnormal/accidental host for the parasite or a condition that helps complete the cycle, like in aquaculture discussed later.

When a large number of individuals harms the host, this relationship is usually referred to as a disease rather than an infection. For example, Lumsden (1979) reports a fibrotic response to an egg of *Schistosoma mansoni* in a mouse liver, a condition that can allow the ultimate passage to the intestinal lumen to be passed externally and does not cause disease unless heavily infected. He also shows an electron-micrograph of *Paragonimus kellicotti* in a cat lung. Responses occur around eggs and the worm; apparently if just 1 specimen obtained from a crayfish infection gets in a lung, it will migrate searching for a mate and create extensive host cellular response and, if the mate is found, the pair becomes protected from further response by fibrotic encapsulation (Lumsden and Sogandares-Bernal, 1970). Many cases of pathological responses are shown to occur in humans (for example, Beaver et al., 1984; Ash and Orihel, 2007) and wildlife (for example, Takashima and Hibiya, 1995; Randall and Reece, 1996; Jacobson, 2007).

In some cases, large numbers of cercariae penetrating into a host or of miracidia initially penetrating and then migrating within a host can cause pathological alterations or even kill the host (for example, Bullard and Overstreet, 2002; 2008). In the case of cercariae of *Diplostomum* sp., its odors did not have

an effect on juvenile rainbow trout; however, when odors—alarm substances—from infected juvenile fish encircled free cercariae, the number of penetrations and length of time spent motionless by the cercariae increased (Poulin et al., 1999).

A different diplostome, *Ornithodiplostomum ptychocheilus*, acts differently in the brain of its fathead minnow host (Matisz et al., 2010). The cercaria can reach the brain within 3 hours by using different nerves, it then utilizes specific nerve tracts to reach the outermost tissue layer of the optic lobes where it grows for 4 weeks, and finally shifts its location to the adjacent meninges where it encysts. Associated with the shift in location occurred a massive inflammation, which lasted about 9 weeks and affected the health of the fish, with the amount apparently depending on the intensity of infection.

Effects on Host Behavior

When a host-parasite relationship is critically examined, one often finds the behavior of the host is altered. Whereas harmful effects often characterize the heavily infected final host, one can more often detect a harmful effect in the second intermediate host. This happens because the alteration influences the ability for the final host to prey on the infected intermediate host and for the parasite to successfully conclude its life cycle than would occur by chance alone. A wonderfully simple example that can be observed along the Gulf of Mexico and Atlantic coastline is infection of the talitrid marsh amphipods *Uhlorchestia spartinophilia, Orchestia grillus*, and *Chelorchestia forceps* by *Levinseniella byrdi.* (Curiously, however, note that this microphallid could not experimentally infect other tested talitrids (Bousfield and Heard, 1986; Overstreet and Lotz, 2016). The outstanding thing about this infection is that about a month after being infected, the amphipod turns from a greenish, grayish, or brownish color to a translucent or bright orangish and becomes negatively phototactic, not always hiding under thatch layers or wracks of dead, dissociated leaves and stems of marsh grass or other of their dietary debris-shelters. The carotenoids in the amphipod with infective metacercariae seem to become unbound from their protein, resulting in free and unmasked pigments and a color that makes the amphipod attractive to predatory birds and an ability to tolerate more direct light than an uninfected amphipod. Moreover, disorientation apparently caused by metacercarial physical effect on the ventral nerve ganglia can make the infected amphipod especially available to the seaside and marsh sparrows, clapper rail, willet, and semipalmated sandpiper definitive hosts. Most species of *Levinseniella* do not cause the orangish coloration, but a similar color occurred in *Austochiltonia australis* in Tasmania when infected with *L. tasmaniae* according to Smith (1981), and in other infections.

The example becomes more complex and ecologically important when researchers examine infections in a large-scale, 11-year, marsh study in Massachusetts, United States where nutrients were added to 3 large marsh areas containing tidal creeks flooded twice a day, but not added to 3 otherwise similar reference locations (Johnson and Heard, 2017). *Orchestia grillus* was the numerically dominant arthropod in the ecosystem, and along with *Uhlorchestia spartinophilia*, which was limited to the low marsh, were the only amphipods infected with *Levinseniella byrdi*. Looking at only *O. grillus,* the authors found similar amphipod densities between the enriched and reference locations during the first 4 years, but the densities were significantly higher in enriched areas in years 5–11. The densities of infected amphipods ranged from 0–3/m^2 with an average prevalence of 2.4% in reference marshes compared with 0–24/m^2 with an average prevalence of 15% across all years in the enriched marshes. The mean intensity of 1–5 metacercarial cysts did not differ among locations, but the prevalence increased each enrichment year, and 1 metacercaria would produce the orange coloration in the amphipod. After a decade, the mean prevalence of infection was up to 30% in the nutrient-enriched marshes compared with 2.4% in reference marshes. The biomass of infected amphipods was 11 times higher in the enriched compared with reference marshes. Infected and uninfected amphipods occurred in the high marshes, but only infected ones inhabited open areas such as vertical creek walls when exposed at low tides in enriched areas where the sandpipers could be observed feeding on them.

A myriad of cases, some critically described, may be found in books, chapters, and articles, some of them cited here (for example, Barnard and Behnke, 1990; Combes, 2001; Moore, 2002), but most cited elsewhere and others waiting to be investigated. Such studies attract readers, and they incorporate many fields of biology and general science for the investigator to adequately assess.

Hyperparasitism

A hyperparasite is a parasite that occurs either in or on another parasite. Dollfus (1946) reviewed literature at that time on hyperparasites of helminths as well as added further information. Examples include an ectoparasite copepod attached to the hemiuroid *Derogenes varicus* in the buccal cavity of what is known today as the American plaice (*Hippoglossoides platessoides*) located in Northumberland, England, United Kingdom, and studied by Marie Lebour.

Box 10. Personal Recollection from the Author Robin Overstreet (from 2018)

I had considerable data on infections in the Atlantic croaker and Gulf killifish collected since 1969 from a variety of locations, a few the same stations on a continual basis. Presentations on some aspects of analyzed data were given, but I had continued collecting samples for intended long-term studies until 2005 and maintained them in various freezers at University of Southern Mississippi and at commercial freezers in Biloxi. As it turns out, in 2005 Hurricane Katrina destroyed all properties containing those freezers, and lack of power for weeks allowed those frozen fish in salvaged freezers to spoil. Nevertheless, my student Andrew Claxton and I hope to report on additional materials and on materials that had been analyzed periodically over the years, and those are substantial. Sometimes even carefully protected host and parasite specimens as well as data can still be destroyed by floods, tornadoes, or fire.

In the pharyngeal cavity of a puffer near Woods Hole, Massachusetts, United States, a trichodinid ciliate was noted by Edwin Linton infesting *Lintonium vibex*. Probably more likely not accidental are a multitude of internal 'protozoans.' He covered a variety of microsporidians from all stages of trematodes, including members of a few different families; a few haplosporideans; a flagellate; opalinids from amphistomes in frogs; and even a nematode. Canning (1975) provided more information on the same and additional microsporidians. She also described others, and Overstreet described yet more with Yuliya Sokolova (Sokolova and Overstreet, 2018; 2020).

Overstreet (1976b) found a flagellate species of *Hexamita* in the cecum of *Crassicutis archosargi* different than one from an acanthocolpid by Hunninen and Wichterman (1938) and others mentioned (Overstreet, 1976b). Overstreet has searched for ciliates and flagellates in digeneans from herbivorous fish like mullets and rabbitfishes without success, but opportunities exist for future researchers.

The myxosporidian *Fabespora vermicola* infects *C. archosargi* (see Overstreet, 1976a) and probably more digeneans will be infected by myxosporidians. A microsporidian has even been described from a myxosporidian in a rabbitfish (Diamant and Paperna, 1985). The haplosporidean *Urosporidium crescens* infects cercariae and metacercariae of microphallids in grass shrimp and the blue crab causing a condition called blackspot in the crab and shrimp when the metacercariae become greatly hypertrophied (for example, Overstreet, 1978; 1983).

Whether an accidental infection or not, Graham (1969) reported an alarid mesocercarium in *Styphlodora magna*. Overstreet has often witnessed these mesocercariae rapidly invade helminths in a stender dish containing saline, but has never seen an infected helminth when immediately transferred into saline.

Bacterial infections can occasionally be seen in digeneans. Overstreet has often seen the Brownian movement of a bacterium in the excretory vesicle of some haploporids from mullets. He tried unsuccessfully to obtain and culture specimens with a drawn out capillary tube and regular tryptic soy broth. Others are encouraged to use a similar technique with a micro-manipulator and a combination of different culture media and sequencing procedures.

As discussed elsewhere in this chapter (Curran and Overstreet, 2004; Bullard and Overstreet, 2008), the diplostomatid *Bolbophorus damnificus* has caused millions of dollars of loss of cultured catfish annually. Infections can be associated with nephrotic pathological alterations in the catfish host. However, when as few as 4 metacercariae of *B. damnificus* are experimentally hyperparasitised by the bacterium *Edwardsiella ictaluri,* the commercial channel catfish (*Ictalurus punctatus*) died (Labrie et al., 2004). About 10% died by day 8 and cumulative mortality of 85% by day 21 compared with 45% mortality when exposed with the bacterium only (without the digenean) and 0% with controls and just the digenean group at day 21. Other studies reveal that different bacterial strains and different fluke genotypes influence host mortality, and interactions affect virulence and host health in surprising ways (Louhi et al., 2015).

A Few Notes on Ecological Methods in Parasitology

Although ecological studies take a long time to complete, they attract a lot of students and their mentors. With careful planning, a parasitologist can accompany an entomologist, ichthyologist, mammologist, ornithologist, or herpetologist, and gather material—hopefully fresh—so it can be examined

under a microscope and fixed properly. Of course, the parasitologist will probably spend the days collecting hosts and the nights collecting parasites. Studies can involve those parasites inhabiting specific hosts, those comparing infections in different hosts or the same host or hosts in different localities or under different conditions.

For a chapter on patterns and processes in parasite communities, Esch and colleagues (1990a) introduced the historical aspects by saying that perhaps most ecological parasitologists agree that the earliest body of ecological studies was conducted by the Russian academician V. A. Dogiel and colleagues (for example, 1966), that H. D. Crofton (1971a; 1971b) introduced quantitative approaches to population dynamics, and that J. C. Holmes (for example, 1979) initiated a quantitative approach to helminth community dynamics. That chapter (Esch et al., 1990b) and other books (for example, Combes, 2001; Bush et al., 1997; 2001) can be used separately or in conjunction to understand terms and approaches. There exist a variety of books and publications that treat different aspects of ecology. For example, Poulin and Morand (2004) wrote a good general book on parasite diversity and models. Chapters should encourage readers to ask themselves many questions regarding their research and course topics. Diversity of trematodes in freshwater fishes is poorly understood and requires more research (Choudhury et al., 2016).

General Digenean Ecology

Marcogliese (2004) presented an opening address to a group of fish researchers entitled "Parasites: Small players with crucial roles in the ecological theater." He told how parasites could have pronounced or subtle effects on the behavior, growth, fecundity, and mortality of the host as well as regulate host population dynamics and influence community structure.

Digeneans seldom kill their definitive host. They occasionally harm their intermediate hosts but seldom kill them unless the hosts are being reared, such as in aquaculture. The majority of the commercial channel catfish (*Ictalurus punctatus*) grown in the United States comes from ponds in Mississippi. Eggs from *Bolbophorus damnificus* are deposited with the feces of its host, the American white pelican, into the ponds where the pelican feeds on the catfish along its flyway. Snail intermediate hosts in the shallow water of the ponds along their borders obtain heavy infections and produce very large numbers of cercariae. Consequently, since fingerling catfish occupy the shallow water, they become heavily infected, and losses of over US $10 million in catfish have occurred annually. As discussed elsewhere in this chapter, hyperinfection with the bacterium *Edwardsiella ictaluri* can kill the catfish when only 4 metacercariae occur per fish. Normally, the hyperparasitized metacercariae kill the fish. The surviving fingerlings usually have about 40 to 50 metacercariae per fish, suggesting that more—and there can be hundreds—kill the fish intermediate host (Overstreet and Curran, 2004; Bullard and Overstreet, 2008). Infected fish often have necrotic kidneys.

In addition to the adult of *Bolbophorus damnificus* in the American white pelican occurs a cryptic species, *Bolbophorus* sp., often just a few centimeters away in the same individual bird's intestine. That digenean uses sunfishes and *Gambusia* spp. as a second intermediate host, and it readily kills them in the same ponds (Overstreet et al., 2002).

As shown elsewhere, infected hosts serve as indicators of many biological activities as well as historical biogeography and phylogenetics (Brooks and Hoberg, 2000). Parasites can indicate trophic interactions over weeks or months as opposed to 24 hours or less when analyzing gut contents. When mullet fry enter the estuary from offshore plankton, the parasites reflect a copepod diet, but when the same sized fry is sampled from the nearby bottom, it adds haploporid trematodes acquired by feeding on the bottom (Paperna and Overstreet, 1981).

On the basis of 1 short collecting trip, Bush and colleagues (1993) collected metacercariae from 2 crab species from a small key in the Florida Keys, found that 1 crab species had 5 different microphallid species, and a few individuals of the other crab harbored 1 microphallid clumped in masses of a few thousand. They suggested that a single definitive host bird briefly feeding on the first crab species may be colonized by 6 different species and that the infrapopulation can increase rapidly by feeding on the other crab species. Consequently, understanding colonization processes in definitive hosts may be a critical underpinning to many community level studies. Consequently, community-level studies on invertebrate hosts (intermediate hosts as source communities) may be easier and more informative than conducting such studies on definitive hosts.

Long term studies on 1 or more parasites are important in understanding many aspects of ecological relationships. Esch and colleagues (1986) and Marcogliese and colleagues (1990) investigated *Crepidostomum cooperi* in the burrowing mayfly for 16–20 years and determined the dynamics were driven by eutrophication.

Digeneans as Indicators

Several studies have involved parasites as indicators, or tags, and most involve marine fishes because of the

difficulties answering many fisheries questions. Some studies deal with specific fishes (Gibson, 1972). A few of the many recent studies include those by MacKenzie and colleagues (1995), MacKenzie (1999; 2002), and Marcogliese and Jacobson (2015). Others are cited elsewhere in the chapter.

Feeding Behavior of Hosts

The same approach can provide information about feeding habits and other biological parameters of the hosts. For example, studies on parasites of 21 species of grebes worldwide by Storer (2000), and those by Overstreet and Curran (2005c) investigating the American white pelican and brown pelican, relate digeneans and other parasites to specific feeding habits. Both studies also show how the digeneans, digenean hosts, and other parasites show evolution of the hosts, evolution of the parasites, health of bird hosts, health of intermediate hosts, public health risks, migratory patterns, and other aspects.

Variations in results from sampling hosts for digeneans obviously differ when the presence of necessary hosts differ. However, when compared ecosystems have variation in temperature and other environmental factors, the prevalence of infection (percentage of hosts infected divided by those examined in a sample) and mean intensity of infection (the number of a specific parasite divided by the number of hosts infected by the specific parasite) may also exhibit variation. Note that high prevalence and mean intensity of the digeneans indicate a healthy host and environment.

Collections made during different seasons from the same locations will usually reflect differences in infections of some of the parasites, depending on the longevity of the infection and other factors. There are also unusual conditions such as collections from near a nuclear power plant discharging hot water. Cercariae shed a month earlier in that water than those not in the heated location (Höglund and Thulin,1988).

Overstreet (1993) discusses a variety of natural and anthropogenic cases involving temperature as well as other environmental factors on host-parasite relationships. Another example reveals dynamics of infections of *Metadena* cf. *spectanda* in the Atlantic croaker (*Micropogonias undulatus*) during subsequent similar seasons. This worm may be the same as *Metadena spectanda* in Brazil (Overstreet, 1971a). However, sequencing a few Brazilian specimens and comparing them with the larger specimens from Mississippi will probably show that the specimens from the northern Gulf of Mexico represent a new species. Both the prevalence and mean intensity reached high values in the early 1970s in Mississippi. The fish fed on a wide variety of prey, but crustaceans, annelids, molluscans, and small fishes serve as the principal diet, at least in inshore water (Overstreet and Heard, 1978). Prevalence of infection with *M.* cf. *spectanda* became increasingly higher in fish over 60 mm-long (standard length) demonstrating when the croaker fed more on fishes. These trematode infections probably differ seasonally and annually because when the temperature and salinity is high, anchovies are abundant, and they are a favorite prey for the croaker but not a host of the trematode. In contrast, when the salinity and temperature are low, anchovies are rare or absent, and the croaker is more energy efficient when searching out gobies as their fish prey. A few different gobies serve as the second intermediate host for *M.* cf. *spectanda*, and during these periods, the croaker served as a super host for that parasite (Overstreet, 1973; 1982; personal observations).

Feeding studies provide a good background for studies dealing with indicators, zoogeography, diversity, and other fields. In a presentation at a symposium, Marcogliese (2003) asked whether parasites were the missing link to food webs and biodiversity. He also pointed out the need for integrating several disciplines (as was done in classical parasitology) and how these fields are no longer highly regarded. This is a shame considering the importance of using digeneans as indicators as discussed elsewhere in this chapter.

Digeneans, especially when in combination with nematodes represent an ecological link between mesozooplankton and relatively large pelagic animals (Noble, 1973; Campbell, 1983; 1990; Marcogliese, 1995; Klimpel et al., 2010; Andres et al., 2016a).

Health of Ecosystem (Including Toxicology)

Using digeneans as monitors of environmental health requires selecting the appropriate animal host. Considerable work has been conducted with fish model systems (for example, Overstreet, 1997). Criteria for a good fish model include having a restricted home range, serving as host for a relatively large number of digenean species, and being common and easily sampled. Depending on how good a model fish is will determine whether it will answer questions and solve problems. Additional features are usually needed to support and refine a study such as parasites other than digeneans, histological findings, or genetic markers.

Overstreet (1997a) used the western mosquitofish, *Gambusia affinis,* in Mississippi as an indicator of parasitism because it was host for many different metacercarial and other larval species that showed that the environment contained many specific teleosts, birds, mammals, turtles, snakes, and the alligator as well as many specific gastropods and bivalves. It shows this because specific harsh conditions can eliminate

Table 4. List of parasitology textbooks that cover digeneans. See the References for the full citations.

First-listed author(s)	Year	Pertinent topic(s) covered, with respect to digeneans
Smyth	1962	Biology
Dogiel et al.	1966	Revised classic tome on biology and ecology
Olsen	1974	Life cycles and ecology
Nickol	1979	Host-parasite interfaces
Smyth and Halton	1983	Physiology
Ginetsinskaya	1988	Life cycles, biology, evolution
Noble et al.	1989	Biology
Esch et al.	1990	Communities
Barnard and Behnke	1990	Parasitism and host behavior
Toft et al.	1991	Coexistence or conflict?
Williams and Jones	1994	General helminthology of fishes
Halton et al.	2001	Practical exercises
Bush et al.	2001	Diversity and ecology
Combes	2001	Diversity, genetics, ecology
Littlewood and Bray	2001	Interrelationships among flatworms)
Moore	2002	Behavior of hosts and ecology
Combes	2005	Ecology
Thomas et al.	2005	Parasitism and ecosystems
Maule and Marks	2006	Molecular biology, biochemistry, immunology, and physiology
Woo	2006	Fish diseases
Poulin	2007	Evolutionary ecology
Schmid-Hempel	2011	Integrated study of infections, immunology, ecology, and genetics
Roberts and Janovy	2013	Foundations
Goater et al.	2014	Diversity and ecology
Loker and Hofkin	2015	Concepts and principles

1 of those specific hosts (break a link in the parasite life cycle) and consequently the associated digenean. He determined that metacercaria of many different species remain in the fish for periods of over a year. Consequently, the relative number of animals in the environment can be determined by sampling the model fish just once or maybe twice a year, whereas sampling the biota requires numerous collections and a variety of biologists to identify the different animals.

If all the parasites in the model in addition to the digeneans are sampled, the number of non-parasitic invertebrate and vertebrate hosts in the ecosystem can be detected, making assessing parasitic data much more economically valuable than sampling animals monthly or bimonthly because many of those animals may remain in the environment for just a short time. Of course, reference stations are necessary for comparisons. When trying to evaluate specific areas, a variety of similar locations containing the model fish with and without the suspected conditions have to be sampled as those reference locations.

Anthropogenic contaminants

Anthropogenic contaminants can act in a distinct manner relative to host, parasites, and each other as well as being influenced by natural environmental conditions. When a sample of a specific fish host from a specific area exhibits a lower number or mean intensity of 1 or more digenean species than in samples from nearby localities, that finding suggests contamination. Further assessment of the samples for bacterial contamination, histopathological alterations, and other parasites can often pinpoint the source of contamination. Multiple samples of the western mosquitofish from one Back Bay, Mississippi, United States location designated as a superfund toxic clean-up site revealed a low prevalence and mean intensity of digeneans compared with samples from reference sites. In another nearby site contaminated with specific chemicals used to treat timbers, a low number of only one of the local digeneans occurred, and a myxosporidian with associated histopathological alterations was also unique to that location. When a live sample from that location was transferred

Table 5. List of books that deal with digeneans in the context of public health or veterinary science. See the References for the full citations.

First-listed author(s)	Year	Pertinent veterinary or health topic(s) covered, with respect to digeneans
Beaver et al.	1984	Clinical parasitology
Deardorff and Overstreet	1991	Seafood transmission
Coles	2006	Chemotherapy
Garcia	2007	Diagnostic medical parasitology
Bullard and Overstreet	2008	Human marine trematode diseases
Noga	2010	Fish disease, diagnosis, treatment
Overstreet	2012	Human marine diseases
Bowman	2014	Veterinary medicine

to a laboratory and reared, about 50% died from the myxosporidian infection. No fish from 2 of the reference sites died or exhibited the infections when reared concurrently (Overstreet, 1997).

In another example from a Texas river using the same fish model, the same group of researchers determined that contamination occurred upstream from an integrated pulp and paper mill effluent canal, primarily on the basis of the mean intensity of a digenean metacercaria, which was most prevalent in the effluent canal, and invasion of a usually free-living ciliate and macrophage aggregates in the spleen, both of which occurred at the upstream location. The effluent canal, which had been incorrectly accused of being a toxic site because of the coffee-like appearance, gave the impression of being the healthiest of the 5 sampled locations (Overstreet et al., 1996).

In another study, Sun and colleagues (1998; 2009) were charged with assessing a large number of sampling locations along 2 contaminated rivers in southern Taiwan. As it turned out, because of the pollution, only fish species and hybrids of tilapia could tolerate the rivers and no intermediate hosts of expected parasites could tolerate the conditions. Results had to be obtained from the amount of morphological and histopathological abnormalities in the fishes.

Bioaccumulation

In addition to parasites indicating the presence of toxicants in the ecosystem, parasites can also concentrate toxins from host tissues. Sures (2001) reviewed this problem in fishes where helminths, primarily acanthocephalans and secondarily cestodes and nematodes, can concentrate numerous heavy metals to concentrations several orders of magnitude higher than those in host tissues or the environment. Most digeneans do not concentrate as much as other helminths, but *Fasciola hepatica* inhabiting the bile ducts of cattle has been shown to accumulate lead concentrations 172 and 115 times higher than values in muscle and liver, respectively (Sures et al., 1998). Perhaps this occurs because lead binds to the erythrocytes, is transported to the liver where the majority of lead is stripped from the blood, and is excreted into the intestine by means of bile. Apparently the site of *F. hepatica* with high concentrations of lead allows the worm good access to it. As a point of interest, this ability of many helminths protects hosts from acquiring too high of concentrations of many heavy metals shows that parasites/digeneans can be good guys!

Catastrophes

By using similar methods for determining biological richness, Overstreet (2007) sampled a variety of locations and known hosts continually for digeneans after a hurricane to assess habitat recovery. Hurricane Katrina in August 2005 reached gusts of 433 km/hour and surges penetrating 20 km inland along bays, rivers, and bayous of coastal Mississippi, Louisiana, and Alabama in the United States. Resulting devastation covered a landmass of about the same size as that of the island of Great Britain, United Kingdom. They investigated a variety of situations involving hurricanes, but regarding digeneans, they noted how long it took various digeneans to become reestablished following Hurricane Katrina. Loss of biota resulted from perturbations of sediments and surge of high salinity water into estuarine and freshwater habitats. Clay and sandy sediments were lost from some areas and added to others, with the storm's energy being most influential offshore and at a depth of 25–30 m, where 1 m of sediment was scoured from the bottom and re-suspended, with the corresponding loss of the infauna. The surge of over 9 m in some locations with water of 32 ppt replacing water of 15–0 ppt saline, flushing out and killing nearly all of the biota.

The reader must keep in mind that it may take 1 or more years for the invertebrates serving as intermediate hosts to become reestablished and additional years for those invertebrates to become infected by their digenean parasites. Of course, migrating fishes that acquire infections in Texas or Florida in the United States do not show a loss of infections nor do local fishes that migrated to avoid the effects of the storm. In the latter case, the authors considered reestablishment as infections in juvenile fish that had not been born until after the storm.

By the time of the first scientific presentation on reestablishment (Overstreet, 2007), only a few fish species became infected, and with a low mean intensity of digeneans. Sampling continued, and updated results on specific digeneans and other parasites were presented at various scientific meetings, and finally the compiled data were published (Overstreet and Hawkins, 2017), showing that reestablishment can take a short period for some species and many years for others.

Climate Change

Parasites, and digeneans in particular, allow researchers to investigate large scale events. Since change takes place over evolutionary and ecological time scales resulting from natural and anthropogenic causes, Marcogliese (2001) considered temperature and parasites of boreal regions of North America as a good focal point for investigations of climate change. Because different hosts in a cycle follow range constrictions, the presence of a parasite will also become modified in unpredictable ways since the host-parasite systems are intricately interwoven with the environment, and changes in physical processes at different temporal and space scales will affect parasite populations differently.

Introduced Species

Occasionally when a megafaunal organism becomes introduced outside its typical location, other organisms are included or the range of the organism spreads. Also, a parasite can be included in the transfer or spread. As an example, tropical fishes are reared in outside facilities and are shared with other growers. This has happened probably on numerous occasions and has involved vegetation and the invasive snail *Melanoides tuberculatus* (common name, red rim melenia). The snail became introduced at least in the 1970s into southern Florida, United States (Roessler et al., 1977). Unfortunately, the heterophyid *Centrocestus* cf. *formosanus* infects the snail and follows it around the southeast United States, and probably elsewhere. This parasite has an unusual characteristic of promoting proliferation of cartilage surrounding the metacercarial cyst, usually in the gills of the host. This abnormal proliferation occurs extensively in a few of the many fishes the cercariae can infect. Some of the fishes are rare, such as the federally listed endangered fountain darter (*Etheostoma fonticola*), which is highly susceptible to and easily killed by the infection. Mitchell et al. (2005) reported on the history of the introduction and the life cycle of the worm.

Digenean species that had once been considered to be introduced are occasionally determined by molecular comparisons to be sister species. For example, what had thought to be *Bolbophorus confusus* introduced from Europe appears to be *B. damnificus* or *Bolbophorus* sp. of Overstreet and colleagues (2002), who discuss the introductions.

Migration of Model Host

Using parasites of pelicans and grebes as a variety of indicators, including migration, was mentioned elsewhere in the chapter. Most species of these are useful to examine because they host many digeneans. However, the use of digeneans and of other parasites has also been very useful for determining migration of fish hosts and stock separation. For example, Blaylock and colleagues (1998) examined Pacific halibut from 15 localities from northern California to the northern Bering Sea for all parasites, including many digeneans. The fish clustered into 3 groups on the basis of parasites, and these depended on temperature and geography, features that have a large effect on digeneans. These and associated data suggest 3 separate stocks of this commercially important fish.

Host Stocks

Not all fishes make good models for using parasites to separate or distinguish fish stocks, and often digeneans do not provide the best parasite indicator. The sablefish, *Anoplopoma fimbria*, off Canada's west coast is an example of a good model (Kabata et al., 1988). These fish contained 7 digeneans, and their prevalence, mean intensity, relationship with host age, and locations (13) differed enough to show the seamount and slope host populations constituted separate stocks. That development of the localized fisheries provided a significant yield to Canadian fishermen.

There are other cases where salmonids infected with a single freshwater digenean species that has a lengthy longevity in both freshwater and marine phases, such as the metacercaria of *Nanophyetes salmincola* and adult of *Plagioporus shawi* in juvenile trout from the United States Pacific Northwest, then tag the fish. They allow researchers to know from which specific or group of freshwater sources the infected individuals arose.

Dalton (1991) reported tagged steelhead trout 5,000–5,500

km from their area of origin in the central North Pacific Ocean. Monitoring of chinook salmon smolts from the Trinity River, California, United States detected annual differences, possibly because of differences in temperature and the resulting shed of cercariae (Foott et al., 1997). In this study, fish and snails were placed in a shallow trough, and 10 fish were examined and sectioned. In a wet mount of the most infected tissue, the mid-kidney, the most infected individual contained 10,220 cysts/gram, and the mean number of metacercariae in sections of the posterior kidney was 28.0 ± 14.7. The Puget Sound Steelhead Marine Survival Workgroup (Berejikian et al., 2018) reported abstracts on various projects on *Nanophyetes salmincola,* including cumulative mortality of fish at 46 days in seawater (mortality leveled at 7% after day 12 for infected individuals versus 0% for uninfected ones), susceptibility of waterborne cercariae to chemotherapeutics (100 ppm hydrogen peroxide, Perox-Aid®, and various doses of formalin), plus others.

Detective Work/Forensics

Many of the findings resulting from using parasites, primarily digeneans and other helminths with complicated life cycles, as indicators can be considered detective work. However, some cases clearly can be defined as detective work in the literal sense. An example concerns a truckload of red drum (*Sciaenops ocellatus*) that had been stopped and examined by different authorities, including United States Customs officials. The fishermen operating the truck said the fish, which they planned to sell, came from the Carolinas, from where the catch would have been legal. Professionals had Overstreet examine a sample of the fish, and he found a bucephalid endemic to the northern Gulf of Mexico where limits and seasons were stricter than along the Atlantic coast. Neither Overstreet nor other researchers who had examined the red drum from the Carolinas found any infection with that worm. That evidence was used to find the fishermen guilty of illegally catching and trying to sell Gulf fish (Overstreet et al., 2009).

Ichthyologists considered the Pascagoula River in the late 1960s to be free of striped bass. Consequently, a few hatchery-reared individuals fed commercial feed were released in the area, and 1 year later Overstreet (1971b) discovered several specimens of a new digenean species, *Neochasmus sogandaresi*, in a specimen of the fish. Then and later, a great deal of effort was unsuccessfully spent trying to see if the parasite also occurred in another host. None was discovered, suggesting that a small wild stock of striped bass had occurred in the area and represented at least enough striped bass to maintain a population of the digenean.

Digeneans as a Human Food Source

Numerous books and articles treat public health. Overstreet (2003) took the other point of view. He wrote about people eating parasites on purpose, with the assumption that there was no public health risk. For example, different people eat, or have eaten in earlier times, the giant liver fluke of various species of deer, *Fascioloides magna*: Hunters eating what they call little livers, Cajuns eating double-fried puffed flukes, Native Americans of the southeast United States eating what they call little flapjacks, and some members of the Sioux Nation in North America eating them and other liver flukes as a portion of their game or domesticated mammal with the intention to transfer the life force. Some indigenous people in Africa eat the paramphistomes from the stomach lining of hippopotamus calling them the juicy part of the hippo, and members of the tribes of Meghalaya, India relish paramphistomes from the rumen of cattle and buffaloes. Lots of parasites other than flukes are eaten fresh or cooked with smiles on the face of the consumers.

A Note on the Literature on Digeneans

Some early literature is intentionally being presented because it, mixed with recent studies, allows a good starting point for a variety of studies that can be readily tackled. These older approaches include those by Paul Nollen, Bernie Fried, Robin Overstreet, and others. Several general parasitology textbooks treat various aspects of digeneans, some in more detail or from a different point of view than presented in this chapter. Examples of some of those include are listed in Table 4.

Some students will address this chapter with public health or veterinary medicine viewpoints. A few of the many references treating such information are listed in Table 5.

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Part IV

ENDOPARASITIC NEMATODES

48

Nemata

Introduction to Endoparasitic Nematodes (Phylum Nemata)

Scott L. Gardner

Phylum Nemata

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Chapter 48

Introduction to Endoparasitic Nematodes (Phylum Nemata)

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General Shape and Structure of Endoparasitic Nematodes

Nematodes are relatively small, mostly dioecious, non-segmented worms that generally lack any sort of well-developed external structures for locomotion. Many of the animal parasitic species possess external cuticular structures that enable them to move and maintain their position in the host and—depending on the host—this could include the gut, mesenteries, sub-cutaneous tissues, or other organs. The external structures of parasitic nematodes that enable them to detect their environment include **amphids** on the anterior end, **deirids** (also called **cervical papillae**) near the level of the **nerve ring**, **phasmids** near the tail, and various kinds of **sensory sensillae**. As far as is known, no animal parasitic nematodes have eye spots, and only a few nematodes that live in marine intertidal interstitial environments have eye spots. Some nematodes have complex lips surrounding the mouth (Figure 1) and these lips facilitate feeding. The lips and associated structures posterior to the mouth may enable the nematode to attach to the host intestine. Not all nematodes have all of these structures and different combinations of characters are used for identification and classification into different groups.

Examples of species that attach firmly in the small intestine are species of Ancylostomidae (Figure 2) which, among others, includes the hookworms (genus *Ancylostoma*). Anisakids are also known to attach to the submucosal layer of the gastrointestinal tract of their hosts. This includes various species in the genera *Anasakis*, *Terranova*, and *Pseudoterranova.* These nematodes usually use marine mammals as their definitive hosts.

On the posterior end of nematodes, males of some groups, such as species of the order Strongylida (Figures 3–5), have a well-developed and complex apparatus called the **copulatory bursa** that is used to grasp the female to facilitate mating. Other nematodes, such as species of *Physaloptera*, Oxyurida, and Filarioidea have various combinations of papillae (sensillae) and cuticular ornamentations that serve a similar purpose.

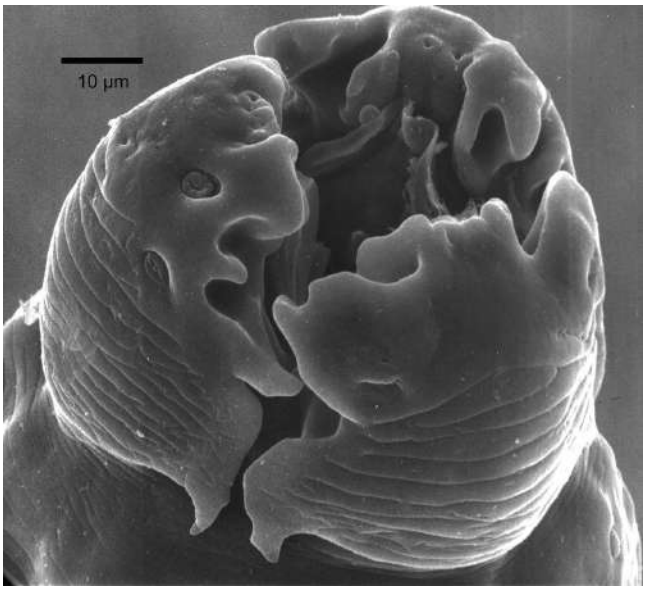

Figure 1. Scanning electron micrograph of the anterior end of a species of *Paraspidodera* from *Ctenomys* in Bolivia showing 3 large lips with sensory papillae, also known as sensillae (plural) or sensillum (singular) on each lip. Source: S. L. Gardner. License: CC BY 4.0.

Figure 2. Image of anterior end of *Ancylostoma ctenomyos*, a parasite of rodents of the genus *Ctenomys* from the eastern lowlands of Bolivia. The stoma with the cutting teeth and plates are clearly visible. The villi of the small intestine are pulled into the stoma and the teeth abrade the villi. Blood then is pumped into the intestine from the abraded villi via the esophagus. Source: S. L. Gardner. License: CC BY 4.0.

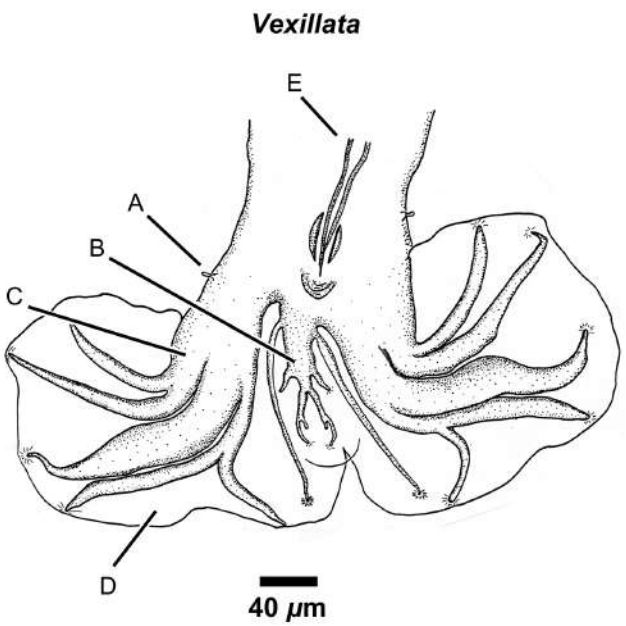

Figure 3. Drawing of the posterior end showing a ventral view of the copulatory bursa of *Vexillata armandae* (order Strongylida, family Ornithostrongylidae), a parasite of the coarse haired pocket mouse (*Chaetodipus hispidus*). Labels: A) Papillae (ray 0); B) Dorsal ray; C) Lateral rays in the 2-1-2 arrangement; D) Vellum (thin cuticular membrane) of bursa; E) Setacous spicules retracted. Source: S. L. Gardner. License: CC BY 4.0.

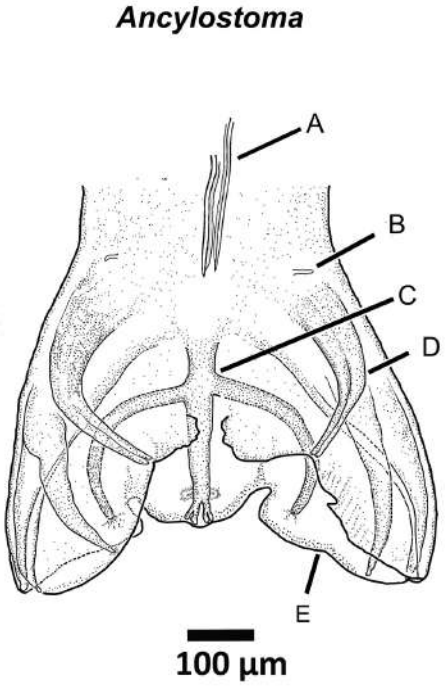

Figure 4. Drawing of the posterior end of a species of *Ancylostoma ctenomyos* a parasite of rodents of the genus *Ctenomys* in lowland Bolivia. Labels: A) Posterior ends of the spicules; B) Ray 0 or pre-bursal ray; C) Dorsal ray; D) Lateral rays; E) Vellum of bursa. Source: S. L. Gardner. License: CC BY 4.0.

Depending on the species, the body of parasitic nematodes usually has a greater diameter (when viewed in transverse section) near the middle of the body of the animal relative to the anterior end. All nematodes have a round shape when viewed from either end of a transverse section through mid-body (Figures 5A–D). The body wall of a nematode is covered with flexible acellular cuticle that is mostly translucent and is secreted by a cellular hypodermis. The circular or round nature of the transverse sections is why these animals are sometimes called roundworms.

The **external cuticle** of the nematode may be smooth, have longitudinal striations, or have well-developed wing-like structures called **alae** (= wings; Latin) or ridges that are situated on the lateral surfaces of the body (Figure 5D). Some species have well-developed lateral alae near the anterior end where they are called **cervical alae**. The tiny wings, or alae, can also run the entire length of the body on the lateral cuticle of the body, in which case they are termed **lateral alae**, and if only on the posterior, they are called **caudal alae**. It is thought by some researchers that the alae are utilized by nematodes to orient themselves somehow in the host and there is conclusive evidence that the alae are species-specific, and can be used in identifying and classifying the nematodes.

The cuticle of nematodes can be extremely thin and fragile, or it can be thick and extremely strong and even very resistant to digestion, as shown in those species that live in the acid environment like that found in the stomach of a carnivore. For example, filarioid nematodes have a very thin cuticle. They occur in the tissues of vertebrates and are able to exist only within these osmotically balanced habitats and if they are removed and placed in water, they usually quickly explode due to osmotic pressures.

Nematodes are sometimes casually called **pseudocoelomates** because in most species that have been studied, their body cavity does not appear to be completely lined with cells derived from embryonic mesoderm. All coelomate animals that are termed eucoelomates—or true coelomates—have a peritoneum that lines both the body cavity and the internal organs and the peritoneum is a layer of tissue that is derived from embryonic mesoderm. Most nematodes do not have an obvious or visible lining of the body cavity, although, as Armand Maggenti (1981, p. 10) pointed out: "Nematodes have a well-developed body cavity filled with fluid and with some evidence of mesodermal lining, if one considers the muscle sheath as mesoderm and the epidermal layer around the gonads and the basal lamella of the intestine as being of mesodermal origin."

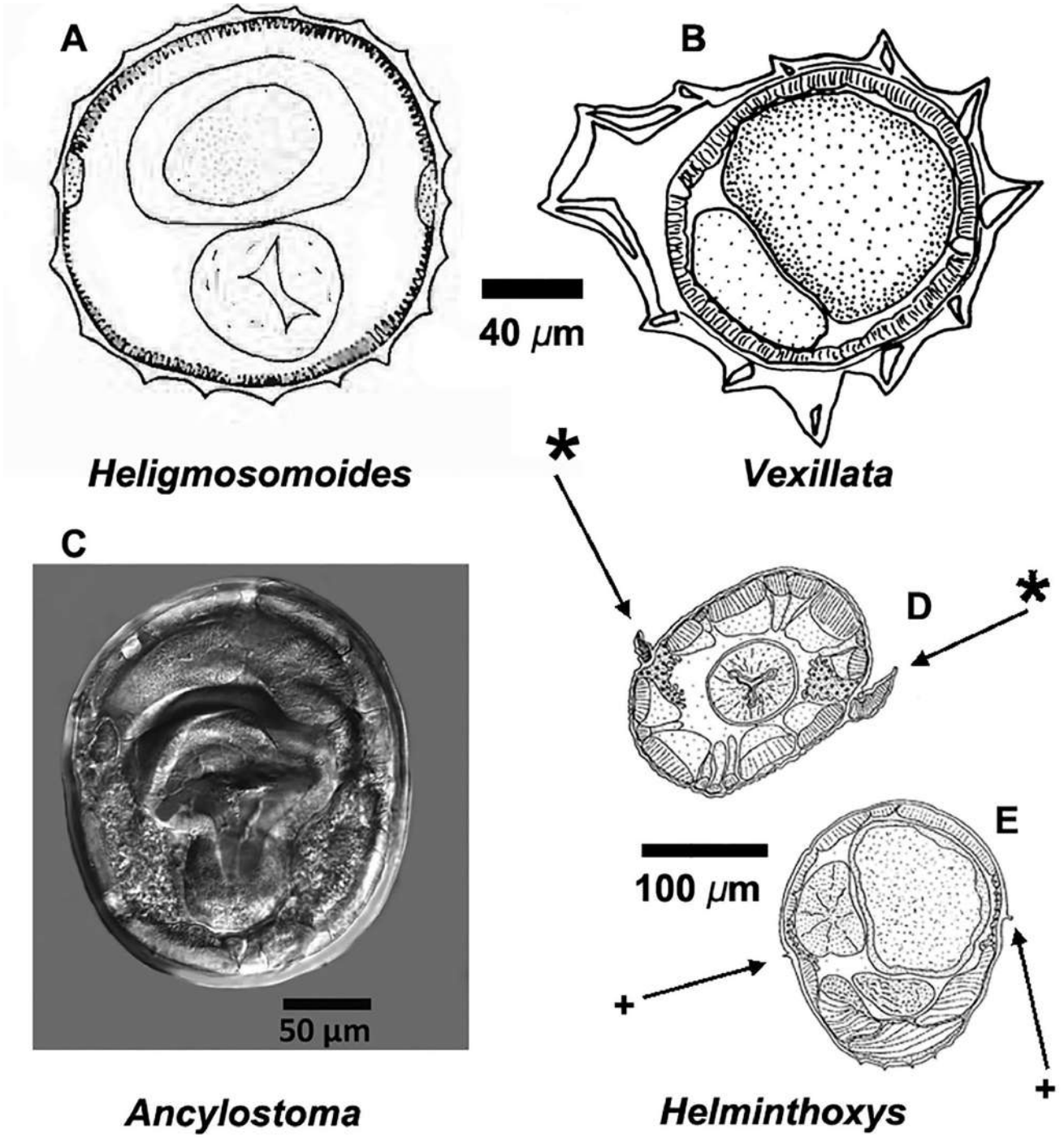

Figure 5. Drawings and photographs of transverse sections from 3 species of order Strongylata (Figures A–C), including: A) Transverse section at midbody of *Heligmosomoides thomomyos* showing the lack of cuticular aretes within the cuticle; B) Transverse section at midbody of a species of *Vexillata* showing the well-developed carene and well-developed cuticular aretes; C) Digital image of a transverse section of *Ancylostoma ctenomyos* showing a relatively smooth cuticle; D and E) Transverse sections through a species of *Helminthoxys* (order Oxyurida) with D) Cephalic alae (*) via a cut through the area of the esophagus. E) Transverse cut through the posterior part of the nematode showing the small lateral alae (+). Source: S. L. Gardner. License: CC BY 4.0.

Another unique feature of all nematodes is the fact that they have no circular muscles. Therefore, movement is accomplished by contraction and relaxation of **longitudinal muscles** in apposition, or antagonistic to, their **hydrostatic skeleton**. The nematode moves as the muscles contract and the cuticle flexes thus enabling nematodes to writhe around, moving through the organs and tissues of their hosts, and in some cases, into the external environment. Nematodes maintain their form in a way analogous to a water balloon because their body fluids are under a positive pressure in the **hydrocoel** relative to their environment.

Individuals of most animal parasitic species of nematodes have a complete **digestive tract** with an anterior **stoma** lined with cuticle, followed by a **pharynx**, then a tri-radiate **esophagus** (Figure 5D) that can be muscular (Figure 6) or glandular in form, or the esophagus may have a combination of both muscular and glandular sections as in some species of the superfamily Filarioidea, and others. The tubular **intestine** (Figure 6F) is usually a single cell in thickness and is lined on the body cavity side with a thin collagen-like material. Internally, the single layers of cells are lined completely with microvilli (Grassé, 1965; Maggenti, 1981; 1991a). The **gastrointestinal tube** extends from the esophagus to the anus, or **cloaca**, with some species possessing out-pouched **cecae** or **diverticulae** near the esophageal end (Figure 7).

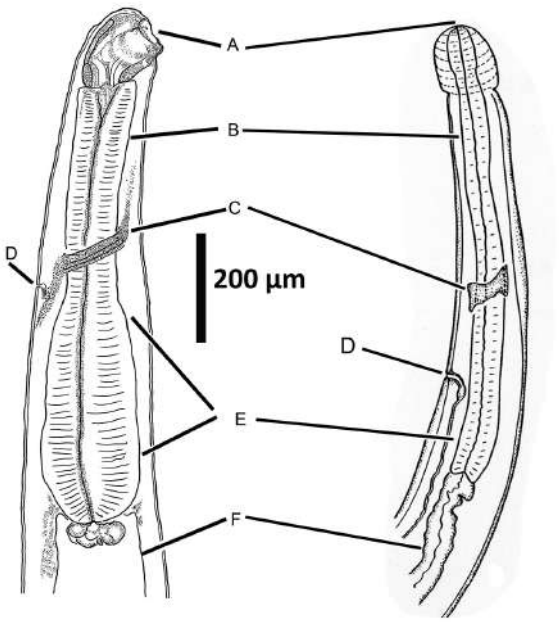

Figure 6. Anterior end of 2 strongylid nematodes showing homologous structures of the anterior end. A) Mouth; B) Anterior end of muscular esophagus; C) Nerve ring, also called the circum-esophageal commissure; D) Excretory pore; E) Base of esophagus; F) Intestine. Source: S. L. Gardner. License: CC BY-NC-SA 4.0.

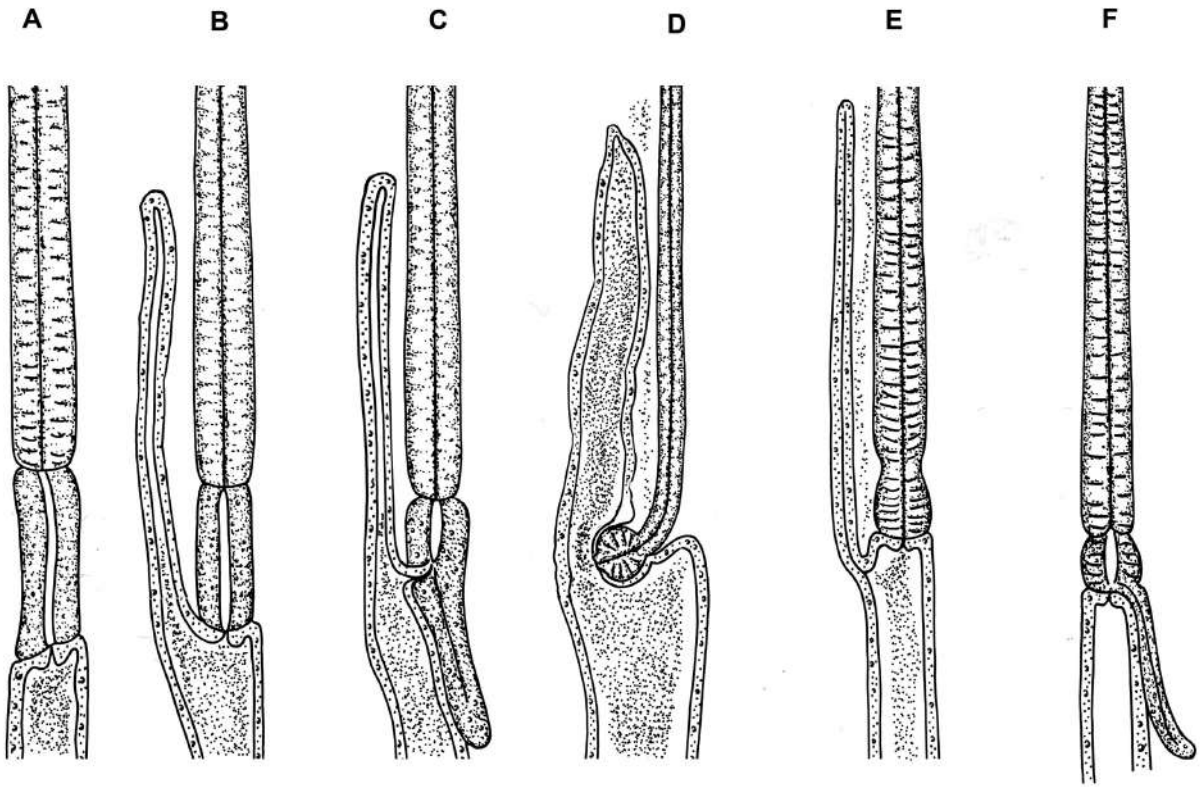

Figure 7. Intestinal cecae or diverticulae that may occur in nematodes of the order Ascaridida. A) *Anasakis*; B) *Porrocaecum*; C) *Contracaecum*; D) *Dujardinia*; E) *Aguticaecum*; F) *Raphidascaris*. Source: Adapted from Maggenti, 1981; Yamaguti, 1961. License: CC BY 4.0.

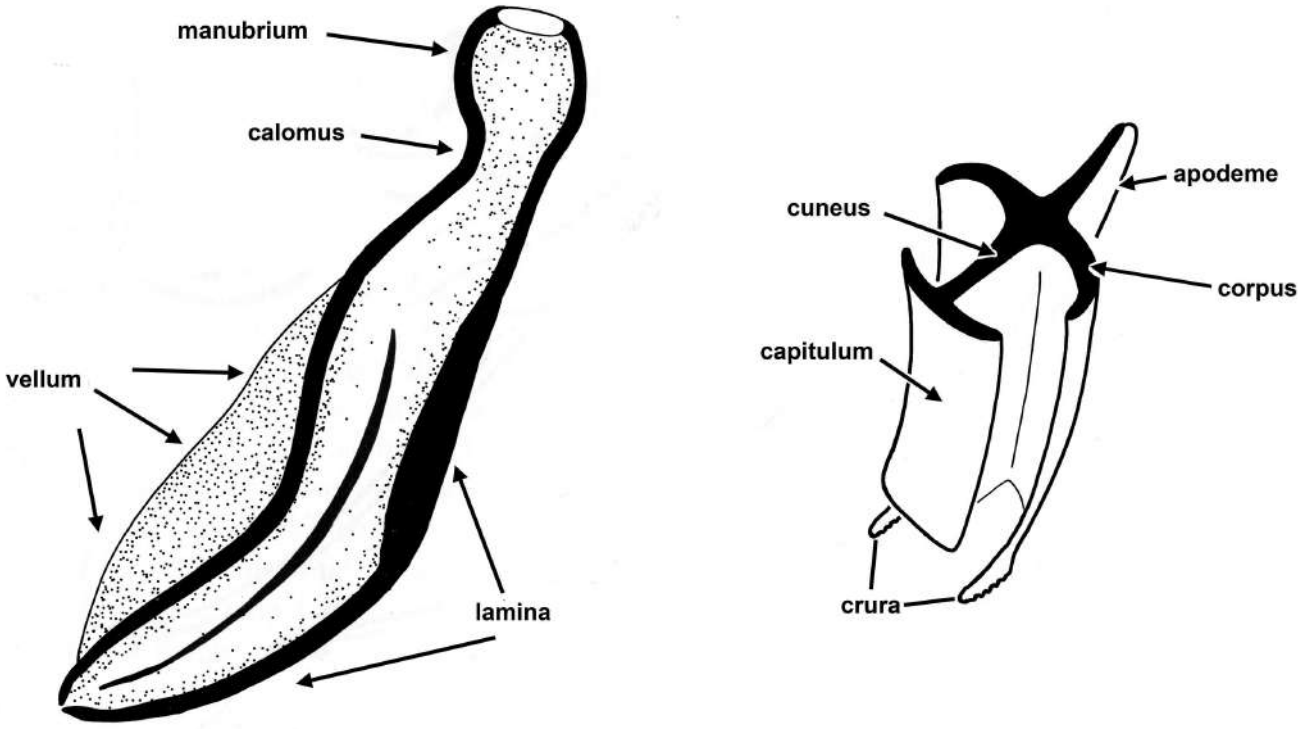

Figure 8. A) Spicule, showing major parts. B) Gubernaculum, transverse section showing how the spicule can slide through the opening between the capitulum and the corpus. Source: Adapted from Maggenti, 1981. License: CC BY-NC-SA 4.0.

Most nematodes are **sexually dimorphic** with definite male and female individuals, and they are usually **dioecious**, meaning that they have to mate to produce viable eggs. In those species that are dioecious, males usually have a pair of cuticularized **spicules** that are used to assist in the transfer of sperm to the females (Figure 8A).

There are some pinworms in which the males have no spicule at all, such as species of *Aspiculuris*. Many male nematodes also have a **gubernaculum** (Figure 8B) that serves to guide the spicules during copulation. Some species are hermaphroditic and in these cases the nematode produces both sperm and ova from the **ovotestis** of the same individual at different times during their life stage, or ontogenetic development (Maggenti, 1981).

A major **synapomorphy** (also called a shared-derived character) for the Nemata is the presence of non-contractile, **myo-neural processes** that extend from the contractile portion of the muscle cell to the neural junctions of the dorsal or lateral **nerve cords** (Figure 9). Figure 9 shows the contractile portion of the muscle below and the neural part with the nucleus above with a laterally extending neural process that extends to the nerve cord.

Nematodes vary greatly in size and the diameter of most species is usually much less than 2 mm, even in the longest of the long nematodes such as *Placentanema gigantissima*, which is a 9 m-long parasitic nematode that lives in the reproductive tract of whales. The thickest ones, or those individuals with the greatest body width of all nematodes known so far, is the giant kidney worm, *Dioctophyma renale.* This nematode lives in mustelids, such as minks, badgers, and weasels, and has a diameter of up to 15 mm with a length of more than 1 m. The eggs of nematodes are also very similar in size with eggs of most species having eggs that range from 50–100 μm-long by 20–50 μm-wide.

External Covering: The Cuticle

An example of diversity in shape and structure of the cuticle is that of the complex cuticular aretes found in most species of the superfamily Trichostrongyloidea that are parasitic in the small intestines of many species of vertebrates, especially mammals, in which the exocuticle is modified into a series of cuticular aretes called the synlophe. In these forms, it is thought that the ridges running down the length of the body of the nematode are used in maintaining their position in the intestine of their hosts (Figure 10). In the latter part of the 1960s and 1970s, an evolutionary schema of nematodes classified in the superfamily Trichostrongyloidea was developed by Marie Claude Durette-Desset and Alain Chabaud in the Laboratoire des vers, Muséum national d'Histoire naturelle in Paris, France (currently named Laboratoire de biol-

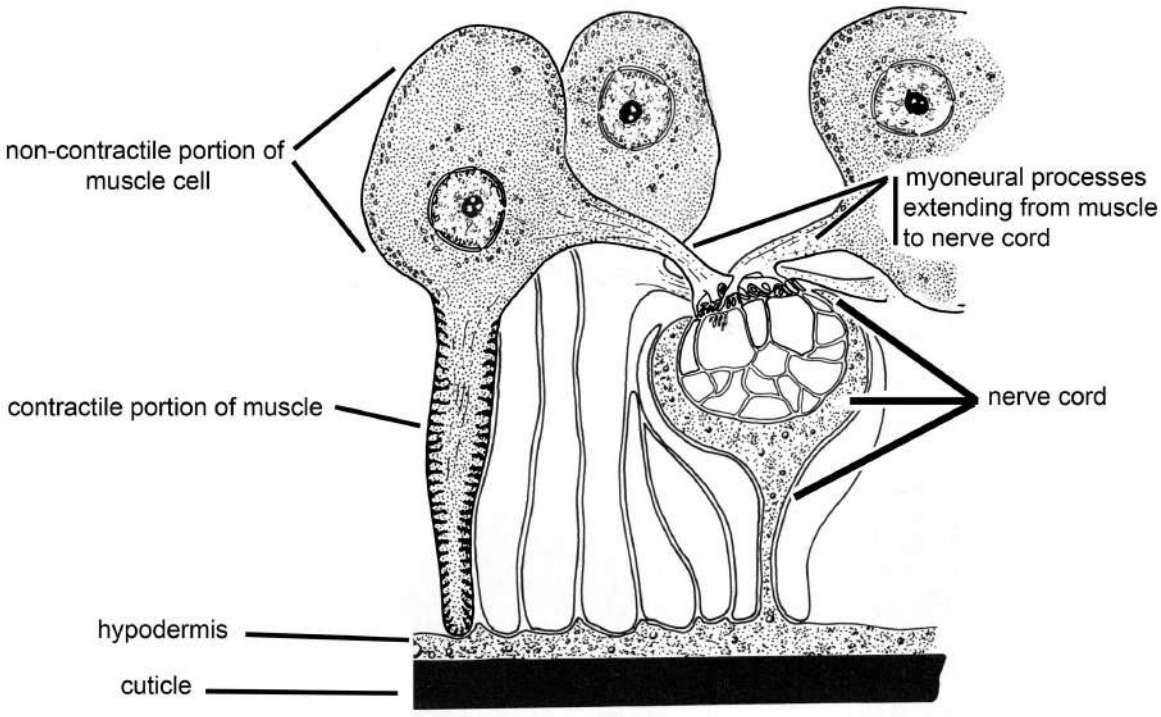

Figure 9. Schematic of the contractile and non-contractile portions of the muscle cells seen in the Nemata. Source: Adapted from Maggenti, 1981. License: CC BY-NC-SA 4.0.

ogie parasitaire, protistologie, helminthologie). They worked out the biogeographical and morphological evolution of these nematodes using a combination of the structure of the **synlophe** (Figure 10) and the rays of the bursa (Figure 3) that allowed an understanding of the evolutionary relationships of one of the most speciose groups of parasitic nematodes (Durette-Desset, 1971).

The external cuticle may be smooth without alae or external lines, or the cuticle may have complex rings. In the posterior part near the cloaca of many male nematodes (Physalopteridae, Filarioidea, and others), and in addition to sensory papillae or sensillae, there may be a rough area of the external cuticle called the **area rugosa**, or the rough area. A hypothesis about why this is found in males is that the rough cuticle may enable the male to locate and attach to the female more easily for mating purposes. Some nematodes have an **exocuticle** (external cuticle) composed of serrated ridges or bumps, or the cuticle may be very smooth. Some groups, such as species comprising the superfamilies Heterakoidea and Subuluroidea, possess cuticularized suckers situated anterior to the cloaca that are surrounded by sensory papillae and evidently enables the male to find and attach to the female in the intestinal tract of the host (Figure 11).

Host Range and Diversity of the Nemata

All species of vertebrates and many species of beetles examined by scientists thus far serve as hosts for at least 1 species of parasitic nematode. Some nematodes have a very **narrow host range**, surviving and reproducing successfully only in a single species of host, or perhaps in a phylogenetically- or ecologically-related group of species. Other nematodes show a wide host range, being much more likely to jump from one suitable host to another during opportune times during their life history, exhibiting what is termed **ecological fitting** (Janzen, 1985; see also Brant and Gardner, 2000; Brooks et al., 2019).

Within a single free-living animal, myriad habitats may be occupied by nematodes. Organs and tissues of a mammal have different hormone levels, different levels of pH, various levels of exposure or isolation from the immune system, and more. To illustrate the diversity of habitats in a single mammal host, for example in humans, *Trichinella* species can occur as juveniles, encysted in various muscles like the diaphragm or the tongue, *Strongyloides* species may be found in the mucosa of the intestine or other tissues, *Ascaris* may be found migrating in blood and lungs, juveniles called microfilariae of filarioid nematodes may be found in blood or lymph, or adult *Ancylostoma*, *Necator*, and *Ascaris* species may be found in the

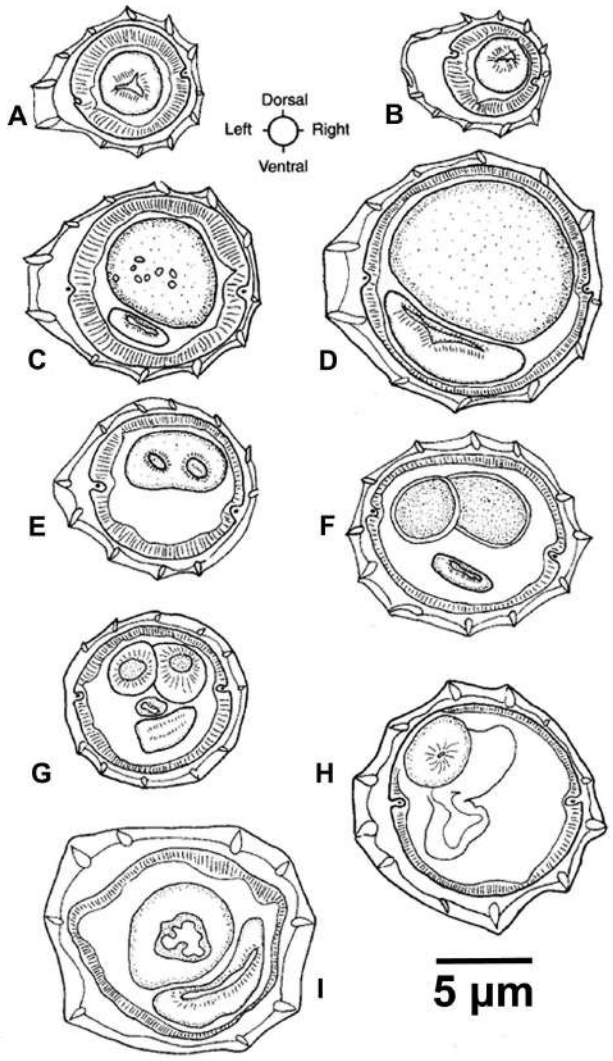

Figure 10. Transverse sections of *Vexillata armandae*. A) Section through esophagus of male; B) Section through esophagus of female; C) Midbody of male; D) Midbody of female; E) Posterior 1/4 of body of male through the spicules; F) Posterior 1/4 of body of female through eggs in the uterus; G) Posterior 1/16 of body of male through the spicules just anterior to the cloaca; H) Posterior section of female at level of ovijector infundibulum I) Posterior section of female at level of ovijector vestibule. Source: S. L. Gardner. License: CC BY 4.0.

small- and large intestines. Individuals of these same species, as well as *Onchocerca, Loa*, and *Wuchereria*, can occur as juveniles in muscle or connective tissues, and as adults in mesenteries and subcutaneous tissues, and *Enterobius vermicularis* may be found in the large intestine and cecum.

In the early part of the 20th century, Nathaniel Cobb (1915) recognized that nematodes are extremely biodiverse. He was well acquainted with animal parasitic nematodes, and his familiarity with the Nemata led him to estimate that well

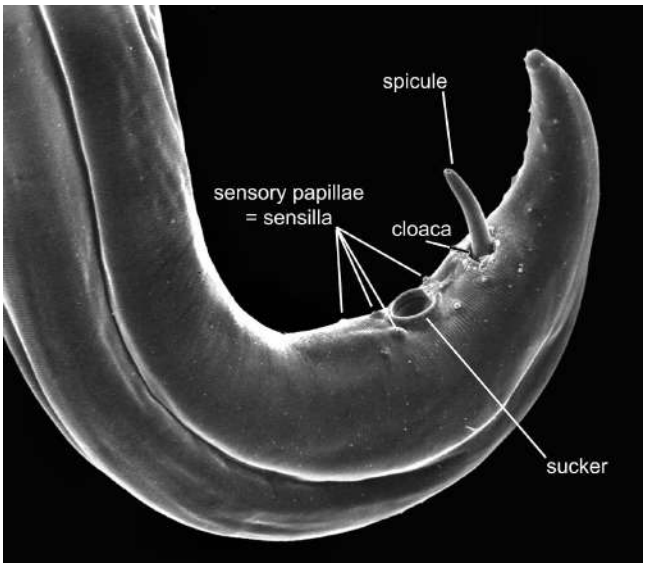

Figure 11. Posterior end (tail) of a male *Paraspidodera* showing the relatively smooth cuticle, a sucker just anterior to the opening of the cloaca, and 1 spicule protruding from the cloaca. Source: S. L. Gardner. License: CC BY 4.0.

over 80,000 species of nematodes would eventually be found parasitizing vertebrates alone. Falling short of Cobb's estimate, to date only about 20,000 species of parasitic nematodes have been described from all species of 48,000-plus recognized species of vertebrates. The natural history, development, and transmission parameters of more than 550 species of animal nematodes are well known (Anderson, 2000). As parasites of vertebrates, Anderson (2000) estimated that there were about 2,300 described genera distributed among 256 families comprising about 33% of all nematode genera. If each of the approximately 6,526 known species of mammals (as of July 2023; see https://mammaldiversity.org) were each infected with only 2 species of nematodes with narrow host range, there would be expected to be a minimum of 13,052 species of parasitic nematodes only in the class Mammalia.

Pinworms, which are nematodes of the order Oxyurida (Figure 12), have a relatively narrow host-range and it is well known that almost all species of rodents, lagomorphs, and primates have their own species of pinworm. Both recent and historical studies have shown that pinworm nematodes exhibit relatively narrow host range, and many have been shown to have cospeciated with their primate and rodent hosts (Hugot, 1999). Currently, a total of about 900 species of pinworms have been described, with vertebrates hosting about 500 species and invertebrate species hosting only about 400.

Because pinworms tend to have a narrow host range, their numbers correlate approximately with the number of host species there are. By way of example, pinworms that are para-

sitic in arthropods are classified in the superfamily Thelastomatoidea. There are currently about 4,500 described species of cockroaches with more than 20,000 to 30,000 additional species expected to be eventually described (Ghosh, 2017). There are around 17,000 species of millipedes known with more than 60,000 species expected yet to be described. If each species of cockroach and millipede harbors its own species of pinworm, huge numbers of thelastomatoid pinworms will eventually be described from just these 2 arthropod groups alone.

The greatest diversity in the superfamily Oxyuroidea of vertebrates expected to be found in the future may result from examination of the catfishes of the Amazon basin (Rodrigues et al., 2020). During studies of the catfishes of the Amazon basin, Rodrigues (personal communication, 2020) stated that every fish examined was infected with oxyurids.

It is estimated that approximately 138 species of nematodes have been reported from humans (Crompton, 1999), with 32 to 36 being host-specific. Estimates of the number of human infections in the year 2000 by species of parasitic nematodes are shown in Table 1.

Large numbers of species of Oxyuroidea are also expected to be described from Neotropical rodents of the family Muridae. As of 2022, only around 8 species of pinworms have been described from Neotropical murids, while there may be 400–800 undescribed species of Oxyuroidea, given that 1–2 new species of oxyuroid nematode are found in each new species of rodent examined.

Classification

In this book, the use of the phylum name **Nemata** (= **thread**; German) for the nematodes partially follows Hodda's (2022) work, as well as the older work by Maggenti (1981; 1991a) who considered Chitwood's (Chitwood and Chitwood, 1977) emendation of the name Nemates to Nemata a correct and robust move because this followed the Pearse system for nomenclatural endings (Pearse, 1936). Maggenti (1981) points out that the old phylum name Nematoda is a leftover class level name from previously discarded classifications that were developed when the nematodes were considered to be a class in a larger group called the now-superceded phylum Aschelminthes. However, not all biologists who study nematodes adhere to this system; see, for example, the contrasts in older classifications in the work by Libbie Hyman (Hyman, 1953), the massive work by the French zoologist Pierre-Paul Grassé (1965), and the book General Nematology by Maggenti (1981) for the variations.

Hodda's (2022) work includes an explanation about why the upper-level classification of the Nemata is confusing and difficult. Maggenti (1981) and Anderson (2000) are generally followed for the higher order names for the animal parasitic nematodes and Hodda (2022) is generally followed for the names lower in the classification; however, in this work, no great effort has been made to synchronize the classifications with Hodda (2022) and others since changes are being made daily as more data from genomic sequencing efforts roll into the databases holding information on the Nemata.

All told, above the level of the order, confusion reigns relative to the classification and systematic arrangement of the nematodes, although Hodda's (2022) efforts should bring some measure of stability to the classification of the group. Maggenti (1981) is usually followed in the upper levels of the classification and the phylogeny presented by Anderson (2000; see Figure 12) provides the main groups; however, there are some points of agreement between Maggenti's and Anderson's with Hodda's more recent (2022) classification.

The classes recognized in this book include the Secernentea and the Adenophorea (Figure 13). Recent work shows that these groups are mostly substantiated both in morphological and molecular analyses although competing phylogenetic hypotheses and associated classifications have also been proposed (Adamson, 1989; Dorris et al., 1999; Blaxter et al., 1998; Brooks and McLennan, 1991; Anderson, 2000; Hodda, 2022).

Table 1. Estimated numbers of common nematode infections in humans worldwide. Data from Crompton, 1999.

Species	Number of humans infected	Geographic distribution
Ancylostoma duodenale and *Necator americanus*	1,298,000,000	Worldwide
Brugia maylayi and *B. timori*	13,000,000	South Pacific, Southeast Asia, and India
Dracunculus medinensis	Estimated to be fewer than 100 human cases	Sub-Saharan Africa
Loa loa	13,000,000	West Sub-Saharan Africa, central Sub-Saharan Africa, and Yemen
Onchocerca volvulus	17,660,000	Central America, South America, and Sub-Saharan Africa
Strongyloides stercoralis	70,000,000	Temperate regions

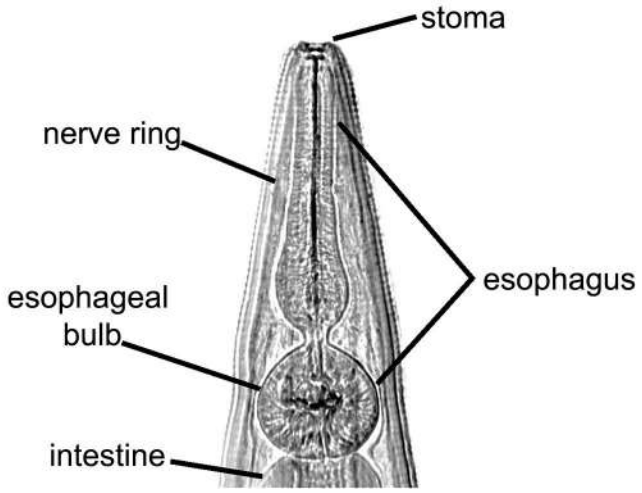

Figure 12. Anterior end of *Didelphoxyuris*, a pinworm nematode of South American marsupials, showing: A) The small stoma at the anterior end, followed by the well-developed muscular esophagus with a large posterior bulb. The esophagus acts as a muscular pumping organ, pumping food into the intestine. Source: S. L. Gardner. License: CC BY 4.0.

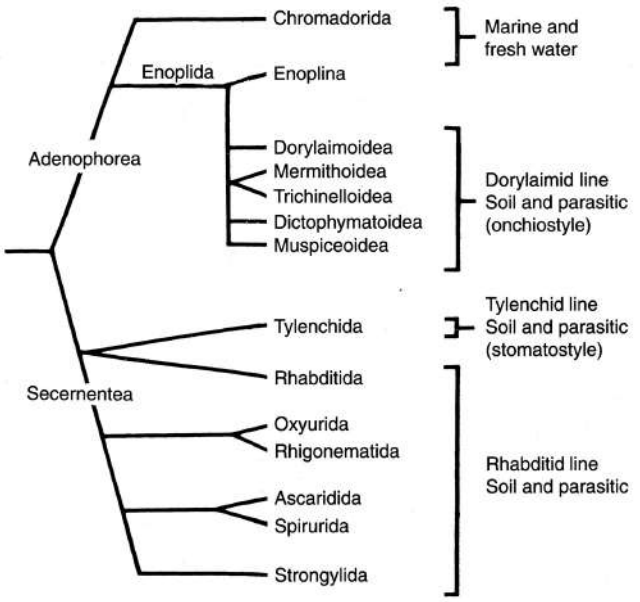

Figure 13. Phylogenetic hypothesis of main groups of the phylum Nemata. Sources: Adapted from Anderson, 2000; Maggenti, 1991. License: CC BY-NC-SA 4.0.

Relation to Other Animal Groups

One analysis grouped the nematodes, gastrotrichs, priapulids, kinorhynchs, and the loriciferans into a superphylum group called the Cycloneuralia based on the circular shape of the nerve ring that loops around the esophagus in most groups and functions as the central part of the nervous system in these animals (Nielsen et al., 1996). Other studies have shown that the Nemata share a common ancestor with the Nematomorpha (see Zrzavy, 1998). Mayer and Whitington (2009) and Nielsen (2012) show that the Nemata share a common ancestor with the Nematomorpha in a different superphylum designation called the Nematoida.

Ancient History of Nemata

The oldest written account of the giant intestinal nematode *Ascaris lumbricoides* in humans dates to approximately 4,750 years ago, from China. In this work, foods to avoid and a description of the symptoms of humans infected with these worms was accurately given (Hoeppli, 1959; Maggenti, 1981). In the area of the Nile River Valley, early Egyptian physicians first recorded the presence of both *Ascaris* and the guinea worm, also called the fire worm, *Dracunculus medinensis*, in an ancient papyrus manuscript written by Egyptian physicians around 3,550 years ago, which was obtained and translated by the Egyptologist Georg Ebers in 1872 (see Chitwood and Chitwood, 1977; Maggenti, 1981). About 2,400 years ago, Hippocrates first wrote about nematodes infecting other animals besides humans when he recorded his finding of pinworm nematodes of horses. In the 13th century, Albertus Magnus and Demetrios Pepagomenos recorded nematodes from falcons (cited in Rausch, 1983; see also Chitwood and Chitwood, 1977).

Physical evidence of nematodes date from much earlier than the written accounts. Eggs of the pinworm of humans, *Enterobius vermicularis*, and the whipworm of humans, *Trichuris trichuria*, occur in coprolites dated to about 7,000 years old from Peru, and eggs of the human-specific hookworm, *Ancylostoma duodenale*, have been reported from coprolites dated to around 7,230 years old, collected from caves in eastern Brazil (Araújo et al., 2008). Eggs of *Ascaris lumbricoides* have been positively identified from coprolites of human origin dated to about 28,000 years old from caves in France, but these are the only occurrence of a record this old (Bouchet et al., 1996). The dearth of other nematodes from human remains older than about 7,000 years appears to be due to the fact that organic material comprising the coprolites themselves do not preserve well enough to last that long (Karl J. Reinhard, personal communication, 2022).

Trace or Fossil History

The only fully fossil nematodes that are known thus far are insect parasitic or plant parasitic forms that occur very rarely in amber inclusions (Poinar et al., 1994). One pinworm egg was found in a fossilized fecal pellet from a cynodont around 250 million years old (Hugot et al., 2014). Because there are no fossil records of nematodes of Cambrian or Precambrian

ages, estimates of the age of the Nemata have been only speculation up to the present time and without fossils, it is difficult to calibrate molecular clocks for the nematodes. An estimate of the time of divergence of the nematodes from the rest of the animal groups appears to be about 1,177 ± 79 million years (Wang et al., 1999).

Most authors consider the ultimate origin of nematodes to be from a marine ancestor (Maggenti, 1991; Malhakov, 1994). The tri-radiate esophagus and the tubular body indicate an initial possible primarily sedentary existence of the ancestral forms of the Nemata, with the posterior end attached to the substrate and the anterior end freely encountering the marine environment from all sides, thus the somewhat radial symmetry may be secondarily-derived from selective advantage of the animal experiencing the environment from all sides simultaneously (Armand Maggenti, personal communication, 1992).

Nematode Chapter Organization

In the following sections in this chapter, the superfamilies Trichuroidea and Trichinelloidea will be covered under class Adenophora and several representatives from class Secernentea will be discussed following those, including order Ascarida and superfamily Heterakoidea, and the orders Camallanida, Filariata, Oxyurida, Spirurida, and Strongylida.

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Nemata

Trichuroidea and Trichinelloidea (Superfamilies)

María del Rosario Robles and Rocío Callejón Fernández

Phylum Nemata

Class Adenophorea

Order Trichocephalida

Superfamily Trichuroidea

Superfamily Trichinelloidea

doi:10.32873/unl.dc.ciap049
2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 49

Trichuroidea and Trichinelloidea (Superfamilies)

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Introduction

Maggenti's (1981) classification of nematodes will mainly be followed in this section and will include a description of the morphology and molecular attributes of nematodes of the superfamilies Trichuroidea and Trichinelloidea. In recent years there has been a significant advancement in knowledge on the phylogenetic relationships of many of the species that are included in these superfamilies (see Hodda, 2022 for a summary). These results and others that help illuminate the ecological and epidemiological aspects of these nematodes will be described. The primary species of medical and veterinary importance will also be discussed.

Taxonomy

The upper level classification of this group is still unsettled and several authors have provided different proposals of infraclass hierarchies, such as from the orders Enoplida, Trichinellida, and Trichocephalida, the superfamily Trichinelloidea and Trichuroidea, and different combinations of families and subfamilies, such as Trichinellidae/Trichuridae, Trichurinae/Capillariidae, Capillarinae/Trichosomoididae, and Anatrichosomatidae/Trichosomoidinae (see, for example, Maggenti, 1981; Anderson, 2000; Moraveč, 2001a; Roberts and Janovy, 2009; Anderson et al., 2009; Hodda, 2022).

Based on Moraveč (2001a), Table 1 includes the genera included in each family considered in this section. Trichuridae includes more than 80 species, Capillariidae includes more than 300 species, Trichosomoididae includes fewer than 10 species, and Trichinellidae is a monotypic family (containing a single genus) and considered for many years to have only 1 species; however, recently 9 species have been identified (Robles et al., 2006; 2008; Fugassa et al., 2014; Krivokapich et al., 2012).

Morphological Characteristics

These nematodes have very few observable differences and, therefore, represent one of the most difficult groups to classify with respect to their taxonomy and systematics, since the genera and species are distinguished only based on their morphology (Moraveč, 2001a). For example, their cephalic structures and details of the posterior end are difficult to observe with a light microscope. The details of the anterior ends are too small to observe readily using standard microscopy so, reliable data can only be obtained by the use of scanning electronic microscopy (SEM). Using SEM, features such as the papillae and the stylet of the oral aperture, the bacillary band, and accessory genital organs may have taxonomic value, and these may otherwise be easily overlooked.

Specimens from the class Adenophorea are characterized by their lack of both phasmids and lateral excretory canals.

Table 1. Genera in each family considered in this chapter, based on Moraveč (2001a).

Superfamily	Family	Genus/Genera
Trichuroidea	Trichuridae	*Trichuris*
	Capillariidae	*Amphibiocapillaria*, *Aonchotheca*, *Baruscapillaria*, *Calodium*, *Capillaria*, *Capillostrongyloides*, *Crocodylocapillaria, Echinocoleus*, *Eucoleus*, *Freitascapillaria*, *Gessyella*, *Liniscus*, *Paracapillaria*, *Paracapillaroides*, *Paratrichosoma*, *Pearsonema*, *Piscicapillaria*, *Pseudocapillaria*, *Pseudocapillaroides, Pterothominx*, *Schulmanela*, *Tenoranema* (among others, depending on the classification used)
	Trichosomoididae	*Anatrichosoma*, *Huffmanela*, *Trichosomoides*, *Trichuroides*
Trichinelloidea	Trichinellidae	*Trichinella*

Selected Sub-groups of Trichocephalida

Following are descriptions of characteristics that help distinguish specimens among a few select groups of Trichocephalida.

Overview of Superfamilies Trichuroidea and Trichinelloidea

In general the differences in species assigned to either superfamily, as well the families included in Trichuroidea, are based on certain diagnostic characteristics (synapomorphies) that are given in Table 2. The main characters that serve to define the groups include: Relative widths of anterior and posterior portions, sexual dimorphism (body size) (Figure 1), the position of the rows of stichocytes, particular characteristics of the bacillary glands, and the number and positions of the associated bacillary bands (Figure 2). In the realm of reproductive characters, the important synapomorphies here include: Characteristics of accessory genital organs in the male, for example, caudal alae, copulatory bursa, papillae, and caudal lobes (Figure 3), as well

Table 2. Trichuroidea and Trichinelloidae: Comparison of morphological characters.

Maggenti (1981)	**Trichuroidea**			**Trichinelloidea**
Moravec (2001a)	**Trichuridae**	**Capillariidae**	**Trichosomoididae**	**Trichinellidae**
Width of body	Thin and long anterior portion, shorter and broader posterior portion	Filiform, similar throughout the extension	Filiform, similar throughout the extension	Filiform, similar throughout the extension
Sexual dimorphism	Little difference in size between sexes	Little difference in size between sexes	Large size difference between sexes	Large size difference between sexes, females twice the size of males
Position of stichocytes	Regularly aligned with similar size	Regularly or irregularly aligned with similar size	Irregularly aligned with different size	Regularly aligned with similar size
Number and position of bacillary bands	1 lateral in anterior portion, with cuticular inflations bordering the bacillary band in the proximal part	1–4 with variable positions in anterior and posterior portions	1–4 with variable positions in anterior and posterior portions	Without bacillary band
Male: Characteristics of genital organs	Spicule sclerotized and spicular sheath cylindrical with spines. Caudal papillae present	Spicule sclerotized or not observable and cirrus with morphology and ornamentation. Variable structures: caudal alae, copulatory bursa, papillae, caudal lobes	Spicule and cirrus vestigial or absent	Spicule and cirrus absent
Female: Characteristics and position of the vulva	Opening near the end of the esophagus May have protruding lips and spines	Opening near the end of the esophagus	Opening near the end of the esophagus	Opening in the middle of the esophagus
Eggs	Polar plugs slightly protruding above the shell surface	Polar plugs not protruding above the shell surface, although with variable forms and ornamentations	Polar plugs not protruding above the shell surface, sometimes dark surface	Without eggs

The classification below superfamilies follows Moravec (2001a). The morphological characterization was obtained from Moravec (2001a), Anderson et al. (2009), and contributions of authors of this chapter (Figures 1–6).

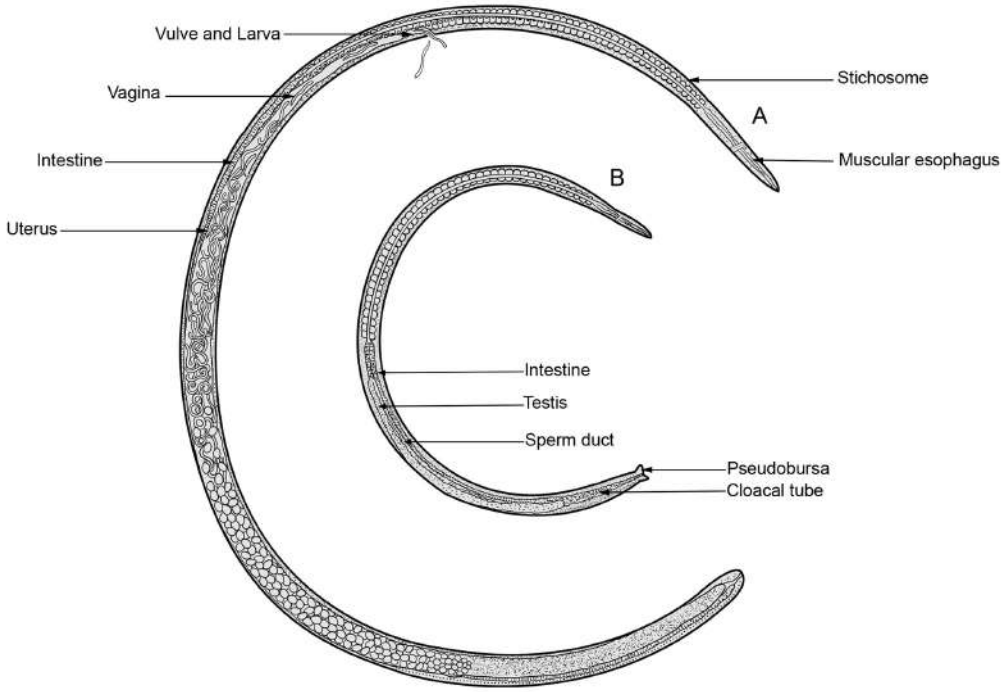

Figure 1. Diagrammatic representation of morphological structures of male and female specimens of *Trichinella spiralis*, as an example of Trichinelloidea. Source: K. Solas. License: CC BY-NC-SA 4.0.

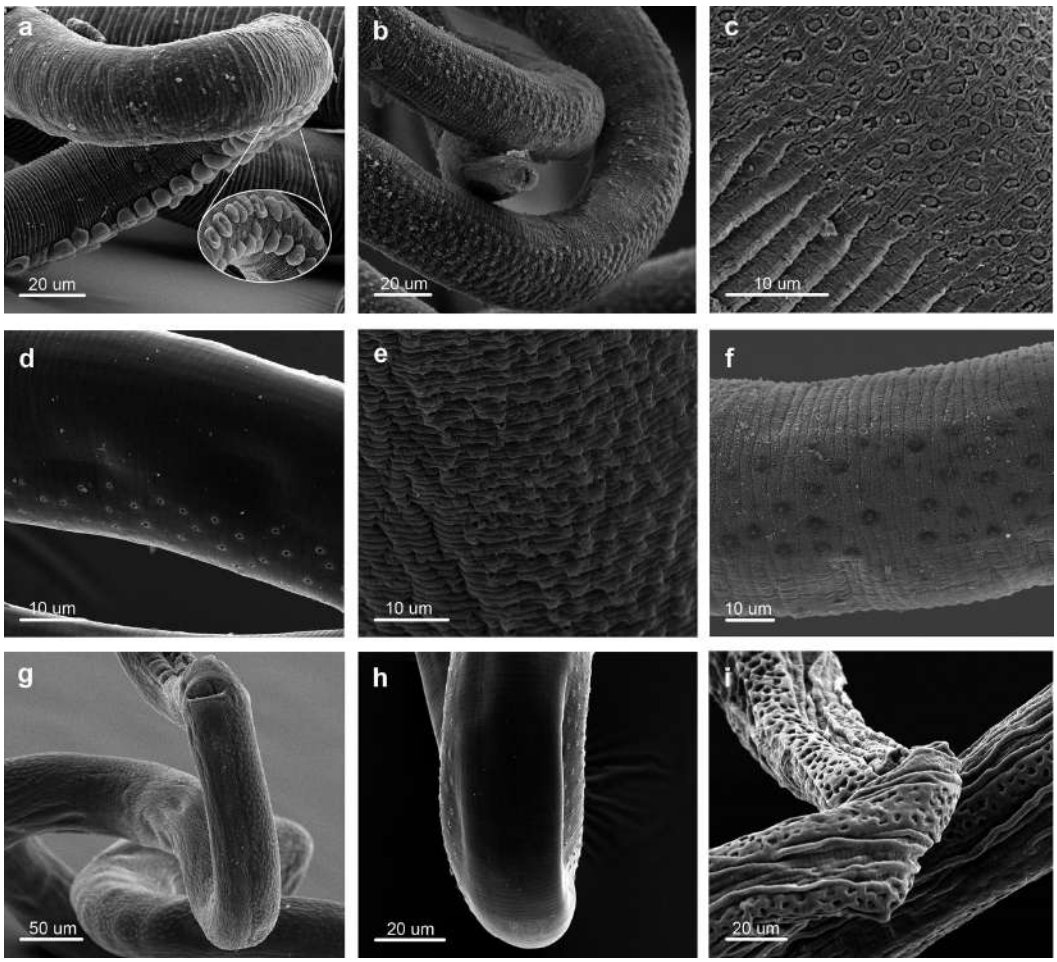

Figure 2. Scanning electron micrographs of bacillary bands from trichuroid species in rodents:

a) Cuticular inflations bordering the bacillary band in *Trichuris navonae* Robles, 2011.

b) Bacillary band located after the inflations, with detail of the oral aperture in *T. laevitestis* Suriano & Navone, 1994. Detail of bacillary glands

(c) in *T. bainae* Robles et al., 2014, (d) in *Eucoleus* sp.,

(e) in *Echinocoleus* sp.,

(f) in *Anatrichosoma* sp. Number and position of bacillary bands,

(g) 1 lateral bacillary band,

(h) 2 lateral bacillary bands,

(i) 1 ventral and 2 lateral bacillary bands.

Source: M. de R. Robles and R. Callejón Fernández. License: CC BY-NC-SA 4.0.

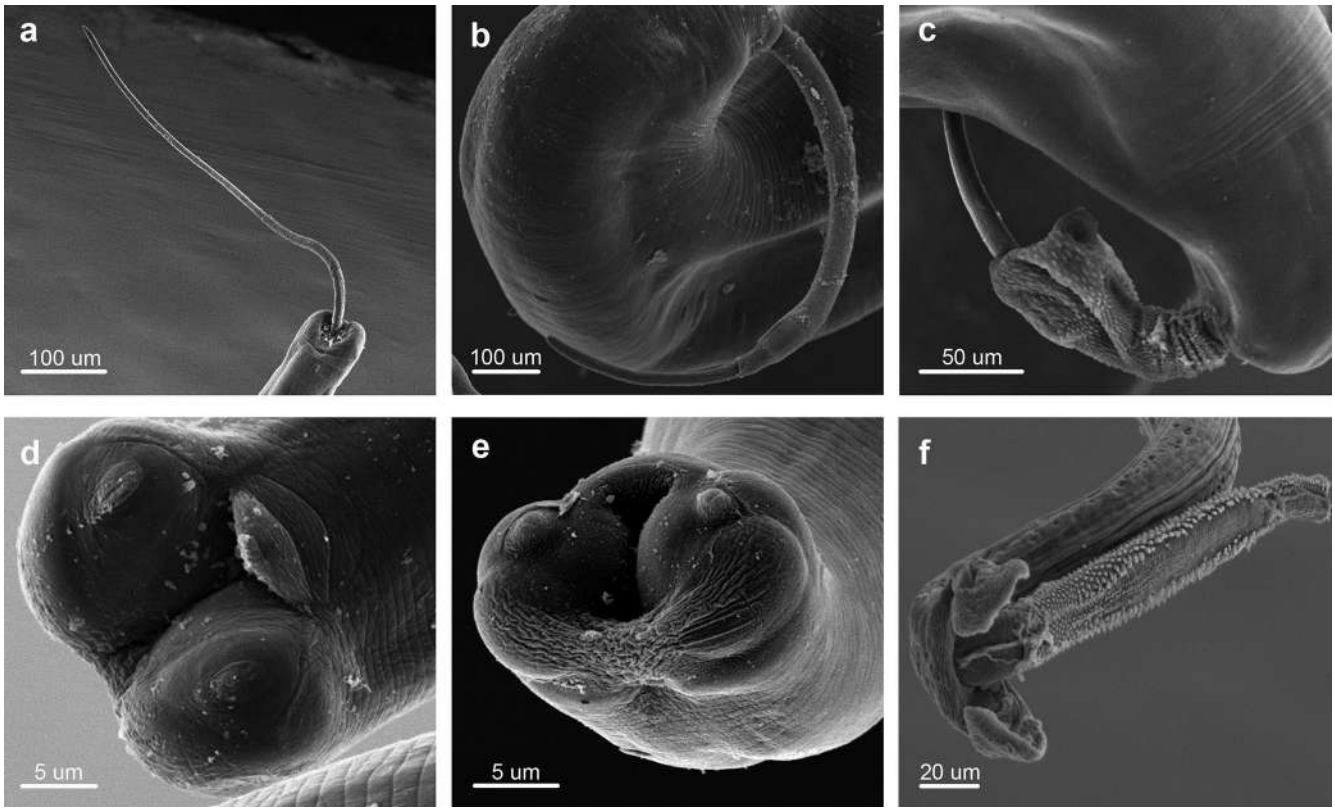

Figure 3. Scanning electron micrographs of accessory genital organs of males from trichuroid species in rodents: a) Ventral view of tail, spiny spicular sheath cylindrical and spicule everted in *Trichuris* sp. b) Lateral view of tail, spiny spicular sheath forming a distal spherical bulge in *Trichuris* sp. c) Ventral view of tail, spiny spicular sheath forming a distal spherical bell in *Trichuris* sp. d) Ventral view of tail, 2 lobes terminally expanded with 2 central papillae in *Pseudocapillaria* sp. e) Ventral view of tail, 2 lobes terminally expanded forming a pseudobursa with 2 lateroventral papillae in *Eucoleus* sp. f) Dorsal view, with spiny cirrus everted and lobes terminally expanded with 2 projections in *Echinocoleus* sp. Source: M. de R. Robles and R. Callejón Fernández. License: CC BY-NC-SA 4.0.

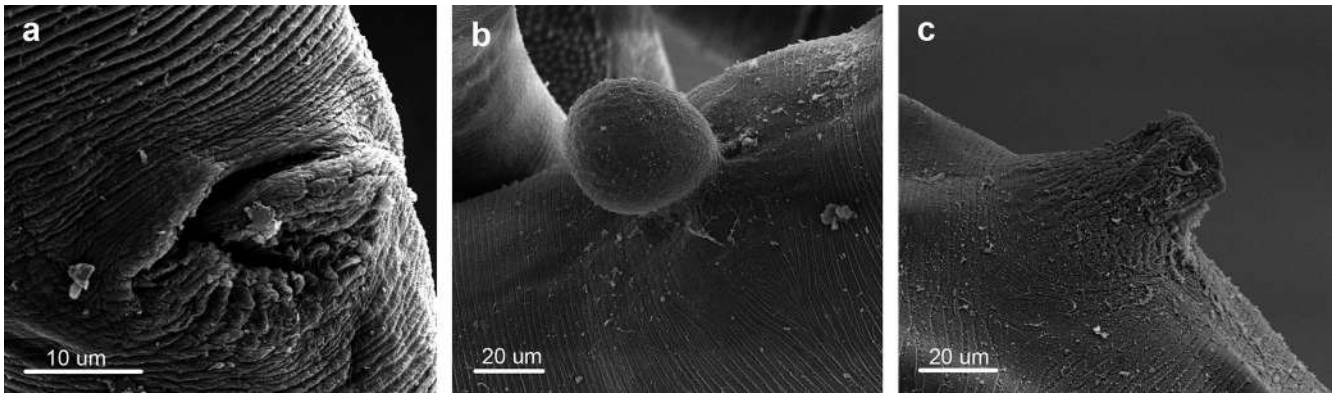

Figure 4. Scanning electron micrographs of characteristics of the vulva of the female from trichuroid species in rodents: a) Detail of non-protusive vulva in *Trichuris navonae* Robles, 2011. b) Detail of spherical protusive vulva in *Trichuris* sp. c) Detail of cylindrical protusive vulva in *Trichuris* sp. Source: M. de R. Robles and R. Callejón Fernández. License: CC BY-NC-SA 4.0.

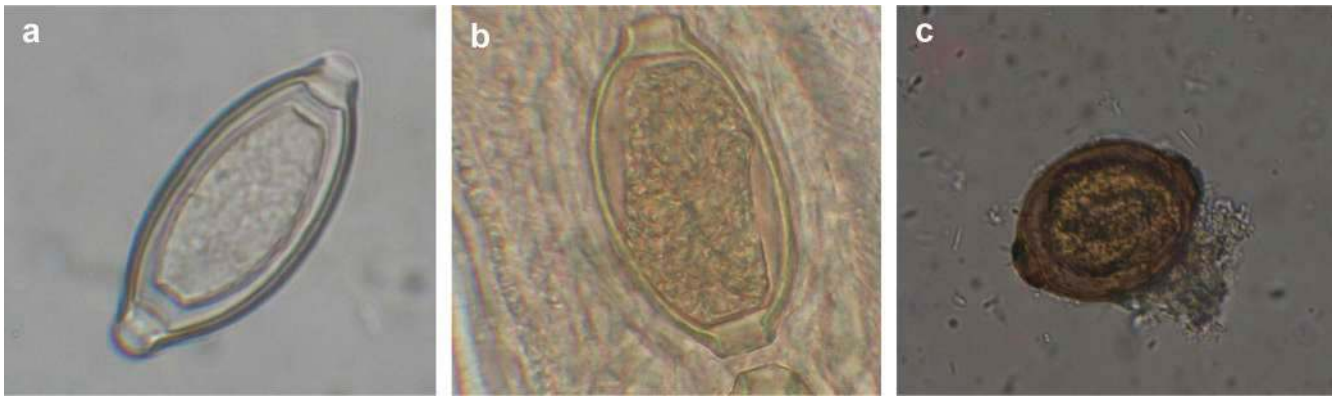

Figure 5. Micrographs of characteristics of eggs from trichuroid species in rodents: a) Detail of *Trichuris* sp. egg. b) Detail of *Eucoleus* sp. egg. c) Detail of *Anatrichosoma* sp. egg. Source: M. de R. Robles and R. Callejón Fernández. License: CC BY-NC-SA 4.0.

Table 3. Comparison of molecular data between the superfamilies Trichuroidea and Trichinelloidea.

Maggenti (1981)	**Superfamily Trichuroidea**			**Superfamily Trichinelloidea**
Moravec (2001a)	**Family Trichuridae**	**Family Capillariidae**	**Family Trichosomoididae**	**Family Trichinellidae**
Number of studied species by genus	Genus	Genus	Genus	Genus
	Trichuris 20 species	*Aonchoteca* 6 species; *Balluscapillaria* 1 species; *Calodium* 2 species; *Capillaria* 11 species; *Eucoleus* 3 species; *Paracapillaria* 1 species; *Pearsonema* 1 species; *Pseudocapillaria* 1 species	*Anatrichosoma*	*Trichinella* 9 species
Genes studied	SSU rDNA, ITS1, 5.8S, ITS2 rDNA, *cox1* mtDNA, *cytb* mtDNA, TPI rDNA, *16S* mtDNA, mitochondrial complete	SSU rDNA, *cox1* mtDNA	SSU rDNA, c*ox1* mtDNA	SSU rDNA, 5S-ISR, ITS rDNA, *cox1* mtDNA, mitochondrial complete

as the characteristics and opening position of the vulva in the female (Figure 4), and the shape of the eggs, especially the characteristics of the polar plugs (Figure 5) (see Maggenti, 1981; Moravec, 2001a; Anderson et al., 2009; Robles et al., 2012).

Molecular Characteristics

DNA-based methods are powerful tools for synthetic studies, providing a basis for a better understanding of poorly understood aspects of the biology, epidemiology, pathogenesis, and taxonomy of trichocephalid nematodes. Note that the systematic literature often contains references to specific regions of comparable DNA, termed **markers**. Markers indicate any region of DNA sequences that are used across different species and are not always genes, although genes are a type of marker. See Table 3 for a comparison of molecular data between the families of Trichuroidea and Trichinelloidea.

Table 4. Comparison of biological aspects between superfamilies of Trichuroidea and Trichinelloidea.

Maggenti (1981)	**Trichuroidea**			**Trichinelloidea**
Moravec (2001a)	**Trichuridae**	**Capillariidae**	**Trichosomoididae**	**Trichinellidae**
Host groups	Some families of mammals	Fishes, amphibians, reptiles, birds, and mammals	Fishes and mammals	Reptiles, birds, and mammals
Infection location	Cecum	Different tissues and organs	Different tissues and organs	Different tissues and organs
Source of eggs	Feces	Feces, urine, skin, and from predators	Feces and urine	Without eggs
Maturation of the laid eggs	Uncleaved or in morula	Uncleaved, in morula, or larved	Larved	-
Place of hatching of the juveniles	Female lays eggs, juveniles hatch as J_1 (oviparous)	Female lays eggs, juveniles hatch as J_1/J_2 (oviparous), or larvae hatch inside uterus (ovoviviparous)	Female lays eggs, juveniles hatch infective (oviparous)	Female releases juveniles inside uterus (ovoviviparous)
Complexity of the life cycle	Direct (1 host)	Direct and indirect (1 or more hosts). With paratenic hosts	Indirect (more than 1 host)	Autoheteroxenous (same individual is both definitive and intermediate host)
Environment	Terrestrial	Terrestrial and aquatic	Terrestrial and aquatic	Terrestrial

Box 1. Interesting Facts

- Mammals are the most important hosts for the genus *Trichinella*, with infections known to occur in 150 species belonging to 12 orders (Marsupialia, Insectivora, Edentata, Chiroptera, Lagomorpha, Rodentia, Cetacea, Carnivora, Perissodactyla, Artiodactyla, Tylopoda, and Primates).
- Humans are the only species of primate that can be infected in natural conditions by any of the species of the genus *Trichinella*, except for *T. zimbabwensis*.
- Mammals are susceptible to all *Trichinella* species, whereas reptiles are only susceptible to *T. papuae* and *T. zimbabwensis* and birds are only susceptible to *T. pseudospiralis*.
- *Trichinella spiralis* is found in 87% of samples of domestic pigs, 67% of wild boar, 88% of equines, 79% of synanthropic rats, and 100% of synanthropic armadillos (data obtained from the samples from the International Center for Research of Trichinellosis) (Pozio and Murrell, 2006).
- Data have surfaced suggesting that Trichinellidae and Trichuridae diverged from a common ancestor 250–300 Ma (= million years ago) using the variation in 3 genes (SSU rDNA, mitochondrial large subunit rDNA, and cytochrome oxidase I (*cox1*) mitochondrial DNA (mtDNA) (Zarlenga et al., 2006).

Biological Aspects

Trichocephalids occur in various organs of all groups of vertebrates. Species of *Trichuris* occur in various species of mammals, especially, but not exclusively, in rodents, carnivores, and primates while species of Capillariidae occur in all vertebrate groups. Interestingly, species allocated to the Trichosomoididae occur in fishes and mammals, while species of Trichinellidae parasitize reptiles, birds, and mammals.

Direct and indirect life cycles have been observed among nematodes of the families Trichuridae, Capillariidae, and Trichosomoididae. For those species that have complex life cycles, a large number of animal groups have been shown to

function as intermediate hosts (for example, molluscs and annelids). Also, in many cases, the life cycles may involve paratenic hosts (Miyazaki, 1991; Anderson, 2000; Moravec et al., 1987; Moravec, 2001a). A paratenic host is a host in which the parasite does not develop further while it is in that host but remains infective to the next definitive, or final, host. The definitive host is the host in which sexual reproduction occurs.

All adult trichurids that have been studied bury a part of the anterior portion of their body (the stichosome) in the base of the cecal villi in the mucosa. The stichosome winds around in this area in a convoluted path. Most species of Capillariidae and Trichosomoididae are able to embed their whole body in different tissues, burrowing through the epithelial and subepethelial tissues and into the organs themselves, such as the stomach, intestine, liver, spleen, musculature, bladder, kidneys, and other organs (Yamaguti, 1961; Anderson, 2000; Moravec, 2001a). See Table 4 for a comparison of biological aspects between the superfamilies of Trichuroidea and Trichinelloidea.

Both superfamilies include species of epidemiological importance, especially *Trichinella spiralis* due to the large number of reported human cases.

Superfamily Trichuroidea

Morphology of Trichuroidea

The body is divided into 2 regions: The narrow anterior part contains the esophagus, with a stichosome (= a series of large gland cells—called stichocytes—attached to the posterior region of the esophagus), and the posterior part

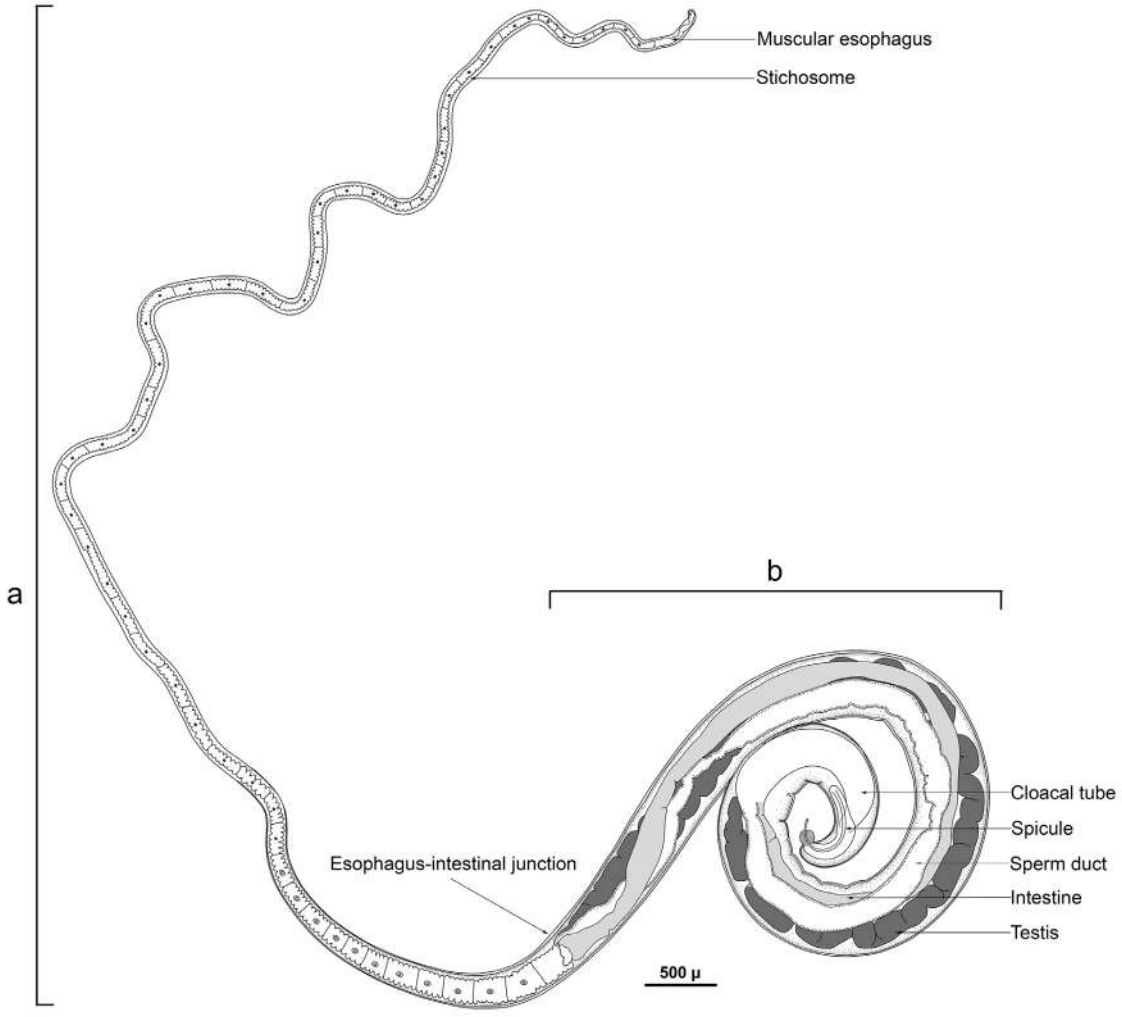

Figure 6. Diagrammatic representation of the morphological structures of a male specimen of *Trichuris muris* as an example of Trichuoidea. a) anterior part, b) posterior part. Source: A. Panti May. License: CC BY-NC-SA 4.0.

of the body contains the reproductive system and begins at esophageal-intestinal junction. A bacillary band is present (= extreme modifications of the hypodermis which can form between 1 to 4 chords of a complex of glandular and non-glandular cells). Males and females each have a single gonad, males with 1 spicule, and females with 1 ovary, and are oviparous. The eggs are bi-operculate, with an opercular plug or opening in each end. The life cycle may be direct or indirect (see Figure 6) (Yamaguti, 1961; Maggenti, 1981; Moraveč, 2001a; Anderson et al., 2009).

Molecular Characteristics of Trichuroidea

Within the superfamily Trichuroidea, several ribosomal and mitochondrial DNA markers from around 20 species allocated to the genus *Trichuris* have been identified. The full mitochondrial genomes of *T. trichiura*, *T. ovis*, *T. discolor*, and *T. suis* are available on GenBank (Liu et al., 2012a; 2012b; see also https://www.ncbi.nlm.nih.gov/genbank/). In the case of the Capillariidae, species of a few genera (around 25) have been studied using 2 DNA markers. Conversely, just a few species of *Anatrichosoma* have been reported (members of the family Trichosomoididae) using sequences from 2 markers.

Biological Aspects of Trichuroidea

Adult Trichuroidea are dioecious (having 2 sexes) and, after mating, females produce eggs that are diploid, a result of mating and the combining of gametes. In what appear to be morphological adaptations to increase the probability of dispersal to new hosts, various innovations in the eggs of trichuroids are evident. Host lifestyle is probably one of the main factors in the development of evolutionary innovations in this group as morphology of the eggs varies depends on the kinds of habitats within which the host resides.

Since the site of infection within the host species of adult trichuroids is extremely variable and the eggs are the only means of making it to the next host, it is reasonable to assume that morphological characteristics of the eggs may increase the probability that the eggs will make it to subsequent host individuals. For species of nematodes living in the gastrointestinal or respiratory tract, eggs are naturally passed with the host's feces. Some species inhabit the bladder or kidneys, and so, the eggs pass in the urine. Also, eggs can occur in the epidermis and they are released to the external environment when the outer layers of skin are shed. Other locations include the liver, spleen, and muscles in which the eggs are encapsulated by host tissue. In these cases, the only way to the external environment can be through predation (the eggs are dispersed by a predator's feces) or from the decomposing host's body, which is an interesting way to get around! (Pence and Little, 1972; Anderson, 2000; Moraveč, 2001a; Robles et al., 2008; 2012; 2014; Fantozzi et al., 2018).

The degree of maturation of the eggs inside the host is also variable among species in the families and genera of trichuroids. In several cases, the eggs are laid uncleaved (that is, without any development of the blastomeres), requiring a certain period in the environment to reach the development of the juvenile form. However, in other cases, the eggs can hatch when laid, making them instantly infective for another host. First-stage juveniles (J_1), and probably other stages, possess a stylet. Despite the majority of surveys suggesting that trichuroids always infect the final host in the first-stage, some authors have observed the second molt of juveniles (J_2) in their intermediate hosts (Moraveč et al., 1987; Moraveč, 2001a).

Family Trichuridae

Genus *Trichuris*

The phylogenetic relationships of *Trichuris* species from different host groups have been explored based on rDNA (ITS and SSU) and mtDNA (*cox1* and *cytb*), showing separate clades. In this context, *Trichuris* from rodents are a sister of *T. vulpis* from canids; while *T. trichiura* and *T. suis* form a separate clade. Both clades are the sister of a clade that includes *T. ovis*, *T. leporis*, and *T. skrjabini*, all parasites from herbivores (Cutillas et al., 2009; Callejón et al., 2013) (Figures 7 and 8).

Other analyses have used different markers (ITS2 rDNA, *cox1*, and *cytb* mtDNA), to explore each distinct clade. For example, a series of papers has been published showing dif-

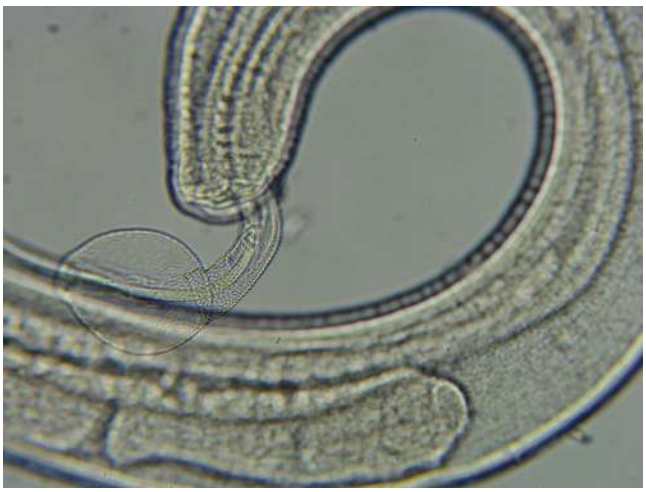

Figure 7. Posterior of a male of the genus *Trichuris* from a rodent. The spicule can be seen inside the spinose spicule sheath. The sheath is expanded at the distal end in this specimen. Source: S. L. Gardner, HWML. License: CC BY.

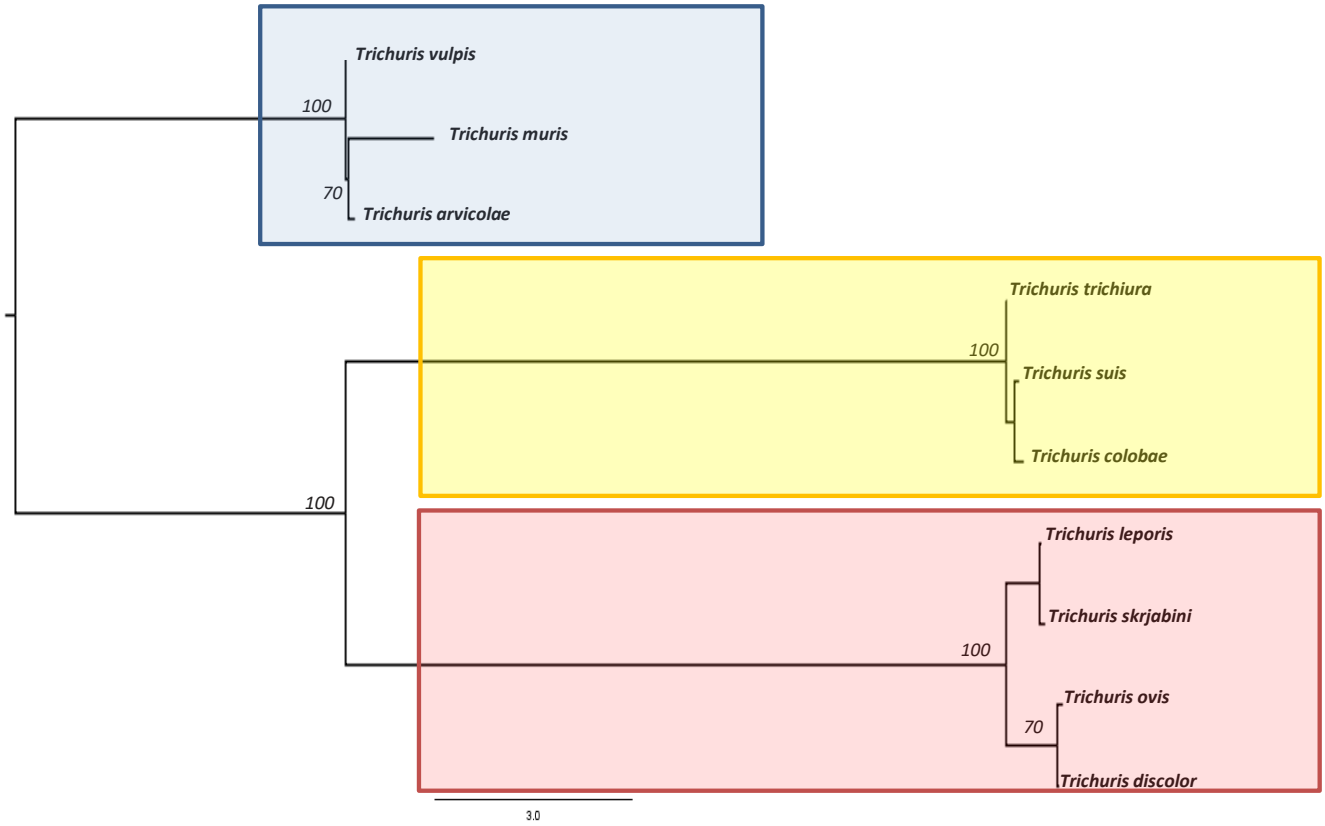

Figure 8. Dendrogram based on ITS1 sequences corresponding to different species of the genus *Trichuris*, with detail of host group. Source: Adapted from Cutillas et al., 2009. License: CC BY-NC-SA 4.0.

ferent phylogenetic hypotheses, including regarding various species of *Trichuris* from rodents of different continents (Robles et al., 2014; 2018; Rylková et al., 2015; Callejón et al., 2016). Notably, species of *Trichuris* seem to accompany their host clades (Robles et al., 2014; 2018); however, some studies also indicate a certain relationship with the history of the geographic areas in which they are now found (Eberhardt et al., 2018).

In a similar way, molecular studies based on ribosomal (SSU, ITS1, ITS2) and mitochondrial (*cox1*, *cytb*) markers have revealed 2 distinct lineages of *Trichuris trichiura* within human and non-human primates (NHP), showing some level of a narrow host range (relationship between each taxonomic level of the host and parasite) (Ravasi et al., 2012; Nissen et al., 2012; Doležalová et al., 2015; Cavallero et al., 2015; Callejón et al., 2017). This is an interesting finding because parasites occuring in many species of hosts versus a parasite species occurring in only 1 host species may have different epidemiological implications, since host reservoirs of the parasites may serve as sources of reinfection for other populations in which the parasite had previously been lost or eliminated. Information on prevalence and occurrence of parasites in hosts in various geographic regions is necessary for design and implementation of effective parasite control systems (Betson et al., 2015).

Family Capillariidae

Genus *Capillaria* sensu lato (s. l.) and other genera

Zhu and colleagues (2000) provided an analysis of genetic variation (*cox1* mtDNA) within and among morphologically-identified species of *Capillaria* s. l. from different host species and from different tissue sites within a host species. Their results showed that, among the species of *Capillaria* s. l. examined, these nematodes showed a relatively high degree of specificity at the level of the host genus.

Little molecular work has been done on species across the genera within the Capillariidae. However, some sequence data are available from capillariids from vertebrates, including sequences of the SSU rRNA gene from birds (Honisch and Krone, 2008; Tamaru et al., 2015), SSU rRNA from capillariids in *Rattus* (Buńkowska-Gawlik et al., 2017), SSU rRNA of human capillariids (El-Dib et al., 2015), *cox1* mtDNA of capillariids from rodents and marsupials (Zhu et al., 2000), and *cox1* mtDNA from capillariids of canine and feline ori-

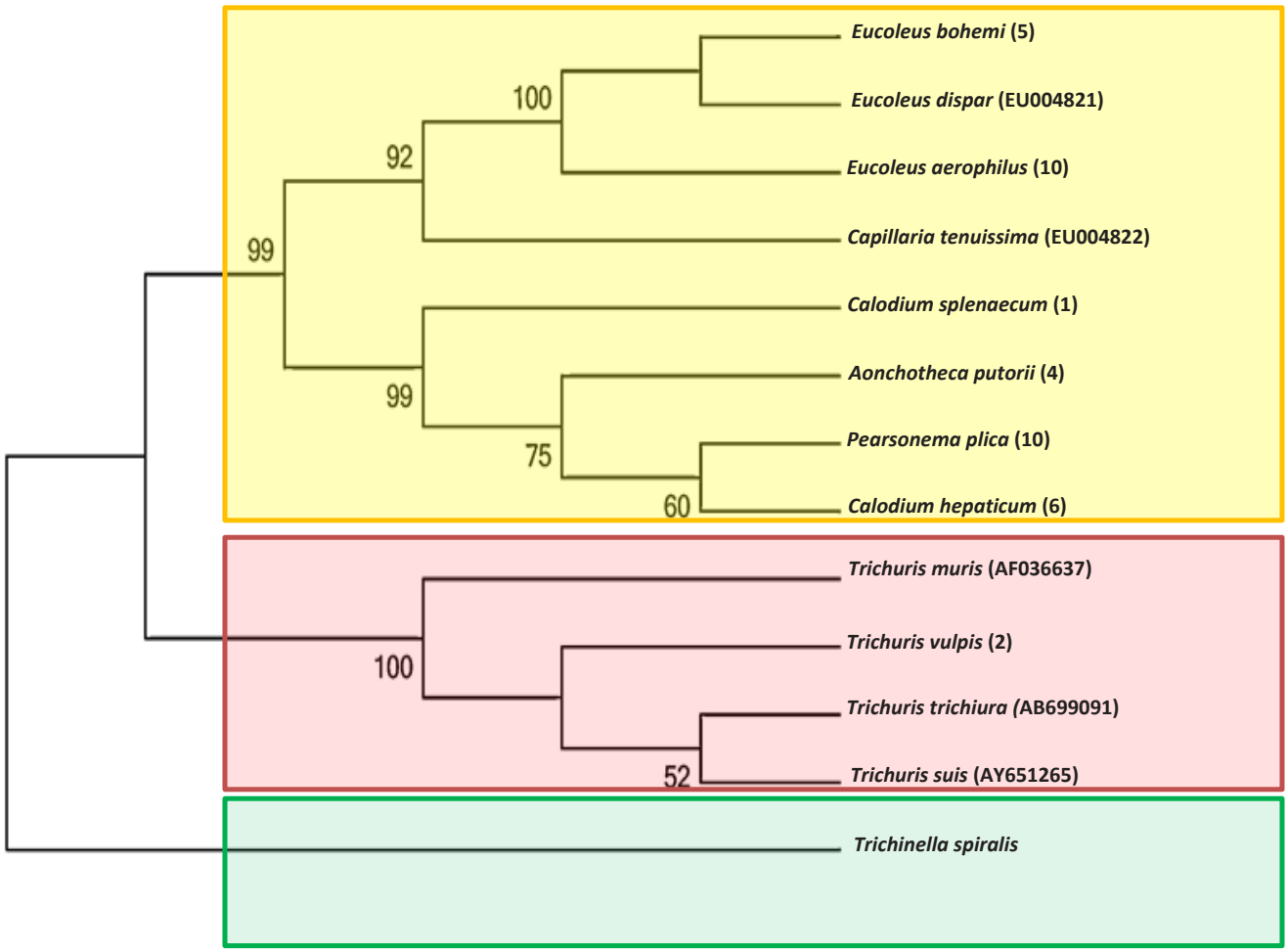

Figure 9. Dendrogram based on the partial sequences of the small subunit rRNA (18S rRNA) gene from species of the families Trichuridae and Capillariidae, with *T. spiralis* as outgroup. Source: Adapted from Guardone, 2013. License: CC BY-NC-SA 4.0.

Box 2. … Building Hypotheses

Many authors have provided phylogenetic hypotheses based on molecular data to try to clarify the relationships of the species in the phylum Nemata including the order Trichocephalida. These molecular analyses mainly have used small subunit ribosomal DNA (SSU rDNA) sequence data.

De Ley and Blaxter (2002; 2004) and Meldal et al. (2007) built trees based on SSU rDNA and based on these data they classified *Trichuris*, *Trichinella*, and *Capillaria* as members of the subclass Dorylaimia (class Enoplea). Holterman et al. (2006) also based on SSU rDNA showed other possible hypotheses in which Trichuris and Trichinella form the sister group of species allocated in the Dorylaimida, Mononchida, and Mermithida. Subsequently, Van Megen et al. (2009) extended the phylogenetic analysis to include members of the Capillaridae, revealing a closer relationship with Trichuridae, and showing both families to be a sister group of Trichinellidae. A recent phylogenetic study has shown that the family Capillaridae seems to be monophyletic and can be clearly separated from Trichuridae (Guardone et al., 2013; Figure 7). In addition, phylogenetic analyses based on mitochondrial DNA suggest that the species included in the genera *Trichuris* and *Trichinella* are members of the order Trichocephalida, separate from other enoplean nematodes including Dorylaimida and Mermithida (Liu et al., 2012; Callejón et al., 2013).

gin (Di Cesare et al., 2012). Guardone and colleagues (2013) show the relationship among different species of nematodes from 5 genera, *Eucoleus*, *Calodium*, *Capillaria*, *Aonchoteca*, and *Pearsonema* (Figure 9).

Many of the early studies must be re-examined since molecular data from species of Capillaridae have not been reconciled with original morphological analyses, and few morphological/molecular voucher specimens have been deposited in recognized parasite collections in established museums. Thus, there appears to be a paraphyletic distribution of species representing genera among different clades that show an incorrect placement of species in phylogenies that are based on an insufficient number of characters. The methods to determine the phylognetics of this group have not been robust enough to include enough data to provide a definitive phylogenetic estimate. More work on the phylogeny combining both morphological and molecular data is necessary to establish well supported trees.

Superfamily Trichinelloidea

Morphology of Trichinelloidea

The body is divided into 2 regions: The anterior part is more slender than the posterior section, the transisition from anterior end to posterior is not clearly visible unless the specimen is cleared in a clearing reagent. The anterior part contains the esophagus with a stichosome comprising stichocytes. The posterior part of the body contains the reproductive system, and the posterior part begins at the esophageal-intestinal junction. This group of nematodes lacks a bacillary band. Both males and females have single gonads and, while males lack spicules and have a large copulatory pseudobursa at the posterior end of the body, in the female, the vulva, or female genital opening, is far anterior in the body, usually just posterior to the region where the stichosome joins the rest of the body. Females are viviparous (Yamaguti, 1961; Maggenti, 1981; Anderson, 2000; Anderson et al., 2009).

Molecular Characteristics of Trichinelloidea

Trichinella spiralis, representing the superfamily Trichinelloidea, has been widely studied using different markers and its full genome is now available. The other 8 species of *Trichinella* in Table 2 have been studied with mitochondrial large subunit rRNA (*lsu* rRNA), *cox1* mtDNA, and ITS rDNA markers (Table 4). Notably, *T. spiralis* occupies a strategic position in the evolutionary tree of nematodes, which helps fill important knowledge gaps in the evolutionary history of this species.

Family Trichinellidae

Biological aspects

Species of Trichinellidae have a very unusual life cycle since the same individual animal serves as both the definitive and intermediate host, with the juveniles and adults located in different organs (Roberts and Janovy, 2009). The transmission of *Trichinella* occurs through a predator-prey cycle and depends on the ability of the juveniles encysted in the muscles to withstand environmental conditions during the interval between host death and ingestion by the next host. Distinct life cycles may be observed in both domesticated and wild animal hosts (Pozio, 2000; Pozio and Zarlenga, 2005).

Genus *Trichinella*.

In general, the members of the genus *Trichinella* are geographically and ecologically restricted to different biogeographic regions. This is due to the adaptations that allow each species to survive in various climates (Pozio, 2016). Eight large areas have been established: *T. nelsoni* (E = encapsulated) and *T. zimbabwensis* (NE = non-encapsulated), and the genotype *Trichinella* T8 in the Afro-Tropical region; *T. britovi* (E) and *T. nativa* (E), and the genotype *Trichinella* T9 in the Palearctic region; *T. nativa* and *T. murrelli* (E), and the genotype *Trichinella* T6 in the Neartic region; *T. papuae* (NE) in the Australasia and Indomalayan region; and *T. patagonienis* (E) in the Neotropical region. *Trichinella spiralis* and *T. pseudospiralis* present a cosmopolitan distribution. The wide localization of *T. spiralis* is evidently the result of anthropogenic activity, while the distribution of *T. pseudospiralis* is linked to spread by birds, except for its presence in the Neotropical region, where it appears to have been introduced though European colonization (Pozio et al., 2009; Krivokapich et al., 2012).

As currently recognized, the genus *Trichinella* comprises a complex of 9 species and 3 genotypes infecting mammals, birds, and reptiles across a broad geographic range (Pozio, 2016). The juvenile stages represented in species of this genus can be distinguished from one another; some are encapsulated (E) and some are non-encapsulated (NE), which refers to the presence or absence of a capsule of collagen around the first-stage juvenile (J_1) that is encysted in the muscle. This stage of the parasite preferentially migrates into striated muscles of different regions of the body of the host and most reports from humans are that the muscles in the diaphram and tongue are first infected. The juvenile nematodes that invade and then encyst in the muscles of the mammal host are the causative agent of the disease **trichinosis** (Zarlenga et al., 2006). Different methods have been developed to enable the correct separation and identification of *Trichinella* genotypes

and species, either through employment of variants of conventional single-gene, single-marker PCR, multiplex PCR (which enables amplification of several marker sequences at one time), and variable studies of rDNA and PCR-RFLP (random amplified length polymorphism analysis) of the *cox1* mtDNA gene, or PCR amplification, followed by nucleotide sequencing of DNA in the 5S-intertranscribed spacer region (5S-ISR) region. At the current time, multiplex PCR of sequences from mtDNA is the most popular technique in use for the identification of species of *Trichinella* by the International Trichinella Reference Center in Rome, Italy.

La Rosa and colleagues (2003) published a phylogeny developed from multilocus protein electrophoresis that verified for the first time that those species of *Trichinella* that show unencapsulated juveniles in host muscles form a monophyletic group. The lack of a capsule is thus a morphological synapomorphy, based on the absence of collagen in the muscle cyst, versus those that form a clade and have collegen comprising the cyst of the nematode in the muscle. Also, a sister group of species without capsules has been observed, with species that can infect poikilothermic hosts, and these species grouped separately from parasites of homeothermic animals. A subsequent phylogenetic analysis using molecular markers from the ribosomal D3 rDNA region also grouped species that encyst in the muscules in collagenous capsules and grouped the species that inhabit the Arctic region, indicating that both geographic specificity and morphological synapomorphies are important in determining the patterns of these species over geographic space (Gasser et al., 2004). Another phylogenetic analysis that included the *cox1* mtDNA, the ITS2 rDNA, and the mitochondrial ribosomal major subunit gene (LSU), confirmed results obtained previously, and it was proposed that *T. spiralis* is the species that would have first diversified within the lineage that forms collagenous capsules, and thus is the ancestral form (Zarlenga et al., 2006). This result may be confirmed by using more comparative field-collected samples.

The complete genome of *Trichinella spiralis* was sequenced as a representative of Clade I, a group with encapsulated juveniles in the muscles of their hosts (Mitreva and Jasmer, 2008). The genome sequencing efforts have now been extended to include a non-encapsulated species, *T. pseudospiralis* (Zarlenga et al., 2009).

Trichocephalida Species of Medical and Veterinary Importance

The life cycles of the majority of trichocephalid species are not known. However, species with medical and veterinary importance or species parasitizing economically-relevant hosts have been studied extensively. Therefore, biological data from the majority of species is fragmentary.

Trichuris trichiura is a parasite with a direct life cycle, meaning that it does not require an intermediate host (Figure 7). This whipworm may be present in the cecum and colon of humans and other primates. Fertilizated eggs mature in 10–21 days in the soil. The juveniles do not hatch nor molt until ingestion by a host. There, the first stage juveniles hatch in the upper part of the small intestine, descend the intestinal canal as they develop, repeatedly invading the intestinal mucosa, and arrive at the cecum where they finally settle. The thin anterior part of body is partially inserted into the host's mucosa, the end of which is capable of being drawn into it, and the thick portion of body remains free in the lumen. The complete process requires about 3–4 months. Adults live between 1 to 4 years (Bundy and Cooper, 1989; Miyazaki, 1991). The number of eggs each female produces is estimated to range from about 3,000 to 20,000 per day (Faust et al., 1975).

An intense trichuriasis infection in humans may cause dysentery, anemia, rectal prolapse, and growth retardation. Children are particularly prone to heavy infections (Cooper et al., 1992; Nokes et al., 1992).

Box 3. Doubts … and Mystery Solved

Trichuris suis and *T. trichiura* have been frequently considered to be the same species (see, for example, Schwartz, 1926). However, Soulsby (1982) determined that *T. trichiura* is morphologically similar but biologically distinct from *T. suis*. Furthermore, *T. suis* is not a human parasite, but after ingestion of eggs, the juveniles hatch and are capable of colonizing a human host for several weeks before they are eliminated from the body without any specific therapy (Li et al., 2012). In addition, morphological studies have separated the species based on the existence of 1 pair of caudal papillae, which in fact is present in *T. trichiura* isolated from humans and other primates, but which are absent in *T. suis* (Tenora et al., 1988). In addition, to help clarify the taxonomic status of both species, the ITS1-5.8S-ITS2 fragment of ribosomal DNA was amplified and sequenced by Cutillas et al. (2009; 2014). The morpho-biometric and molecular results support the existence of different species in pigs, humans, and non-human primates.

Trichuriasis is regarded to be the second most common parasitic infection in humans in the tropics (Bundy and Cooper, 1989). This is a cosmopolitan species concerning epidemiological risk, since the appropriate physical conditions exist in several parts of the world, such as a warm climate, high rainfall and humidity, moisture-retaining soil, and dense shade. However, the highest prevalence is observed in populations with poor standards of sanitation (Bundy and Cooper, 1989; Cooper et al., 1992).

Trichuris suis is a parasite with a direct life cycle involving the cecum and colon of pigs. Fertilized eggs mature in the soil between 19 and 21 days, depending on ambient temperatures, and the eggs can survive in the soil for 6 years (Hill, 1957). The juveniles do not hatch nor molt until ingestion by a host, and then they make their way to the cecum, as is similar in all species of *Trichuris*, although different studies have recorded different maturation times (Alicata, 1935; Hill, 1957; Beer, 1973). The complete process requires about 4–5 months.

Common manifestations of *Trichuris suis* infection in pigs include diarrhea, anorexia, and retarded growth. The high prevalence of *T. suis* in pig production systems is one of the major factors constraining global food availability. This severely impacts small scale farmers in developing countries (Li et al., 2012).

Trichuris muris is a parasite with a direct life cycle, present in the cecum and colon of infected mice, rats, and other rodents. Fertilized eggs mature after about 30 days in the soil, depending on ambient temperatures. The juveniles do not hatch nor molt until ingestion by a host, and then they make their way to the cecum as is similar in all *Trichuris* species, although different studies record different maturation times (Shikhobalova, 1937; Fahmy, 1954). This nematode has been extensively utilized as a laboratory model for the study of the human whipworm, *T. trichiura*. This has proven to be an invaluable tool in dissecting the different components involved in immunity to *Trichuris* infection. Moreover, its biology has been used to paradigmatically demostrate cytokine-mediated immunity to gastrointestinal nematodes in general (Cliffe and Grencis, 2004).

Calodium hepaticum (a synonym *Capillaria hepatica*) is a parasite with a special life cycle since it requires no intermediate host, but 2 final hosts are usually needed. This nematode mainly parasitizes the liver of rodents. When this host is eaten by another rat or a carnivore, the eggs are released and passed along with feces of the predator. Also, eggs may be freed when parasitized rodents die and the body degenerates. Notably, the eggs cannot embryonate in the liver; they are embryonated in the soil, and so, are not infective to the predator (Miyazaki, 1991; Roberts and Janovy, 2009; Fantozzi, 2018).

In the life cycle, the female worm deposits eggs in the liver, which become encapsulated within the host tissue. The eggs pass through the digestive tract of the predator with its feces (Roberts and Janovy, 2009). Eggs are susceptible to desiccation or temperatures between 1 ºC and −7 ºC for about 16–19 days, but in temperatures around 25 ºC they can develop in 35–45 days, and new infection occurs by contamination. After hatching in the small intestine, juveniles migrate to the liver, where these mature (Luttermoser, 1938; Spratt and Singleton, 2001).

Humans and other mammals are infected orally through ingestion of contaminated food and beverages containing mature eggs. The presence of this worm in the liver of humans causes serious illness due to necrosis of the parenchyma, and a granuloma is formed eventually as a result of the fibrosis. The afflicted patient may exhibit fever, hepatomegaly, and eosinophilia.

This is a cosmopolitan species with considerable epidemiological risk, since rodents are frequently in the peridomiciliary, and the eggs reach the environment through the decay of the host carcass or when a predator (for example, a dog or cat) ingests the host and releases the eggs through the feces.

Box 4. Curiosities

Eggs of *Calodium hepaticum* have been found in several species of earthworms (Romashov, 1983), resulting in earthworms serving as an important disperser of the eggs.

Calodium hepaticum are a potential biological control agent for rodent populations (Singleton and McCallum, 1990).

Diagnosis of *Calodium hepaticum* infection by molecular techniques has been reported recently (Guardone et al., 2013; Fantozzi et al., 2018).

In the New World, sigmodontine rodents predominate, comprising 381 species (D'Elia and Pardiñas, 2015). Despite the great diversity in species of these rodents, *Calodium hepaticum* has only been recorded in 8 species of this rodent group, and 6 of them from Argentina (Vogelsang and Espin, 1949; Fantozzi et al., 2018). This lack of demonstrated presence within the sigmodontine rodents is probably the result of insufficient sampling in other areas of the Neotropical region.

Box 5. The Importance of Taxonomy with Respect to Medical Aspects

Unfortunately, some taxonomically important morphological features, for example, the structure of the male caudal end, have been inadequately described or are not mentioned at all in studies of the morphology, preventing an appropriate generic assignment of this species in the presently recognized classification system of capillariids. To help rectify this oversight and to correct the systematic status of the medically important capillariid species, the morphology of *Calodium philippinensis* was re-studied by Moravec (2001b). In that study, Moravec (2001b) described the general structure of the male caudal end, particularly the presence of a well-developed membranous bursa supported by 2 lateral, finger-shaped protrusions (rays), each of them bearing a big papilla at its base. These structures are typical among species of *Paracapillaria*, the genus to which *Calodium philippinensis* was transferred.

The eggs then embryonate and may infect a new host. Ingestion of non-embryonated eggs leads to an untrue (or spurious) infection in which the eggs pass through the intestinal tract and exit with the feces without hatching (Juncker-Voss et al., 2000).

However, notably, other studies have shown that people who often eat the liver of wild mammals present a 10-fold higher risk of presenting with spurious infection than those who do not eat the liver of wild animals. There has been speculation by several authors as to the mechanism of transmission, and examination of intradomiciliary rates of spurious infection. In addition, the occurrence of dog feces infected with unembryonated nematode eggs near homes suggests greater risk of new infections without the participation of wild animals in the infection cycle (Wright, 1961; Gonçalves et al., 2012).

Paracapillaria philippiensis has been found to be pathogenic in humans. Its mode of transmission is unknown, but the first human victim (in the Philippines) was known to eat food that contained various internal organs of some small mammals. In *P. philippiensis*, the movement of the worms results in a disruption of the mucosal lining of the small intestine, a degeneration of the lining of the the epithelial cells, and, finally, inflammation of the mucosa. This extensive damage to the intestinal wall induces symptoms resembling malabsorption syndrome. The major symptom is intractable diarrhea that leads to rapid dehydration and emaciation. There is usually some abdominal pain and distension accompanied by a low grade fever. In fatal cases, the loss of nutrition leads to shock, and death is due to this rather than the tissue damage attributable to the parasite.

This highly pathogenic parasite is known to be distributed in eastern, southern, and southeastern Asia, and northern Africa (Philippines, Thailand, Japan, Korea, Taiwan, India, Iran, and Egypt) (for more information on the medical aspects, see, for example, Pradatsundarasar et al., 1973; Hoghooghi-Rad et al., 1987; Chen et al., 1989; Youssef et al., 1989; Lee et al., 1993; Kang et al., 1994; Khalifa et al., 2000). According to Cross (1992), 1,884 confirmed cases of the disease caused by this nematode were documented in humans from 1967 to the end of 1990; 110 cases were fatal.

Anatrichosoma is the genus of nematode parasites known to have an indirect route of infection; however, the life cycle has not been studied in detail. Notably, the eggs have thick-walled and dark shells. These eggs are deposited in tunnels in the epithelium and are presumably released during the sloughing of epithelial cells. These pass out of the host's body in excretory products and are probably infective to the definitive host (Orihel, 1970; Pence and Little, 1972).

Trichinella spiralis is a parasite with an autoheteroxenous cycle; in other words, it is a nematode that is present in an individual animal which serves as both the definitive and intermediate host. It is difficult to know where to begin describing the cycle since it is so complex, and because no stage occurs outside the host (see Figure 10). Transmission occurs when humans or other meat eaters consume raw or undercooked meat of wild animals contaminated with the cysts of *Trichinella*. When raw or rare meat containing cysts is consumed, the infective first-stage juveniles are released from their envelope with the aid of the host's gastric juices. The juveniles then invade the duodenal and jejunal mucosa. In about 36 hours, males and females develop (Gould et al., 1957). Soon after fertilization of the females, the males die. The females subsequently increase to their maximum size and burrow deeper into the mucosa each depositing about 500 juveniles, the majority of which migrate into the intestinal lymphatic and mesenteric veins, eventually reaching the heart and lungs, and then are distributed into the arterial circulation, where they then move to the muscles. The juveniles encyst in the muscles after about 19 days, moving into striated muscles that have low amounts of glycogen and predominantly include the diaphragm, larynx, tongue, abdomen, intercostal spaces, biceps, pectorals, deltoids, and more. Here, the infected muscle cells are transformed into nurse cells (Stewart and Gianini,

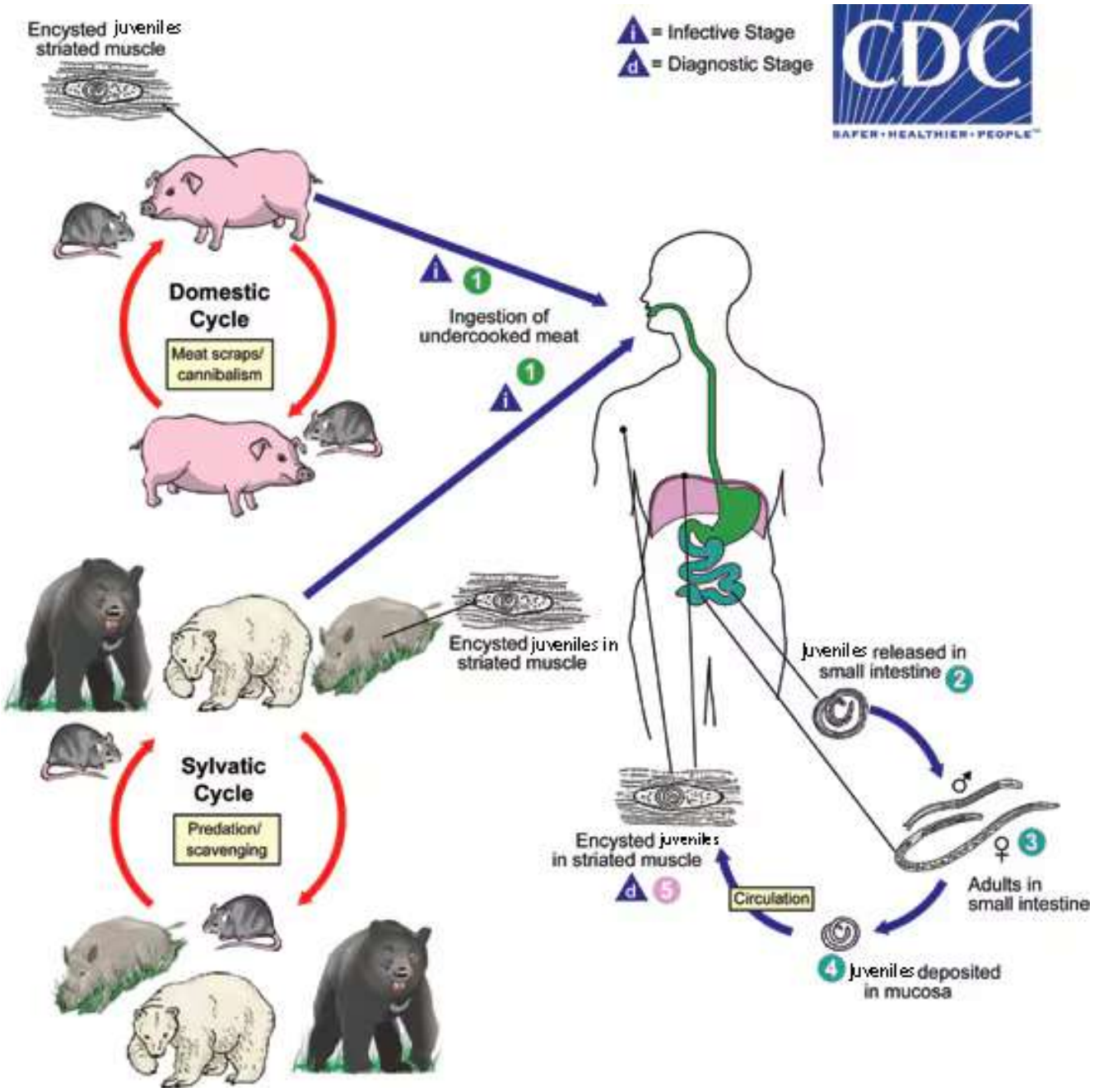

Figure 10. Adult worms and encysted juveniles develop within a single vertebrate host and an infected animal serves as a definitive host and potential intermediate host. A second host is required to perpetuate the life cycle. The domestic cycle most often involves pigs and anthropophilic rodents, but other domestic animals such as horses can be involved. In the sylvatic cycle, the range of infected animals is great, but animals most often associated as sources of human infection are bear, moose, and wild boar. — Life cycle. Trichinellosis is caused by nematodes (roundworms) ingested with undercooked meat containing encysted juveniles of *Trichinella* species (except for *T. pseudospiralis* and *T. papuae,* which do not encyst) (1). After exposure to gastric acid and pepsin, the larvae are released from the cysts (2) and invade the small bowel mucosa where they develop into adult worms (3). Females are 2.2 mm in length; males 1.2 mm. The life span in the small bowel is about 4 weeks. After 1 week, the females release juveniles (4) that migrate to striated muscles where they encyst (5). Diagnosis is usually made based on clinical symptoms and is confirmed by serology or identification of encysted or non-encysted larvae in biopsy or autopsy specimens. — Several species are recognized, including *T. spiralis* (carnivorous and omnivorous animals worldwide), *T. pseudospiralis* (mammals and birds worldwide), *T. nativa* (Arctic bears), *T. nelsoni* (African predators and scavengers), *T. britovi* (carnivores of Europe and western Asia), *T. papuae* (wild and domestic pigs, Papua New Guinea, and Thailand), and *T. zimbabwensis* (crocodiles in Africa), all but the last of which have been implicated in human disease.

1982). In 6 to 9 months the fibrous capsules become calcified. Within the cysts, juveniles may remain viable for more than 5 years. This is the termination of the cycle and the juveniles must await the ingestion of this host to continue.

The list of common hosts is extensive and includes humans, rats, pigs, bears, walruses, seals, and dogs (Maggenti, 1981; Miyazaki, 1991). It has been shown that seal pups can acquire an infection through their mother's milk during the period of parasite juvenile migration. Humans normally become infected by breaking into the pig-rat-pig cycle or by eating uncooked bear meat; and, in North America, bear meat infection is not altogether rare. Five cysts per gram of body weight can be lethal to a human. Therefore, *Trichinella spiralis* is the cause of a serious and often fatal disease in humans known as trichinosis. In mild cases, the symptoms do not differ greatly from the so-called stomach flu, accompanied by stomach upset and general bodily aches and pains. When the invasion is severe, the syndrome includes 3 phases: Invasion, migration, and encystment. Invasion is characterized by stomach flu or food poisoning symptoms. Penetration of the gut by large numbers of juveniles creates symptoms such as vomiting, nausea, dysentery, and colic. Migration and initial invasion and encystment in the muscles is manifested by difficulty in breathing, chewing, swallowing, and speech, and in the limbs there may be spastic paralysis. Encystment is the critical third stage. Often nutritional stress and dehydration are evident. The pulse may at first be fast and strong and then it suddenly drops and cyanosis supervenes; as blood pressure falls, the host collapses as shock ensues. Prior to collapse, nervous disorders include visionary defects, loss of reflexes, disorientation, delirium, and encephalitis. Diagnosis is by biopsy after the juveniles reach the preferred muscle sites (Miyazaki, 1991; Anderson, 2000).

Education is an important part of any control program; however, the most effective measure to avoid becoming infected with these nematodes is by thoroughly cooking meat before ingestion, especially, pork, bear, and rat meat. Freezing is also successful at differing temepratures for different lengths of time. Mainly, prevention requires the cooking of garbage fed to swine, proper freezing and low temperature storage of prepared pork products, and proper inspection. These implementation of pork production and storage regulations have led to a significant lowering of the incidence of trichinosis. Control consists of the destruction of all infected carcasses and viscera, extermination of rats and mice, and heat treating garbage fed to swine (Miyazaki, 1991; Anderson, 2000). However, *Trichinella arctica* has been shown to be infective even after freezing at very low temperatures (Pozio, 2016).

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Nemata

Ascaridoidea (Superfamily): Large Intestinal Nematodes

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Superfamily Ascaridoidea

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Chapter 50

Ascaridoidea (Superfamily): Large Intestinal Nematodes

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Introduction

Ascaridomorpha includes a diverse group of parasites that live in the alimentary tract of their definitive hosts, and includes species that are of veterinary, medical, and economic importance. The life cycles of these parasites are quite variable, ranging from species with simple direct patterns involving the ingestion of eggs containing infective juveniles, to others that use invertebrates or vertebrates as intermediate or paratenic hosts. Species of Ascaridomorpha are familiar to biologists and laypersons alike as the large intestinal roundworms (although, here preferentially called nematodes) that infect pet dogs and cats; however, a much wider range of vertebrates serves as definitive hosts, including elasmobranchs, teleost fishes, amphibians, reptiles, birds, and mammals.

Ascaridomorpha occurring in mammals are typically large, stout nematodes with 3 large lips; however, there is substantial variation in body size and morphological characteristics among genera and species, even though different taxa are superficially similar in structure. Phylogenetic analysis of SSU rDNA sequences has shown that species allocated to this group are not monophyletic (Nadler et al., 2007), whereas certain families and subfamilies in this group are strongly supported as clades by molecular data (Nadler and Hudspeth, 2000). Of several families in this infraorder, this chapter will emphasize Ascarididae (subordinate within the superfamily Ascaridoidea), which includes many species of medical importance. Representative members of certain other superfamilies will be discussed briefly.

Superfamily Ascaridoidea

Family Ascarididae Baird, 1853

Ascaridids are among the largest nematodes, some species achieving a length of 45 cm or more. They are distinguished by having large rounded or trapezoidal **lips**, and cervical, lateral, and caudal **alae** may be present. **Spicules** are equal in length and rodlike or alate. This family contains the cosmopolitan human intestinal parasite, *Ascaris lumbricoides* Linnaeus 1758 (Crompton, 2001).

Ascaris lumbricoides

Because of their great size and high prevalence, these nematodes may well have been among the first parasites known to humans. The ancient Greeks and the Romans were familiar with them and they were mentioned in the Ebers Papyrus, the 16th century book of medical knowledge from Egypt (Hallman-Mikołajczak, 2004). It is probable that *Ascaris lumbricoides* was either a parasite of pigs that adapted to humans when swine were domesticated and began to live in close association with humans—or perhaps it was a human parasite that humans gave to pigs. Populations of *Ascaris* spp. exist in both humans and pigs, but the extent of genetic isolation between these putative species (*A. lumbricoides* and *A. suum*, respectively) has been the subject of much recent research (Leles et al., 2012).

The two forms are so close morphologically that they are now considered to be the same species. Slight differences in the tiny denticles (small "teeth") on the inner edge of the lips were described between these species (Sprent, 1952), but were later found to reflect age-related wear rather than serve as reliable taxonomic characters (Madden and Tromba, 1976). None of the genetic markers examined to date consistently discriminate between pig- and human-source *Ascaris* spp. Experimental cross-transmission studies show that both putative species can reach maturity in humans and pigs. Genetic studies based on microsatellite markers reveal that there is a low level of hybridization between these species that occurs during co-infection. The distribution of maternally in-

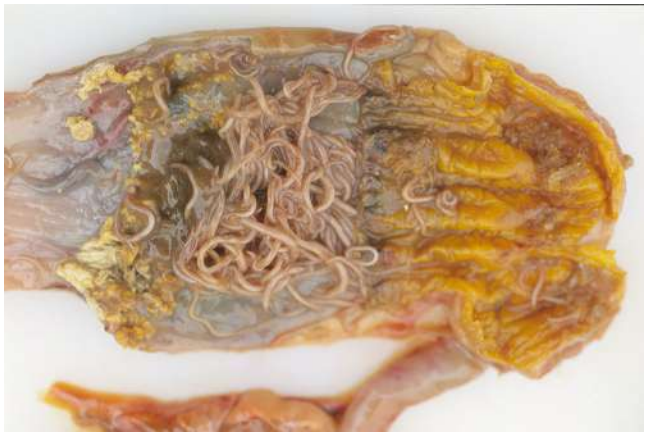

Figure 1. *Contracaecum* sp. (Rhabditida: Anisakidae) in proventriculus and gizzard of a guillemot (*Cepphus* sp.) collected from Scotland, United Kingdom between 1994 and 2013. Source: T. Pennycott, available at Edinburgh DataShare, 2013. License: CC BY 4.0.

herited mitochondrial DNA (mtDNA) haplotypes also reveals patterns that are consistent with low levels of cross-infection, but this interpretation is complicated by the possible retention of ancestral mtDNA polymorphisms between these very recently diverged taxa (Anderson and Jaenike, 1997; Criscione et al., 2007). Leles and colleagues (2012) showed that there are essentially no differences between these two species,so they should be considered to be one species: *A. lumbricoides* Linnaeus 1758.

Morphology

In addition to their great size (Figure 1), *Ascaris lumbricoides* is characterized by having 3 prominent lips each with a dentigerous ridge and no alae. Lateral hypodermal cords are visible with the unaided eye.

Males are 15–31 cm-long and 2–4 mm in diameter at the greatest width. The posterior end is curved ventrally and the tail tip is blunt. Spicules are simple, nearly equal, and measure 2.0–3.5 mm-long. No gubernaculum is present.

Females are 20–49 cm-long and 3–6 mm in diameter. The vulva is about one-third the body length from the anterior end. The ovaries are extensive and uteri may contain up to 27 million eggs with 200,000 being laid per day. When transferred to parasite-naive pigs, female *Ascaris* cease producing eggs after 2–3 weeks (Jungersen et al., 1997). They resume egg production when male worms are transferred into the pig with the females.

Fertilized eggs (Figure 2) are oval to round, 45–75 µm-long by 35–50 µm-wide, with a thick, lumpy outer shell (comprising a mammillated, uterine, or proteinaceous layer) that is contributed by the uterine wall. When eggs are passed in the host's feces, the mammillated layer is bile-stained a

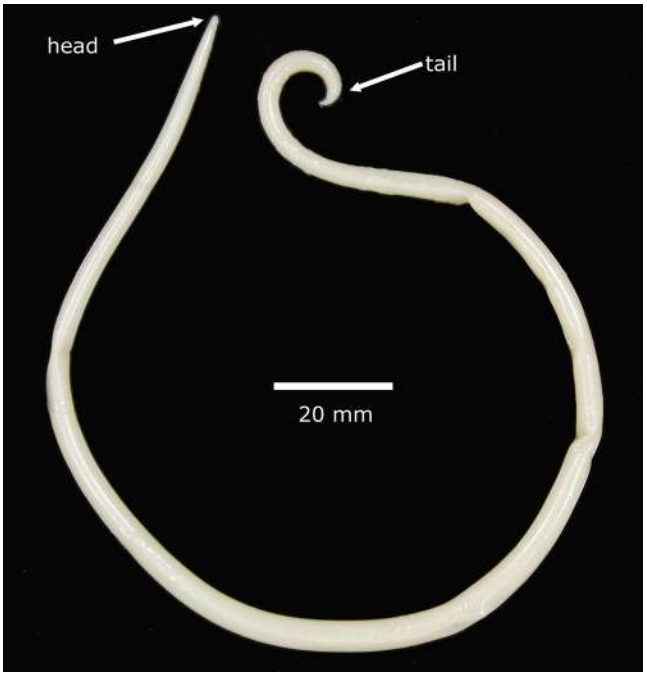

Figure 2. *Ascaris lumbricoides* (Nemata: Ascaridida: Ascarididae), adult male from a human host. Note the tapered head end and the tail that is reflexed (curved) ventrad (meaning, in the ventral direction). These nematodes commonly come out of the anus or the nose of the human host at inopportune times. Source: S. L. Gardner, HWML. License: CC BY.

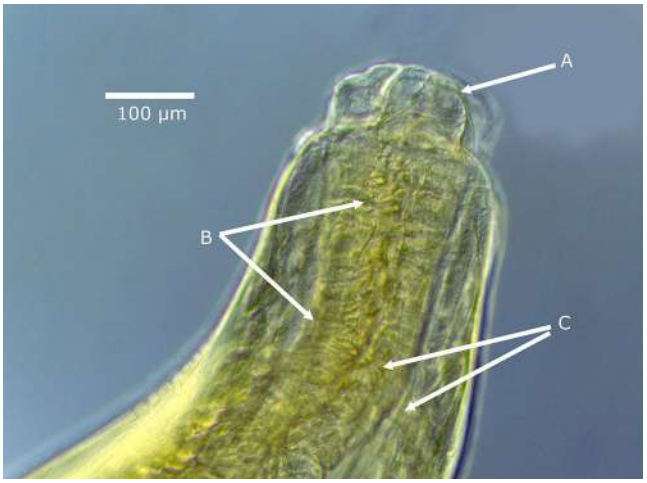

Figure 3. Close up view of the anterior end of an ascarid (Nemata: Ascaridida: Ascarididae) (*Toxascaris procyonis*) showing well, 1 of the 3 lips on the anterior end (A), the anterior part of the esophagus (B), and the nerve ring is seen in arrows pointing from (C). Source: S. L. Gardner, HWML. License: CC BY.

golden brown. The embryos within are usually uncleaved when eggs are passed. An uninseminated female, or one in early stages of oviposition, commonly deposits unfertilized eggs (Figure 3) that are longer and narrower than fertilized ones, measuring 88–94 µm-long by 44 µm-wide. Only the

proteinaceous layer can be distinguished in unfertilized eggs because the vitelline, chitinous, and lipid layers of the eggshell are formed only after sperm penetration of the oocyte.

Biology

A period of 9–13 days is the minimal time required for embryos to develop into active first-stage juveniles (J_1s). Embryos are extremely resistant to low temperature, desiccation, and strong chemicals; however, sunlight and high temperatures are lethal in a relatively short time (for example, 2 days at 47 °C). Human ascariasis does not occur where average land temperatures exceed 37–40 °C. Clearly, global warming may change the distribution of ascariasis and other parasitic diseases (Weaver et al., 2010). Juveniles must molt to the third stage to be infective (Geenen et al., 1999) .

Infection occurs when host animals swallow unhatched juveniles with contaminated food and water. They hatch in the duodenum through an indistinct operculum (Figure 4), where the juveniles penetrate the mucosa and submucosa and enter lymphatic tissue or venules (Figure 5). After passing through the right heart of a pig, they enter the pulmonary circulation and break out of capillaries into air spaces. Many worms get lost during this migration and accumulate in almost every organ of the body, causing acute tissue reactions. In contrast to this classical pattern, Murrell and colleagues (1997) report that juvenile *Ascaris* do not penetrate the mucosa immediately after hatching but rather rapidly transit the small intestine and penetrate the mucosa of the cecum and upper colon. Juveniles then accumulate in the liver for up to 48 hours. Incidentally, this research on *Ascaris* in pigs strongly suggests that the actual migration pattern of these nematodes in humans involves the liver, rather than the pattern observed in experiments with abnormal hosts such as guinea pigs and rats (Crompton, 2001).

While migrating through tissues, juveniles molt to the fourth stage (J_4), and during a period of about 10 days grow to a length of 1.4–1.8 mm. They then move up the respiratory tree of the host to the pharynx, where they are swallowed. Many juveniles make this last step of their migration before molting to the fourth stage, but these J_3s cannot survive gastric juices in the stomach. Fourth-stage juveniles (J_4) are resistant to such a hostile environment and readily pass through the stomach to the small intestine, where they molt again and mature. Within 60–65 days of being swallowed, they begin producing eggs. Genetic markers show that *Ascaris* females may be inseminated by more than 1 male in producing offspring (Zhou et al., 2011).

It seems curious that these worms embark on such a hazardous migration only to end up where they began. One hypothesis to account for it suggests that migration simulates an intermediate host, which normally would be required during juvenile development for species with indirect life cycles. Indeed, molecular phylogenetic hypotheses confirm that indirect life cycles are ancestral for ascaridoids, and that the direct (1-host) life cycle of *Ascaris* sp. and *Parascaris* sp. is the derived condition (Nadler and Hudspeth, 2000). After comparing many nematode taxa having tissue migration with closely related taxa that remain in the gut, Read and Skorping (1995) conclude that tissue migration enables faster growth and larger size, thus increasing reproductive capacity.

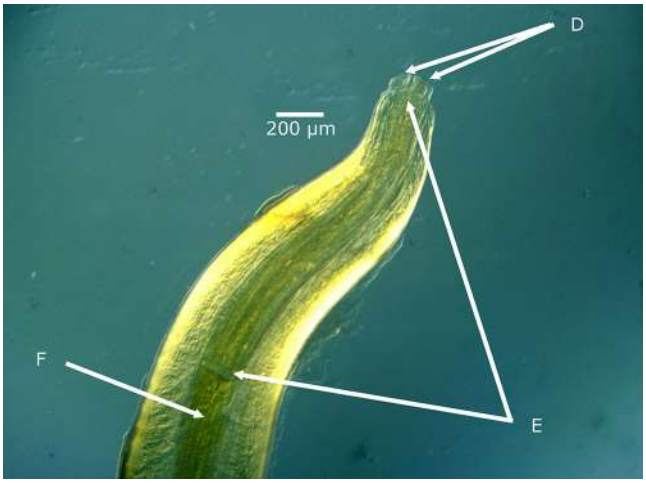

Figure 4. Anterior end of *Toxascaris procyonis* (Nemata: Ascaridida: Ascarididae) showing the three lips on the anterior end (D), the esophagus (E), and the proximal end of the intestine where it attaches to the esophagus (C). Source: S. L. Gardner, HWML. License: CC BY.

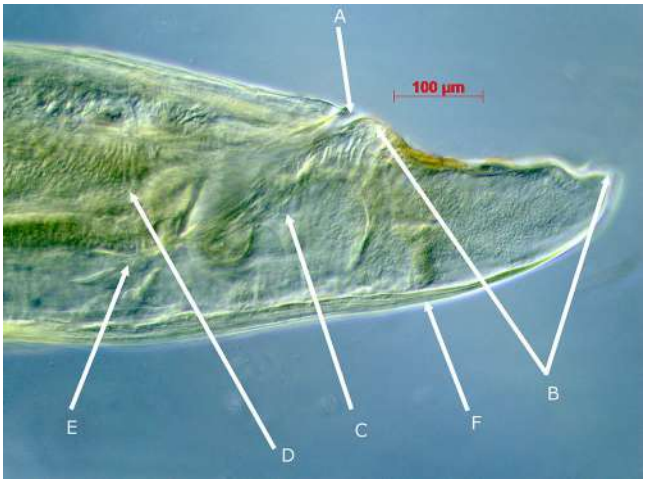

Figure 5. Posterior end of a female *Toxascaris procyonis*. Anus (A), tail (B), muscles that control the rectum and anus (C and E), posterior end of the intestine (D), cuticle (F). Source: S. L. Gardner, HWML. License: CC BY.

Epidemiology

The dynamics of *Ascaris* spp. infection are similar to those of *Trichuris trichiura*. Indiscriminate defecation by hosts, particularly near human or other animal habitations, seeds the soil with eggs that may remain viable for years. Resistance of *Ascaris* spp. eggs to chemicals is in fact legendary. They can embryonate successfully in 2% formalin, in potassium dichromate, and in 50% solutions of hydrochloric, nitric, acetic, and sulfuric acid, among other similar inhospitable substances (Schwartz, 1960). Eggs can survive in anaerobic sewage lagoon sludge for more than 10 years (Rosypal et al., 2007). This extraordinary chemical resistance is a result of the lipid layer of their eggshell, which contains ascarosides.

Longevity of *Ascaris* spp. eggs also contributes to success of the parasite. Brudastov and colleagues (1971) infected themselves with eggs kept for 10 years in soil at Samarkand, Uzbek SSR, Soviet Union. Of these eggs, 30–53% were still infective after all that time. Because of such longevity, it is impossible to prevent reinfection when yards have been liberally seeded with eggs, even when proper sanitation habits are initiated later.

Contamination, then, is the typical means of infection. Children are the most likely to become infected by eating soil or placing fingers and toys in their mouths. Chickens can serve as paratenic hosts (Permin et al., 2000). In regions in which night soil (that is, human excrement) is used as fertilizer, uncooked vegetables become important mechanical vectors of *Ascaris lumbricoides* eggs (Weidong et al., 1998). Experimental support for this hypothesis came from Mueller (1953), who seeded a strawberry plot with eggs. He and volunteers ate unwashed strawberries from this plot every year for 6 years and became infected each year. Cockroaches can carry and disseminate *A. lumbricoides* eggs (Burgess, 1984). Similarly, in some areas, dogs acquire *A. lumbricoides* eggs by coprophagy and spread viable eggs in their feces (Traub et al., 2002). Even windborne dust can carry eggs when conditions permit. Bogojawlenski and Demidowa (1928) found *A. lumbricoides* eggs in the nasal mucus of 3.2% of school children examined in the Soviet Union. Dold and Themme (1949) found *A. lumbricoides* eggs on 20 German banknotes in actual circulation.

Worldwide, 1.27 billion people, about one-quarter of the world population, are infected at any given time (Chan, 1997). Most infections occur in east Asia, China, sub-Saharan Africa, South America, and Central America (WHO, 2006). Morbidity as assessed by disability-adjusted life years (DALYs) totals ~ 10.5 million (Chan, 1997). Severe morbidity occurs in > 100 million cases each year (Chan, 1997); intestinal obstruction, mainly in children, occurs in roughly 1 out of 1,000 infections.

Worms are commonly aggregated in local populations, with a small number of people harboring infections of high intensity. These individuals seem to be predisposed to infection; when they are cured, they tend to become reinfected with large numbers of worms. The reasons for predisposition may be social, behavioral, environmental, and genetic, either alone or in combination. Members of a household tend to have similar infection intensities (household clustering), and individual household risk factors account for much of the variation in household worm counts (Walker et al., 2011).

Pathogenesis

Little damage is caused by penetration of intestinal mucosa by newly hatched worms. Juveniles that become lost and wander and die in anomalous locations, such as the host's spleen, liver, lymph nodes, or brain, often elicit an inflammatory response. Symptoms may be vague and difficult to diagnose and may be confused with those of other diseases. Transplacental migration into a developing fetus is also known. Allergy and immunopathology of ascariasis was reviewed by Coles (1985). The polyprotein allergens (lipid binding proteins) of *Ascaris* spp. are known to elicit IgE antibody responses and appear to be a contributing factor in *Ascaris* pneumonitis (sometimes referred to as Loeffler's pneumonia).

When juveniles break out of lung capillaries into the respiratory system, they cause a small hemorrhage at each site. Heavy infections will cause small pools of blood to accumulate which then initiate edema (swelling) with resultant clogging of air spaces. Accumulations of eosinophils and dead epithelium add to the congestion, which is known as *Ascaris* pneumonitis. Large areas of lung can become diseased, and, if bacterial infections become superimposed, death can result. Once, a student vented his ire on his roommates by seeding their breakfast with embryonated *Ascaris suum* eggs. One roommate almost died before his malady was diagnosed (Newsday, 1970; Phills et al., 1972; Jack Morrison, personal communication, 2023).

Pathogenesis from "normal worm activities"

The main food of *Ascaris* spp. is liquid contents of the small intestinal lumen. In moderate and heavy infections, the resulting theft of nourishment from the host can cause malnutrition, underdevelopment, and cognitive impairment in small children (Crompton, 2001; Levav et al., 1995). Abdominal pains and sensitization phenomena—including rashes, eye pain, asthma, insomnia, and restlessness—often result as allergic responses to metabolites produced by the worms.

A massive infection can cause fatal intestinal blockage (Baird et al., 1986) (Figure 6). Why in one case do large numbers of worms cause no apparent problem, whereas in

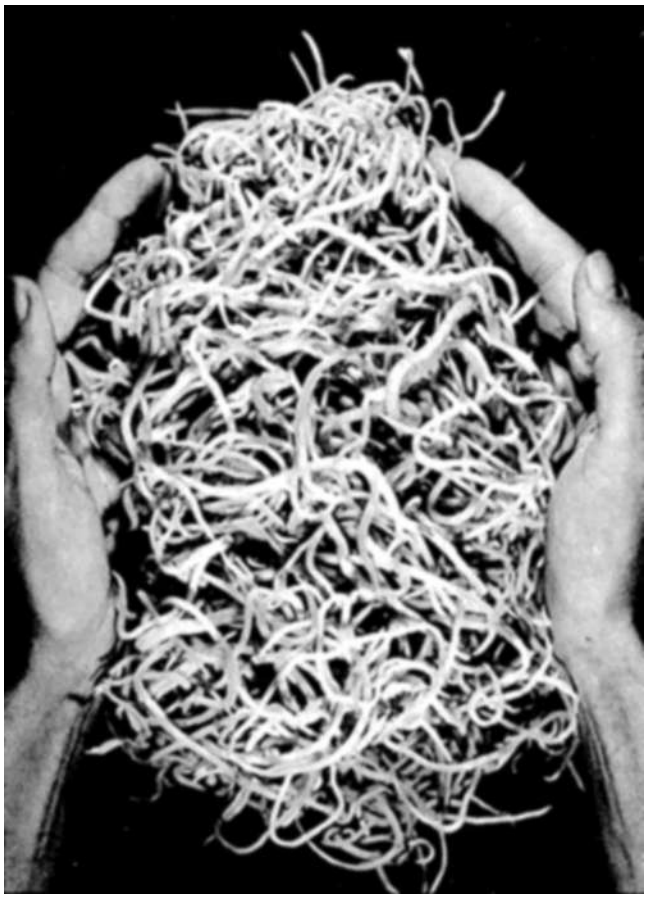

Figure 6. Worms recovered from necrotic small intestine, stomach, esophagus, intrahepatic and extrahepatic bile ducts, and gallbladder of a 2-year-old South African girl. Source: Baird et al., 1986. United States public domain.

another worms knot together to form a mass that completely blocks the intestine? The drug tetrachloroethylene, which was formerly used to treat hookworm, can cause *Ascaris* to knot up, but other factors remain unknown. Penetration of the intestine or appendix is not uncommon. The resulting peritonitis is usually quickly fatal. According to Louw (1966), at one time, 35.5% of all deaths in acute abdominal emergencies of children in Cape Town, South Africa were caused by *Ascaris lumbricoides*.

Wandering worms

Overcrowding in high-intensity infections may lead to wandering of adult worms. Downstream wandering may lead to the host's appendix, which can become inflamed or penetrated, or to the anus, with an attendant surprise found in the toilet of an unsuspecting host. Upstream wandering may lead to the pancreatic and bile ducts, possibly occluding them with subsequent grave results. Multiple liver abscesses have resulted from such invasion (Rossi and Bisson, 1983). Worms reaching the stomach are aggravated by the acidity and writhe around, often causing nausea. The psychological trauma induced in someone who vomits up a 45-cm ascarid is difficult to quantify. Aside from any psychological effects, aspiration of a vomited worm can result in death (Darby and Westphal, 1972). Worms that reach the esophagus, usually while the host is asleep, may crawl into the trachea, causing suffocation or lung damage; they may crawl into eustachian tubes and middle ears, causing extensive damage; or they may simply exit through the nose or mouth.

Diagnosis and treatment

Accurate diagnosis of migrating juveniles is impossible at this time. Demonstration of juveniles in sputum is definitive, provided a technician can identify them. Most diagnoses are made by identifying the characteristic, mammillated eggs in feces or by an appearance of the worm itself. Adults can also be diagnosed by ultrasound and other noninvasive radiographic methods (Goyal et al., 2010). So many eggs are laid each day by one worm that direct fecal smears are usually sufficient to demonstrate eggs. *Ascaris lumbricoides* should be suspected when any of the previously listed pathogenic conditions are noted. Most light infections are asymptomatic, and such infections are typically diagnosed only following spontaneous elimination of adults from the anus.

Benzimidazole-based drugs (for example, mebendazole or albendazole) are often effective in a single dose. Benzimidazoles bind to tubulin in the worm's intestinal cells and body wall muscles (Bughio et al., 1994). Emodepside, a novel anthelminthic so far licensed in combination with praziquantel for use in cats, causes relaxation of body-wall muscle of *Ascaris* and inhibits contraction (Willson et al., 2003). Nitazoxanide and ivermectin are also effective (Doumbo et al., 1997; Marti et al., 1996). In regions endemic for many different soil-transmitted nematodes, certain drugs may be preferable to others due to their broader spectrum of efficacy in cases of multiple-species infections.

Toxocara canis

This species is a cosmopolitan intestinal parasite of domestic dogs and wild canids and it is the chief cause of visceral migrans (VM) in humans, discussed later.

As a result of prenatal infections, even puppies in well-cared-for kennels are typically infected at birth and require anthelminthic treatment. It is not uncommon for 100% of puppies to be infected. The owner of a brand new puppy is likely to be startled by the pet's vomiting up several large, active worms. Puppies tend to have the highest infection prevalence. The infective dose of eggs has a large impact on the success of infection in adult dogs where protective immu-

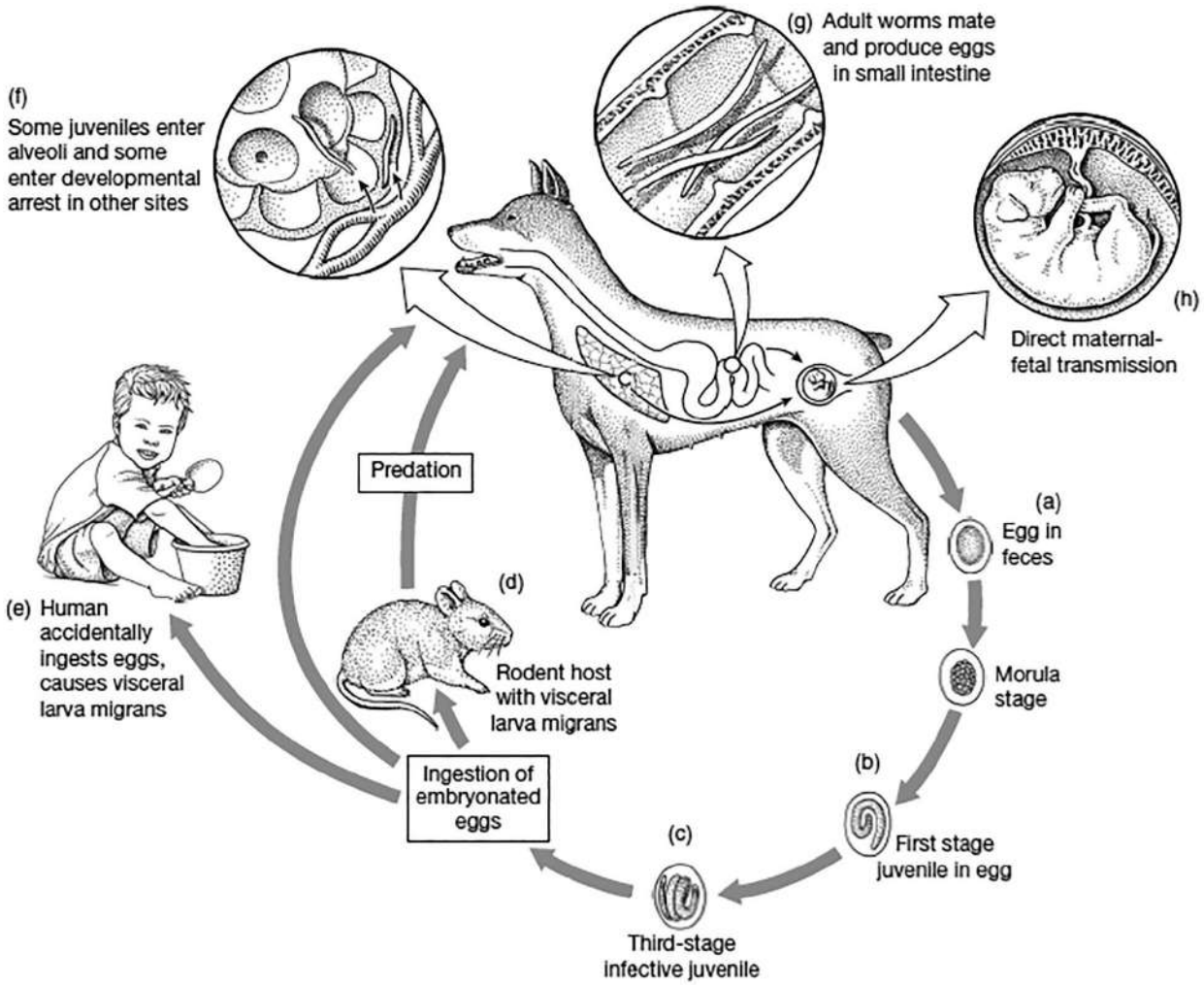

Figure 7. Life cycle of *Toxocara canis*. (a) Shelled embryo passed in feces. (b) J_1 in egg. (c) Infective J_3 in egg. (d) Eggs hatch in rodent host, and juveniles enter developmental arrest in viscera. (e) Eggs hatch in human and juveniles cause visceral larva migrans. (f) After penetration of intestinal wall, some juveniles break out into alveoli, ascend trachea, and finally mature in small intestine. Other juveniles (especially in mature dogs) enter developmental arrest in other sites. (g) Adult worms mate and produce eggs in small intestine. (h) Direct maternal-fetal transmission. Source: W. Ober and C. Garrison in Roberts et al., 2014. License: CC BY 4.0.

nity may have a larger role in the fate of juveniles; a smaller number of eggs administered is more likely to lead to patent infection (Dubey, 1978).

Adult *Toxocara canis* resemble *Ascaris* spp., only are much smaller. Three lips are present. Unlike *Ascaris* spp., however, *T. canis* has cervical alae in both sexes. Males are 4–6 cm-long, and females are 6.5 cm- to more than 15.0 cm-long. The brownish-colored eggs are almost spherical and roughly 75 µm × 85 µm, with surface pits, and are unembryonated when laid.

Biology

Adult worms live in the small intestine of their host, producing prodigious numbers of eggs, which are passed with the host's feces (Figure 7). Development of J_3 within eggs takes 9 days under optimal conditions.

The fate of ingested J_3s depends on host age and immunity. If a puppy is young and has had no prior infection, worms hatch and migrate through the portal system and lungs and back to the intestine, as in *Ascaris lumbricoides*. If the host is an older dog, J_3 fate is variable. Most J_3s will not complete the tracheal migration to become adults, but instead will enter the capillaries and undergo a somatic migration, eventually entering developmental arrest, with most individuals residing in the skeletal muscles.

If a dog harboring encysted, arrested juveniles becomes pregnant, those juveniles are reactivated late in the pregnancy and reenter the circulatory system, where they are carried to

the placenta. There they penetrate through to the fetal bloodstream and migrate to the liver where they reside until birth. Juveniles begin migration to the lungs within 30 minutes following birth, and then undergo a tracheal migration. Thus, a puppy can be born with an infection of *Toxocara canis*, even though its mother has shown no sign of patent infection (meaning, not producing eggs). The puppy may also become infected by the transmammary route (that is, in the mother's milk), but this is probably less common than the transplacental route (Gillespie, 1988). If a lactating dog ingests infective juveniles, they can complete migration to the intestine and produce a patent infection.

Another option in the life cycle of *Toxocara canis* is offered when a rodent or other mammal ingests embryonated eggs. In this host the juvenile begins to migrate but then becomes dormant with arrested development. If the rodent is eaten by a dog, the worms promptly migrate through the lungs to the intestine or into tissues to continue their wait, depending on the dog's age. Thus, rodents are paratenic hosts. Although this adaptability favors survival of the parasite, it bodes ill for paratenic hosts, which may undergo behavioral changes as a result of infection that increases their risk of predation (Hamilton et al., 2006).

Visceral migrans

When nematode juveniles gain access to the wrong host species they do not complete the normal migration but undergo developmental arrest and may begin an extended, random wandering through various organs and soft tissues of the body. The resulting disease is known as visceral migrans (VM), in contrast to cutaneous migrans (CM), which occurs only in skin. Visceral migrans can be caused by a variety of spirurid, strongylid, and other nematodes in addition to ascaridoids. However, *Toxocara canis* is the most common species causing VM in humans.

Epidemiology

Many years ago, it was assumed that dog and cat ascaridoids could not infect humans or were not dangerous to them. In the early 1950s it was discovered that this assumption is not true, particularly for nematodes such as *Toxocara canis*. At any one time, about 2.2% of adult dogs and 98% of puppies in the United States are infected with *T. canis*; with the population of pet dogs in the United States, this means that more than 1 million dogs are currently shedding *T. canis* eggs. Thus, risk of human exposure to infective eggs is very high. However, most human infections are covert, and even overt symptoms may go unrecognized and unreported.

Development of a specific immunodiagnostic test, an ELISA using secretory-excretory antigens collected from cultured juveniles, has been a boon to epidemiological studies of VM (Schantz, 1989). This test can distinguish between *Ascaris lumbricoides* and *Toxocara canis*, but does not distinguish *T. canis* from *T. cati* (see Lynch et al., 1993). In the United States, an extensive survey showed an overall seroprevalence of 13.9% (in people > 6 years-old), but was higher for non-Hispanic blacks (21.2%). Other risk factors included low socioeconomic status, living in rural areas, and geographic region (Hotez and Wilkins, 2009; Overgaauw, 1997). A seroprevalence of 34% has been found among Irish school children, and 31% of the children from Croatia with eosinophilia (Holland et al., 1995; Sviben et al., 2009). Seroprevalence among children in developing tropical countries has been much higher, from 50–80%. Visceral migrans is predicted to have a substantial impact on individuals living in poverty worldwide.

Dogs and cats defecating on the ground seed an area with eggs, which embryonate and become infective to any mammal or bird ingesting them. Small mammals are important paratenic hosts; infected mice undergo behavioral changes that increase their risk of predation, which increases the chance to complete the life cycle (Cox and Holland, 1998; 2001; Dold and Themme, 1949; Hamilton et al., 2006). Considering that the crawling-walking age of small children is a time when virtually every available object goes into the mouth for a taste, it is not surprising that the disease is common in children between 1 and 3 years old. In an urban setting, dog owners look upon the city park as the perfect place to walk a dog, while parents bring young children there to play on egg-seeded grass. Thus, of note is the high risk to children by exposure to the environment of puppies (Schantz, 1989). Finally, a factor to contemplate in light of the foregoing is the durability and longevity of *Toxocara canis* eggs, which are comparable to those of *Ascaris* (discussed above).

Pathogenesis

Juvenile *Toxocara canis* provoke a delayed-type hypersensitivity reaction in paratenic hosts and the degree and timing of the reaction depend on the infecting dose (Schantz, 1989). In experimental hosts, most juveniles eventually end up in the brain; it is unclear whether this is because juveniles have a predilection for the brain or because they are destroyed in other sites but remain in the brain. In sites other than the brain, juveniles may be encapsulated by a granulomatous reaction (Figure 8). The most common site of juvenile residence is the liver (as shown in Figure 8), but any organ will do.

Characteristic symptoms of VM include fever, pulmonary symptoms, hepatomegaly, and eosinophilia. The extent of damage usually is related to numbers of juveniles present

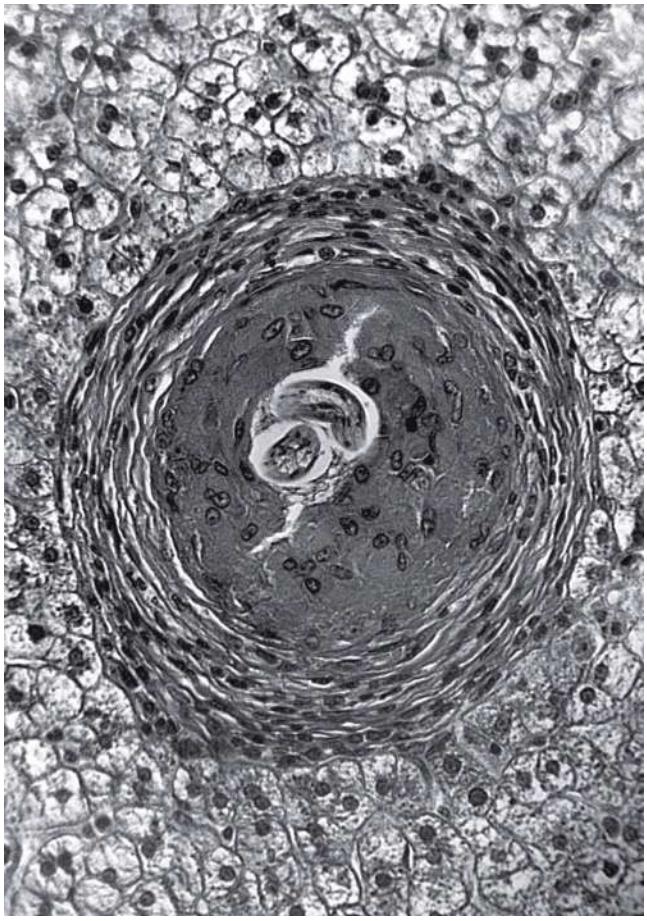

Figure 8. *Toxocara canis* juvenile section in liver of a monkey at 9 months' infection. The juvenile rests in a matrix of epithelioid cells surrounded by a fibrous capsule lacking intense inflammatory reaction. Source: Beaver, 1969 in Roberts et al., 2014. License: CC BY-NC-SA 4.0.

and their ultimate homestead in the host's body. Various neurological symptoms have been reported and deaths have occurred when juveniles were especially abundant in the brain. Presence of juveniles in the spinal cord can lead to inflammatory lesions and sensory or motor dysfunction; treatment with albendazole can yield neurologic improvement in such patients (Jabbour et al., 2011). Rarely, juveniles of *Toxocara canis* cause eosinophilic meningoencephalitis (Vidal et al., 2003). While dire consequences such as these can result from infection, most cases result in rather minor, transient symptoms such as abdominal pain, headache, and cough. This subacute condition is known as covert or common toxocariasis, which is commonly either undiagnosed or misdiagnosed.

Juveniles in a host's eye may cause chronic inflammation of the inner chambers or retina or provoke dangerous granulomas of the retina. These reactions can lead to blindness in the affected eye. The frequency of ocular toxocariasis in the United States is difficult to assess. Ocular toxocariasis was diagnosed in 1% of patients examined for vision loss in Alabama eye clinics in 1987 (Maetz et al., 1987). Generally, ocular damage is the result of invasion of only a single juvenile (Schantz, 1989). It may be that, because heavy infections stimulate a much stronger immune response with low survival rates, juveniles survive longer in light infections, giving them more time to wander into an eye. Other lesions destroy lung, liver, kidney, muscle, and nervous tissues.

Diagnosis and treatment

An ELISA using secretory-excretory antigens has facilitated clinical diagnosis enormously. This test is more sensitive for detecting covert toxocariasis and VM than ocular disease. A high eosinophilia is suggestive of infection with *Toxocara canis*, especially if the possibility of other parasitic infections can be eliminated.

Usually, only patients with severe symptoms are treated (Gillespie, 1988). Diethylcarbamzine and mebendazole appear to be effective treatments (Magnaval, 1995; Smith et al., 2009). An excellent summary of current therapies and preventative measures is given by Magnaval and colleagues (2022). Control consists of periodic deworming of household pets, especially young animals, and proper disposal of the animals' feces. Thus, for toxocariasis, veterinary medicine practices are important to mitigate disease transmission to humans. Some anthelminthics have been reported to be effective against all stages in dogs, including juveniles in arrested development (Altreuther et al., 2009), which presents new options for reducing transmission among dogs. Dogs and cats should be restrained, if possible, from eating available transport hosts. Sandpits in public parks can be protected from contamination by covering them with vinyl sheets when not in use (Uga and Kataoka, 1995; Uga et al., 1996).

Other *Toxocara* Species

Toxocara cati is widely prevalent among domestic cats and other felids (Figure 9). The cervical alae (Figure 10) of *T. cati* are shorter and broader than those of *T. canis*, and the eggs of the two species are slightly different in size. Life cycles are similar, including the use of paratenic hosts, but kittens are infected with *T. cati* only by the transmammary route if mothers are infected during late gestation (Gillespie, 1988). *Toxocara cati* may be an important cause of visceral migrans (VM), but it is difficult to determine the relative importance of each species because the current ELISA test for human infection does not distinguish between *T. canis* and *T. cati*. Adult *T. cati* have occasionally been reported from humans (Eberhard and Alfano, 1998).

Toxocara vitulorum is the only ascaridid that occurs in cattle. Its life cycle is similar to that of *T. cati*, with the young

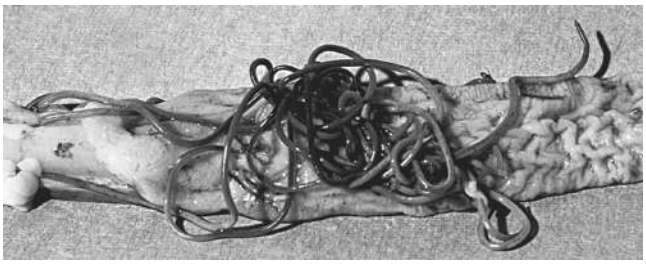

Figure 9. Intestine of a domestic cat, opened to show numerous *Toxocara cati*. Source: R. E. Kuntz in Roberts et al., 2014. License: CC BY-NC-SA 4.0.

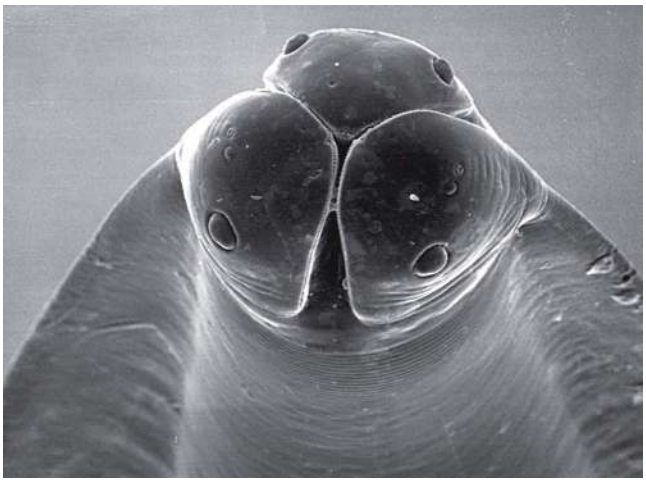

Figure 10. Scanning electron micrograph of *Toxocara cati* en face view. Note the 3 lips with sensory papillae and broad cervical alae on each side. Source: J. Ubelaker in Roberts et al., 2014. License: CC BY 4.0.

being infected by their mother's milk (Roberts, 1990). Adult hosts are refractory to intestinal infection. Young calves may succumb to verminous pneumonia during migratory stages of the parasites. Diarrhea or colic results in economic losses to the animal's human owner.

Parascaris equorum

This large nematode and its congener *Parascaris univalens* are the only ascaridoids found in horses and other equids. *Parascaris equorum* is a cosmopolitan species. It is very similar in gross appearance to *A. lumbricoides* but is easily differentiated by its huge lips, which give it the appearance of having a large, round head. In addition, *Parascaris* spp. individuals are white, whereas fresh *Ascaris* spp. specimens have a reddish color due to their characteristic muscle hemoglobin.

The life cycle is similar to that of *Ascaris lumbricoides*, involving a lung migration. Foals are often infected soon after birth; however, there is no evidence of prenatal or transmammary transmission. Resulting pathogenesis is especially important in young animals, with pneumonia, bronchial hemorrhage, colic, and intestinal disturbances resulting in unthriftiness and morbidity. Intestinal perforation or obstruction is common. Prevalence and intensity of infection decrease with horse age, presumably due to acquired immunity. In several regions *Parascaris equorum* shows strong resistance to the drug ivermectin, although certain other compounds remain viable alternatives for treatment (Lyons et al., 2008). The development and spread of drug resistance in nematodes is of concern because relatively few new anthelminthic drugs are being developed.

Baylisascaris procyonis

This is a very common intestinal parasite of raccoons in North America. Other related species in this genus occur in bears, skunks, badgers, and other carnivores. When embryonated eggs are ingested by a young raccoon, they will hatch in the small intestine, burrow into the intestinal wall, and mature. Older raccoons are typically infected when they eat infected rodent, lagomorph, or bird paratenic hosts that have juveniles encysted in their tissues. More than 90 species of birds and mammals have been reported to be infected (Kazacos, 1986). In these animals, parasite juveniles wander, often invading the central nervous system, resulting in neurological damage and debilitation, or death. This makes infected hosts vulnerable to predation or scavenging by raccoons. Unfortunately, juveniles affect humans in the same way. Neural (juvenile) migrans (NM) caused by *Baylisascaris procyonis* occurs almost exclusively in children younger than 2 years old; risk factors for egg ingestion include geophagia and pica. A substantial fraction of NM cases are fatal. Ocular migrans may occur in association with NM, or independently when it occurs in adult humans. Serological diagnosis of infection in humans has been difficult, but a new ELISA method based on a recombinant DNA antigen appears promising (Dangoudoubiyam et al., 2011).

An important epidemiological factor is close contact between humans and raccoons or raccoon feces. Scavenging raccoons may prowl and feed on pet dog or cat food near human dwellings and outbuildings. Their preferred communal defecation sites are dangerous sources of infection to humans and other animals (Page et al., 1999). Infected raccoons shed approximately 25,000 eggs per gram of feces, and communal raccoon latrines almost always contain infective eggs. Eggs can remain infective for years under ideal conditions, so once an area is contaminated it is nearly impossible to decontaminate using chemical treatments. Methods using heat such as steam generators or a propane flame gun can be effective for small areas (Kazacos, 2001) because juveniles within eggs are killed at 62 °C (Kazacos, 1982; Shafir et al., 2011). Pet kinka-

jous (another procyonid) sold in the United States have been reported to be infected with *Baylisascaris procyonis* (see Kazacos, 2001). Domestic dogs can also serve as hosts of adult *B. procyonis*, and if such infections were to become prevalent, this could alter factors influencing human infection.

Other species of *Baylisascaris* may have similar pathogenicity, but most hosts are not as likely to come in close contact with humans. Skunks infected with *B. columnaris* are potential hazards, however.

Toxascaris leonina

Toxascaris leonina is a cosmopolitan parasite of dogs and cats and related canids and felids. It is similar in appearance to *Toxocara* spp., being recognized in the following ways: 1) The body tends to flex dorsally in *T. leonina* and ventrally in *Toxocara* spp.; 2) alae of *T. cati* are short and wide, whereas they are long and narrow in *T. canis* and *T. leonina* (Figure 11); 3) the egg surface is smooth in *T. leonina* but pitted in *Toxocara* spp.; and 4) the tail of male *Toxocara* spp. constricts abruptly behind the cloaca, whereas it gradually tapers to the tail tip in *T. leonina*.

The life cycle of *Toxascaris leonina* is simple. Ingested eggs hatch in the host's small intestine, where juveniles penetrate the mucosa. After a period of growth, they molt and return directly to the intestinal lumen, where they mature. Alternatively, juveniles in intermediate hosts such as rodents can infect definitive hosts following predation.

Like for *Toxocara* spp., the pathogenicity of *T. leonina* for the definitive host depends on infection intensity, and in severe cases can involve intestinal obstruction or rupture of the intestine. Visceral migrans involving *T. leonina* has been implicated as a possible cause of human eosinophilia on St. Lawrence Island (Bering Sea), where this nematode commonly infects Arctic foxes, working dogs, and voles (rodent genus *Microtus*) as paratenic hosts (Rausch and Fay, 2011).

Figure 11. Anterior end of *Toxascaris leonina*, an intestinal parasite of dogs, cats, and other canids and felids. Note the narrow cervical alae (arrow) as compared with the broad alae of *Toxocara cati*. Source: J. Georgi in Roberts et al., 2014. License: CC BY-NC-SA 4.0.

***Lagochilascaris* species**

Relatively little is known about the natural definitive host ranges of the 5 described species in *Lagochilascaris*, a genus mainly reported from North America, Central America, and South America. The genus name is derived from the prominent cleft on the inner margin of each lip (Figure 12). These nematodes normally mature in the host's gastrointestinal tract but seem to have a tendency to develop in abscesses outside the gut. The life cycle is indirect, with juveniles developing to the infective stage within rodent intermediate hosts that ingest the eggs. Embryonated eggs are not directly infective for definitive hosts. *Lagochilascaris minor* and *L. major* have often been reported from domesticated cats; *L. minor* is typically found in subcutaneous abscesses in the head or neck of such hosts whereas it localizes in the stomach, esophagus, and trachea of wild cats in South America and the Caribbean. Domestic cats have been experimentally infected with the third-stage juveniles (J_3) of *L. minor* from mice (Barbosa et al., 2007). Experimental infections were patent, suggesting that domestic cats may serve as a reservoir for zoonotic infection. The pharynx of domestic cats appears to be the preferred site for *L. major*; this species has also been reported from wild and domestic canids, and raccoons. Wild cats are believed to represent the natural definitive host for both *L. minor* and *L. major* in South America, but host records are few. In North

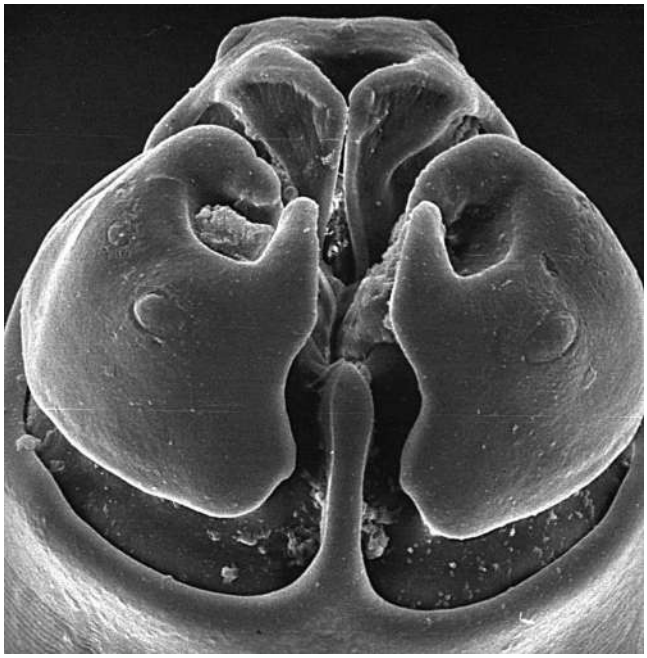

Figure 12. *Lagochilascaris turgida.* Note the prominent cleft in the tip of each lip, typical of the genus (lagos (Greek) = hare; cheilos (Greek) = lip). Source: J. Sprent in Roberts et al., 2014. License: CC BY 4.0.

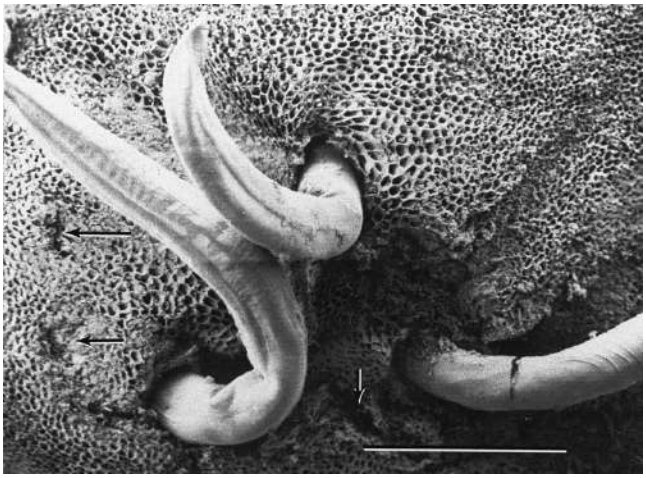

Figure 13. Scanning electron micrograph of *Terranova* sp. juveniles (family Anisakidae) penetrating the stomach of a rat on day three postinfection. Arrows indicate acute lesions caused by juveniles. Scale bar = 1 mm. Source: Deardorff et al., 1983 in Roberts et al., 2014. License: CC BY-NC-SA 4.0.

America, *L. sprenti* uses opossums as its definitive host.

Lagochilascaris minor has been reported in humans at least 8 times, usually found in the tonsils, nose, or neck (Sprent, 1971; Volcan et al., 1982). A fatal brain infection has been reported (Rosemberg et al., 1986). When present, worms cause abscesses that may contain from 1 to more than 900 individuals. Juveniles can mature in these locations, and they produce pitted eggs, much like those of *Toxocara* spp. Human infections may last many years or may kill infected people rapidly. How humans become infected is unknown. Humans are unnatural, accidental hosts for this zoonotic infection.

Family Anisakidae Railliet & Henry, 1912

The many species in the family Anisakidae are stomach parasites of fish-eating birds and marine mammals. Species in the genus *Anisakis*, have a life cycle that involves passage of eggs in feces of their definitive hosts, embryogenesis and hatching of J_3s, ingestion of J_3s by a crustacean, development in the hemocoel of the crustacean, and then either, 1) Ingestion by a definitive host, or 2) ingestion by a fish paratenic host, which is ultimately consumed by a definitive host (Deardorff et al., 1991; Sakanari, 1990). Definitive hosts of *Anisakis* spp. are marine mammals.

Living *Anisakis* spp. juveniles can produce pathological conditions in humans who eat them in raw, salted, marinated, smoked, or pickled fish in preparations such as ceviche, sushi, sashimi, lomilomi, and rollmops. Such conditions may be asymptomatic, mild, or severe (Bier et al., 1987). Symptoms generally commence when juveniles begin to penetrate the stomach lining or intestinal mucosa (Figure 13). Gastric involvement may manifest from 1 to 12 hours after ingestion of infected seafood or after up to 14 days in the case of intestinal penetration. Symptoms may include severe epigastric pain, nausea, vomiting, diarrhea, and hives, but the disease may be confused with other disorders, such as peptic ulcers. Sometimes severe IgE-mediated hypersensitivity reactions occur, and because the allergenic substances present may be heat resistant, even cooking may not render them harmless (Caballero and Moneo, 2004).

Diagnosis of gastric anisakiasis by endoscope and removal of worms with biopsy forceps is effective, although catching lively worms with forceps may be challenging (Deardorff et al., 1991). In intestinal anisakiasis, or cases in which the worm has fully penetrated into submucosa or migrated beyond the gastrointestinal tract, diagnosis is more problematic, and symptoms can mimic a number of other, more common conditions. In such cases serodiagnosis can be helpful and recombinant antigens have made detection of IgE antibodies highly specific (Anadón et al., 2010; Sakanari et al., 1988).

Most cases have been reported from Japan, South Korea, Spain, and Scandinavian countries, where raw or marinated fish is consumed regularly. Approximately 2,000 cases per year have been reported from Japan, where it is a major

foodborne disease, and the number of cases reported from the United States is increasing (Deardorff et al., 1991; Kagei and Isogaki, 1992). Fatalities due to peritonitis have been recorded (Bier et al., 1987).

Anisakis spp. juveniles are the most frequent cause of anisakiasis, but the name of this disease is a misnomer because other anisakid genera, and even species from other families (such as Raphidascarididae), can be responsible. A common feature of the causative organisms is that they are transmitted through aquatic food chains that involve invertebrates and most typically fish paratenic hosts; these paratenic hosts can be infective for humans.

Cooking kills juveniles, but continued popularity of raw or undercooked fish dishes (some examples of which are listed above) ensures a continued risk of human infection. In many cases, commercial blast freezing causes little change in the texture or taste of fish while effectively killing *Anisakis* sp. juveniles (Deardorff and Throm, 1988).

Acknowledgement

This section was adapted with permission from Roberts et al. (2014, p. 411–421).

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Nemata

Heterakoidea (Superfamily): Cosmopolitan Gut-Dwelling Parasites of Tetrapods

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Phylum Nemata

Superfamily Heterakoidea

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Chapter 51

Heterakoidea (Superfamily): Cosmopolitan Gut-Dwelling Parasites of Tetrapods

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Introduction

Heterakoidea is a superfamily of ascaridid nematodes that occur most often in the cecum and large intestine of amphibians, reptiles, birds, and mammals. Some species are very common with several occurring in galliform birds worldwide, while others may be found commonly in various mammals in both North America and South America. Two genera particularly, *Ascaridia* and *Heterakis*, include important parasites of birds, and both impact rearing of commercial poultry (Jansson et al., 2010).

Heterakoids are characterized by a pre-cloacal sucker in males and an esophagus with a posterior bulb and a muscular anterior corpus. The life cycle of heterakoid nematodes is simple: Eggs containing the infective third-stage juvenile (J_3) are ingested by the definitive host, although for some species, paratenic hosts may be involved.

Phylogenetic analysis of SSU rDNA sequences reveals that, as currently defined, this superfamily is not monophyletic and requires taxonomic revision (Nadler et al., 2007).

Morphology

As noted in the introduction, one of the most conspicuous characters of the heterakoid nematodes is the **precloacal sucker**, which is endowed with a well-developed **cuticular rim**. This character is present in all but a few species that are classified in the superfamily. Notable exceptions include some species of *Lauroia* (see Proença, 1938; Jiménez-Ruiz and Gardner, 2003) and *Ascaridia*. Other important features have been highlighted by Inglis (1967) who described the cuticular ornamentation of the **stoma** (also called the **buccal cavity**) and **lips**, and notes their homology or common origin with the cuticular derivatives of the **esophagus** and **body wall**. The typical stoma of the heterakoid is endowed with a fused **esophagorhabdion** and a conspicuous **cheilorhabdion**. Before the advent of molecular techniques, these characters had been used extensively for the description of genera and the classification of the suprageneric taxa. Other important characteristics of these nematodes include **papillae** in the **precloacal rim** and the **preanal papillae** (see Figure 1).

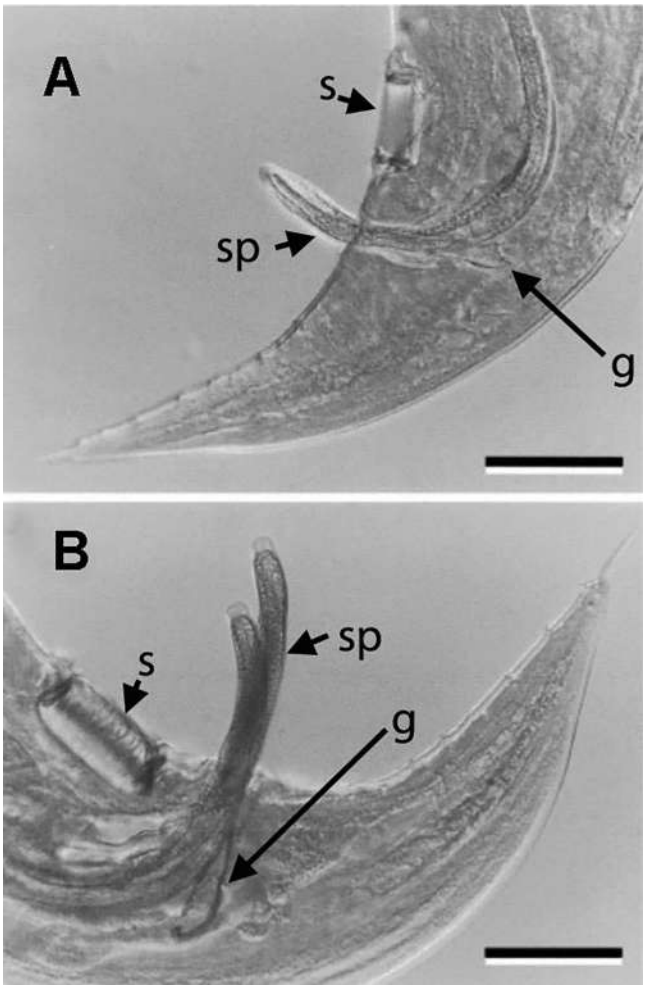

Figure 1. Heterakoid nematodes *Aspidodera* spp. A) Posterior end of *A. sogandaresi* showing pre-cloacal sucker (s) and spicules (sp); B) posterior end of *A. fasciata* showing paired spicules (ps) sucker (s) and proximal end of gubernaculum (g). Scale bars = 100 µm. Source: S. L. Gardner, HWML. License: CC BY 4.0.

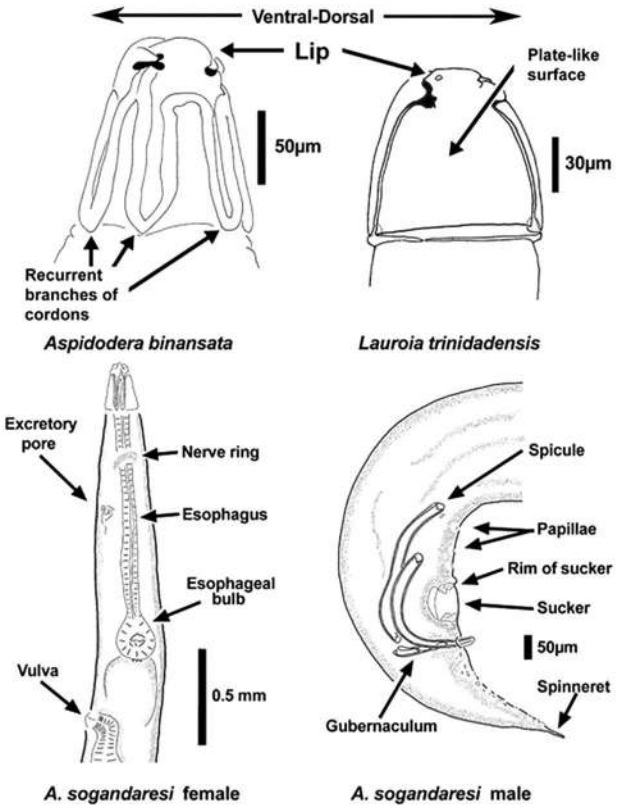

Figure 2. Examples of the structures of the hood in Aspidoderidae (Nemata: Heterakoidea) as seen in the right lip. Source: S. L. Gardner, HWML. License: CC BY 4.0.

Finally, the esophagus is divided into 3 parts, which acquire their final adult configuration in the fourth molt. These include a conspicuous **pharynx**, the **corpus**, and an **esophageal bulb** that is endowed in most species with a **trivalved sphincter** (Figure 2).

Diagnosis

The Heterakoidea are members of the class Secernentea, order Ascaridiomorpha. Three lips are present, with a dorsal lip that is bilaterally symmetrical featuring double papillae and ventral lips, each with a single papilla. There is a conspicuous cheilorhabdion lining the buccal cavity or stoma and a medially located esophagorhabdion. The esophagus is muscular, divided into 3 conspicuous parts, including a pharynx that projects into the lips, corpus, and bulb. There is a heavily cuticularized precloacal sucker with a robust rim, 2 spicules, and a gubernaculum that guides the spicules during copulation. The vulva is usually located at midbody. In the uterus, the eggs are usually unembryonated and the shell is smooth, some with polar pores.

Distribution and Host Associations

Members of the taxon include dwellers of the cecum or large intestine of terrestrial tetrapods, with a single case of infection reported in fish (*Meterakis japonica*, Moravec and Sey). Most of the taxonomic diversity is present in scaled reptiles and birds, followed by several taxa present in frogs and mammals. The heterakoid nematodes have a cosmopolitan distribution being found in the large intestines of reptiles, birds, and mammals on all continents except Antarctica. Very few species appear to be endemic to temperate land masses, with notable exceptions, such as *Hatterianema hollandei* and *Kiwinema gracilicauda*, which appear to be limited to the main islands of New Zealand. Both species occur in endemic tetrapods of these islands such as the tuatara (*Sphenodon punctatus*) and an unidentified species of kiwi of the genus *Apteryx*. Species diversity of these taxa is very low, as only 1 species is known for each genus (Inglis, 1991). This taxon also includes a third genus of limited diversity, since *Mammalakis* includes 2 known species in naked mole rats from South Africa and Europe (Inglis, 1991).

The biogeography and host associations for subfamilies Spinicaudinae and Meteterakinae are in sharp contrast to one another. Prevailing hypotheses posit that Spinicaudinae has a cosmopolitan distribution, yet members of the Meteterakinae show a disjoint and perhaps relictual distribution confined to southeast Asia and the Neotropics (Baker, 1984). These 2 groups are associated with frogs and semiaquatic reptiles. A different pattern is evident in species in the Family Aspidoderidae, for which distribution is chiefly Neotropical with documented dispersions into North America (Jiménez-Ruiz et al., 2012). These parasites chiefly infect mammals of a Neotropical origin and are the only family that predominantly shows this distribution and host association. Interestingly, the relationships of members of the family with other members of the Heterakoidea are yet to be resolved.

The rest of the groups in the family, namely Ascaridiidae and Narsingianellinae, show contrasting patterns. The latter appears to be restricted to toads in southeast Asia and the Indian subcontinent (Rao, 1978; Rizvi, 2009), whereas the former is cosmopolitan, with species occurring in birds, and occasionally in mammals and reptiles.

General Biology

The precise dietary requirements of these nematodes have not been determined, although it has long been speculated that because of their habitat they must feed on cecal or gut bacteria. Experimental manipulations show that varying levels of fiber in the host diet induce conspicuous differences in the survival and fecundity on the cecal-dwelling *Heter-*

akis gallinarum (see Daş et al., 2014). Fiber-rich diets increase the volume of the ceca and the fermentation activity induced by bacteria.

Experimental infections that help illustrate their life cycle have been completed for just 6 species, yet they all seem predominantly to feature direct transmission. In some cases, earthworms and other terrestrial invertebrates are used as vectors for the eggs (Ackert, 1917; Frank, 1953). The thin-shelled nature of the eggs, featuring 2 polar pores, may make them prone to prompt dehydration, thus making them highly dependent on humid environments. There is ample evidence, however, that humidity is the key environmental condition that promotes the development of these worms. Experimental work on *Heterakis gallinarum* has been used to characterize the typical life cycle of members of the superfamily. As such, this can be generalized to be monoxenous (without an intervening intermediate host), with females laying unembryonated eggs that complete embryogenesis in the external environment and juveniles undergoing 2 molts (Araújo and Bressan, 1977). The development of the infective stage is temperature dependent; it takes 7 to 12 days to form the infective stages in temperatures ranging between 17 and 29 °C (Graybill, 1921). When temperature is maintained at 27 °C and 33 °C, development completes in 6 and 4 days, respectively. Usually, eggs become infective 24 hours after the second molt (Roberts, 1937).

Experimental approaches to test the animals' endurance in adverse conditions document their resistance to dry environments and drastic temperature and humidity changes, as well as their prolonged retention of infectivity. The eggs can remain infective after being passed through the digestive system of earthworms and grasshoppers (Ackert, 1917; Frank, 1953). The nematodes are able to complete migration to their target organ 48 hours post-infection (hpi).

In contrast, some of the species appear to be able to complete their development optimally while completely submerged in tap water (Petter, 1968; Bain, 1970). In the case of *Spinicauda freitasi* and *S. inglisi*, the development of the infective stage takes between 14 and 15 days when submerged in tap water at 26 °C. Infective juveniles feature a rhabditiform esophagus. When fed to a definitive host, the nematodes reach their target organ typically 40 dpi (Petter, 1968). Experimental infections of larvated eggs of insects showed that the juveniles of *Strongyluris brevicaudata* can migrate and encapsulate in the thorax of cockroaches and occasionally in mosquitoes (Bain, 1970).

As nematodes develop throughout their life, there is a drastic reconfiguration of both internal organs and external appearance. Detailed accounts of this metamorphosis document the transformation for *Spinicauda inlgisi*, *Spinacauda freitasi* (see Petter, 1968), *Strongyluris brevicaudata* (in Bain, 1970), and *Heterakis gallinarum* (shown by Dorman, 1928). These juveniles undergo changes and molt twice to develop into infective forms, featuring a rhabditiform esophagus that is devoid of a bulb. During the migration through the digestive system of the definitive host, the nematodes mature with the concomitant development of the characteristic esophageal bulb (Petter, 1968; Bain, 1970).

Evolution

Based on their geographic distribution and the features of their anterior end and cuticular ornamentation, and their association with ectothermic tetrapods, Inglis (1967) offered an interpretation of their evolution with emphasis on their changes of association with vertebrates (specifically, host switching) and major morphological transitions. This notion was further elaborated by Baker (1984) who concentrated on a handful of species in 2 subfamilies and speculated on an origin in the Cretaceous for members of Heterakoidea. Studies on the phylogenetic associations between nematodes of this taxon and their hosts using replicable datasets started with the cophyletic approach for South American species of *Paraspidodera* spp. infecting hystricognath rodents (Gardner, 1991). The historical association among the Aspidoderidae and their wide array of hosts was further addressed in work by Jiménez-Ruiz and colleagues (2006; 2008; 2012). A holistic approach addressing the origin of the Heterakoidea has not yet been produced.

Systematics and Phylogeny

Analysis for species included in suprageneric taxa are not clearly defined, and the relationships and even the classification of the families are still in flux (Rao, 1978; Inglis and Harris, 1990; Jiménez-Ruiz et al., 2008; 2012). There are a few proposals of the phylogenetic arrangement for members of the Heterakoidea, yet all of them concentrate on the relationships among species in a genus or a family (Bouamer and Morand, 2008; Jiménez-Ruiz et al., 2013) (see Table 1 for a Linnean classification for the Heterakoidea).

Building on the foundation of the systematic approaches presented by Mozgovoi (1953) and Skrjabin and Shikhobalova (1951), Inglis (1967) proposed an overall classification structure for the group identifying 3 main synapomorphies: 1) Very well-developed **rhabdions** (cuticular structures derived from the esophagus and the body wall that cover the inner lining of the mouth; see Figure 3); 2) **lips**; and 3) a **ventral sucker** with a cuticular rim. This systematic arrangement has survived until the present, although some phylogenetic re-

Table 1. Linnean classification for Heterakoidea (Superfamily) as of 2014.

- Heterakoidea
  - Kiwinematidae Inglis and Harris, 1990
    - Kiwinematinae Inglis and Harris, 1990
      - *Kiwinema* Inglis and Harris, 1990
        - *Kiwinema gracilicauda* Inglis and Harris, 1990
      - *Hatterianema* Chabaud and Dollfus, 1966
        - *Hatterianema hollandei* Chabaud and Dollfus, 1966
    - Mammalakinae Inglis, 1991
      - *Mammalakis* Inglis, 1991
        - *Mammalakis macrospiculum*
        - *Mammalakis spalacis*
  - Heterakidae Railliet and Henry, 1912
    - Heterakinae Railliet and Henry, 1912
      - *Heterakis* Dujardin, 1945
      - *Pseudaspidodera* Baylis and Doubney, 1922
      - *Odonterakis* Skjabin and Schikhobalova, 1947
      - *Musserakis* Hasegawa, Dewi and Asagawa, 2014
        - *Musserakis sulawesiensis* Hasegawa, Dewi and Asagawa, 2014
      - *Neoheterakis* Kumar and Thienpoint, 1974
      - *Haroldakis* Inglis, 1991
    - Meteterakinae
      - *Meteterakis* Karve, 1970
      - *Gireterakis* Lane, 1917
      - *Bufonerakis* Baker, 1980
      - *Cagourakis* Petter, Chermette and Vassart, 1988
    - Narsingellinae Rao, 1978
      - *Narsingiella* Rao, 1978
    - Spinicaudinae
      - *Spinicauda* Travassos, 1920
      - *Africana* Travassos, 1920
      - *Moaciria* Texeira de Freitas, 1956
      - *Strongyluris* Mueller, 1894
      - *Pseudostrongyluris* Guerrero, 1970
    - Aspidoderidae Skrjabin and Shikhobalova 1947
      - Aspidoderinae Skrjabin and Shikhobalova 1947
        - *Aspidodera* Railliet and Henry, 1912
        - *Ansiruptodera* Skrjabin and Shikhobalova 1947
        - *Nematomystes* Sutton, Chabaud and Durette-Desset, 1980
      - Lauroiinae Skrjabin and Shikhobalova 1947
        - *Lauroia* Proença, 1938
        - *Paraspidodera* Travassos, 1914
    - Ascaridiidae Travassos, 1919
      - *Ascaridia* Dujardin, 1845

constructions challenge its monophyly (Nadler et al., 2007). The accelerated rate of species descriptions of *Meteterakis* species from the Southeast Asian archipelago seems to suggest the notion that the groups are diverse, yet the taxonomic impediment (that is, a lack of qualified, trained taxonomists) hinders the documentation of biodiversity.

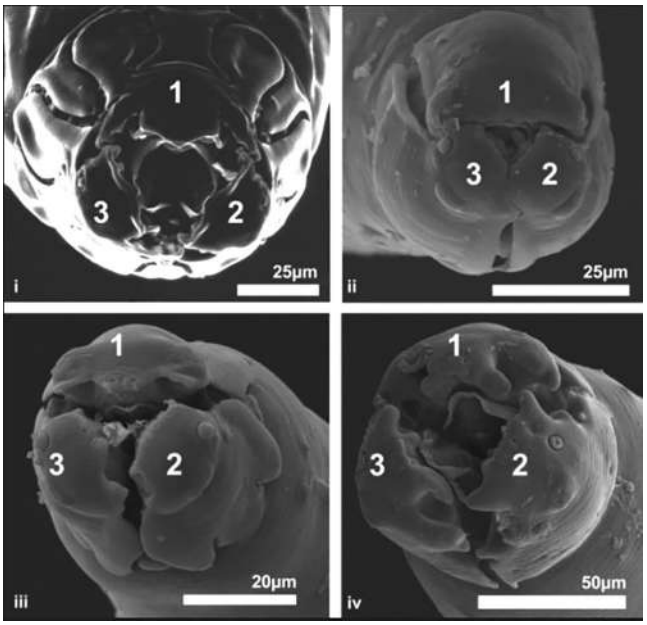

Figure 3. En face view of 4 species of Aspidoderidae showing the positions of the lips. Dorsal lip is labeled 1 in all images; ventral side includes 2 lateroventral lips labeled as 2 (sinistroventral) and 3 (dextroventral). *Aspidodera scoleciformis* (i), *A. bolivari* (ii), *A. scapteromi* (iii), and *Paraspidodera uncinata* (iv). Source: Jiménez-Ruiz et al., 2008. License: CC BY-NC-SA 4.0.

Superfamily Heterakoidea

Family Ascaridiidae Travassos, 1919

It is important to note that this family name is very similar to the family name Ascarididae Baird, 1853, which is included within the superfamily Ascaridoidea (discussed in another chapter). It is unfortunate that they are so similarly named, but be clear that Ascarididae and Ascaridiidae are absolutely separate groups.

One species within the Ascaridiidae of interest is *Ascaridia galli*, which is a cosmopolitan parasite of the small intestine of domestic fowl and game birds. Males reach a length of 77 mm, and females reach 115 mm. Juveniles within eggs hatch after they are ingested with contaminated food or water. The life cycle does not involve extensive tissue migration. Instead, 8 or 9 days after infection, juveniles molt to the third stage (J_3) and begin to burrow into the mucosa, where they generally remain with their tails still in the intestinal lumen. After molting to J_4 at about 18 days, they return to the lumen, where they undergo their final molt. Probably a majority of worms complete their 2 molts and attain maturity without ever leaving the lumen. However, some juveniles burrow their anterior ends into the intestinal mucosa where they remain for up to 2 months before molting and returning to the lumen to complete development to the adult stage.

Those that attack the mucosa cause extensive damage, and *Ascaridia galli* causes production losses in chickens. High-intensity infections can obstruct the small intestine and cause death. In addition, adult *A. galli* are sometimes found in chicken eggs destined for human consumption. This is obviously of concern to egg producers. Improved management practices to control infection through sanitation are important because in some countries few anthelminthics are approved for use in poultry.

Family Heterakidae Railliet & Henry, 1912

Heterakis gallinarum is cosmopolitan in domestic chickens and turkeys. It was probably brought to the United States in imported ring-necked pheasants. The worms live in the cecum, where they feed on its contents. *Heterakis gallinarum* is unusual because in galliform birds it serves as a vector of the parasitic protozoan, *Histomonas meleagridis*, the causative agent of histomoniasis (blackhead). Hence, the curious phenomenon of one parasite acting as an intermediate host and vector of another is revealed.

Several species of *Heterakis* are known from birds, particularly in ground feeders, and one species, *H. spumosa*, is cosmopolitan in rodents.

Three large lips and an esophageal basal bulb as well as lateral alae are found in this genus. Males are as long as 13 mm and possess wide caudal alae supported usually by 12 pairs of papillae (Figure 4). Their tail is sharply pointed, and there is a prominent preanal sucker. Spicules are strong and dissimilar, and a gubernaculum is absent. Females have the vulva near the middle of their body and a long, pointed tail.

Biology

Eggs of *Heterakis gallinarum* contain a zygote when laid. They develop into the infective stage in 12 to 14 days at 22 °C and can remain infective for 4 years in soil. Infection is contaminative: When embryonated eggs are eaten, third-stage juveniles (J_3) hatch in the gizzard or duodenum and pass down to the cecae. Most complete their development in the lumen, but some penetrate the mucosa, where they remain for 2 to 5 days without further development. Then, returning to the lumen, they mature about 14 days after infection.

If eaten by an earthworm, a juvenile may hatch and become dormant in the worm's tissues, remaining infective to chickens for at least a year. Since these nematodes do not develop further until eaten by a bird, an earthworm is a paratenic host. Grasshoppers, flies, and sowbugs can also serve as mechanical vectors of eggs.

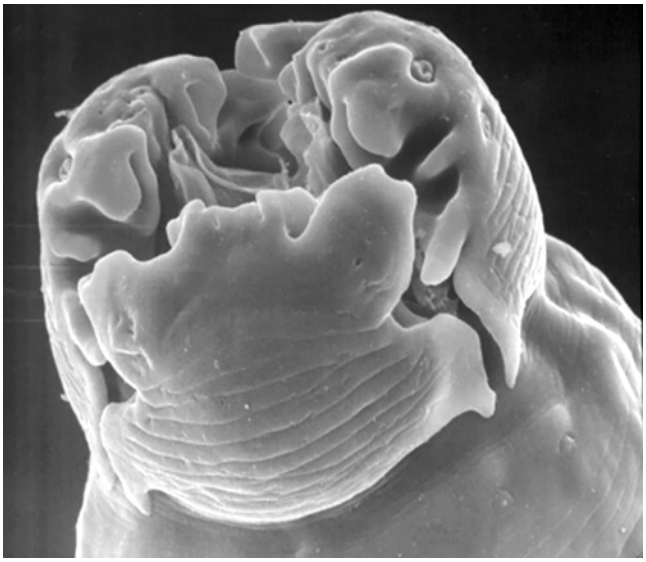

Figure 4. The anterior end of *Paraspidodera* sp. from a rodent from Bolivia, showing the 3 lips. Source: S. L. Gardner, HWML. License: CC BY 4.0.

Epidemiology

As a result of the longevity of the eggs, it is difficult to eliminate *Heterakis gallinarum* from a domestic flock. The many different mechanisms for persistent contamination of poultry farms by eggs remains a challenge to implementing sanitation procedures, such as cleaning and disinfection, without concurrent use of strict hygiene barriers. In addition, wild birds may also serve as sources of infection. Furthermore, as earthworms feed in contaminated soil, they accumulate large numbers of juveniles, which in turn cause massive infections in the unlucky birds that eat them.

Pathogenesis

Generally speaking, *Heterakis gallinarum* is not highly pathogenic in itself. Chickens typically have only minor histopathological lesions when infected, but show localized cellular immune effects, particularly a Th2-dominated response at the site of infection (Schwarz et al., 2011). However, the protozoan, *Histomonas meleagridis*, is transmitted between birds within eggs of *He. gallinarum* (see Long et al., 1987). This protozoan is the etiological agent of blackhead, a particularly serious disease in turkeys where mortality in captive flocks can exceed 85%. Unlike in chickens, blackhead can be directly transmitted between turkeys by fecal contamination. Typically, the protozoan is eaten by the nematode and multiplies in the worm's intestinal cells, ovaries, and finally the embryo within the egg. Hatching of the worm within a new host releases *Hi. meleagridis*. In chickens co-infected with

He. gallinarum and *Hi. meleagridis*, severe ulceration of the cecal mucosa may occur. The protozoan infection elicits a different, Th1-dominated immune response and a higher T-cell infiltration rate than with infection of *He. gallinarum* alone (Schwarz et al., 2011).

Diagnosis and treatment

Heterakis gallinarum can be diagnosed by finding eggs in feces of its host. Birds allowed to roam a barnyard usually are infected. Worms are effectively eliminated with mebendazole. Usually, a flock of birds routinely gets this or other drugs in its feed or water. Other benzimidazole drugs that are effective against juvenile stages, such as albendazole and febendazole, have been shown to be useful for preventing establishment of *Histomonas meleagridis* by preventing nematode infection (Hegngi et al., 1999). Unfortunately, drugs directly effective against *Hi. meleagridis* have been found to be carcinogenic and are no longer registered for use in poultry. Without effective drugs or a vaccine, control of blackhead disease currently relies on management practices, including prophylaxis by regular deworming. In some countries, regulatory bans on keeping laying hens in metal cages have led to husbandry conditions that increase transmission of these nematodes, providing new challenges to their control (Jansson et al., 2010).

Ecology

Heterakoid worms appear to be moderately prevalent in the populations of tetrapods sampled in a systematic manner. Navone (1990) has demonstrated that the prevalence of some species of *Aspidodera* in armadillos from central Argentina was greater than 50% in the wet season and reduced to roughly 30% in the dry season. The prevalence of 2 species of *Aspidodera* in central Florida reach a combined level of 63% (Varela-Stokes et al., 2008). Both *Heterakis gallinarum* and *Ascaridia galli* occur in several wild and domestic galliform birds, posing a problem for wildlife managers in certain regions of the world.

Economic Importance

The species with the greatest known economic impact in the group is *Heterakis gallinarum*, known to infect poultry (domesticated chickens and both wild and domesticated turkeys) and may produce disease from high levels of infection. Yet the pathology induced by these species seldom jeopardizes the survival of the host and the infection by these nematodes alone rarely induces much mortality in a population. Nevertheless, *He. gallinarum* is involved in the transmission of a species of flagellated protozoan of the order Trichomonadida that causes significant mortality in wild and captive flocks of galliform birds. As noted above, *Histomonas meleagridis* is the causative agent of blackhead in chickens. This trichomonad is not known to produce cysts, having only an unflagellated trophozoite stage as well as a flagellated trophozoite stage, thus the trophozoite is the only morphotype in their life cycle. The parasite is transmitted horizontally through cloacal exchange or via contamination with fresh feces. The trophozoites do not live long in feces, and chickens are little affected by this protozoan, but in turkeys it is sometimes 100% fatal to the flock.

An interesting aspect of the biology of the nematode and the protozoan is that the trophozoites of *Histomonas meleagridis* are able to infect the sexual organs of both female and male nematodes. In the body of the females the trophozoites migrate through the uterus and reach the ovary. In that organ they are able to infect the developing embryos before the proteinaceous shell is formed. In this way, the trophozoites colonize a structure that will act as an exterior casing for the trophozoites, isolating them from the external environment outside both the avian host and the parasitic nematode. Several species of earthworms may serve to help complete the life cycle by ingesting nematode eggs and passing the infected eggs on to chickens or turkeys. The earthworms can pick up the eggs of the heterakoid nematodes from deep in the soil. The trichomonads are then able to hatch from the egg once consumed by the definitive host, then to reach the cecum and continue growth (Figure 5). In combination, these unique biological characteristics hinder the efforts to prevent and control the disease caused by these organisms.

Conservation

Prominent conservation biologist Gerardo Ceballos encourages biologists to frame their studies as a conservation activity (Rojas-Bracho et al., 2018). He suggests that there is value in documenting the distribution of all species to establish the consequences of species interactions in the function of any ecosystem. A challenge for future parasitologists is to frame the study of any group of nematodes (and, in fact, all parasites) from this perspective. Although most efforts in the conservation of parasites deal with the problem of the stress maintained on the hosts (including the pathological consequences of the effects that these parasites have on their hosts), there are more possibilities including using parasites with indirect life cycles as probes for biodiversity, which refers to the fact that discovery of a single species of parasite that uses a complex life cycle in a host immediately reveals several layers of biological complexity (Gardner and Campbell, 1992) or listing the parasites that cycle through sympatric animals and identifying the factors that determine this distribution.

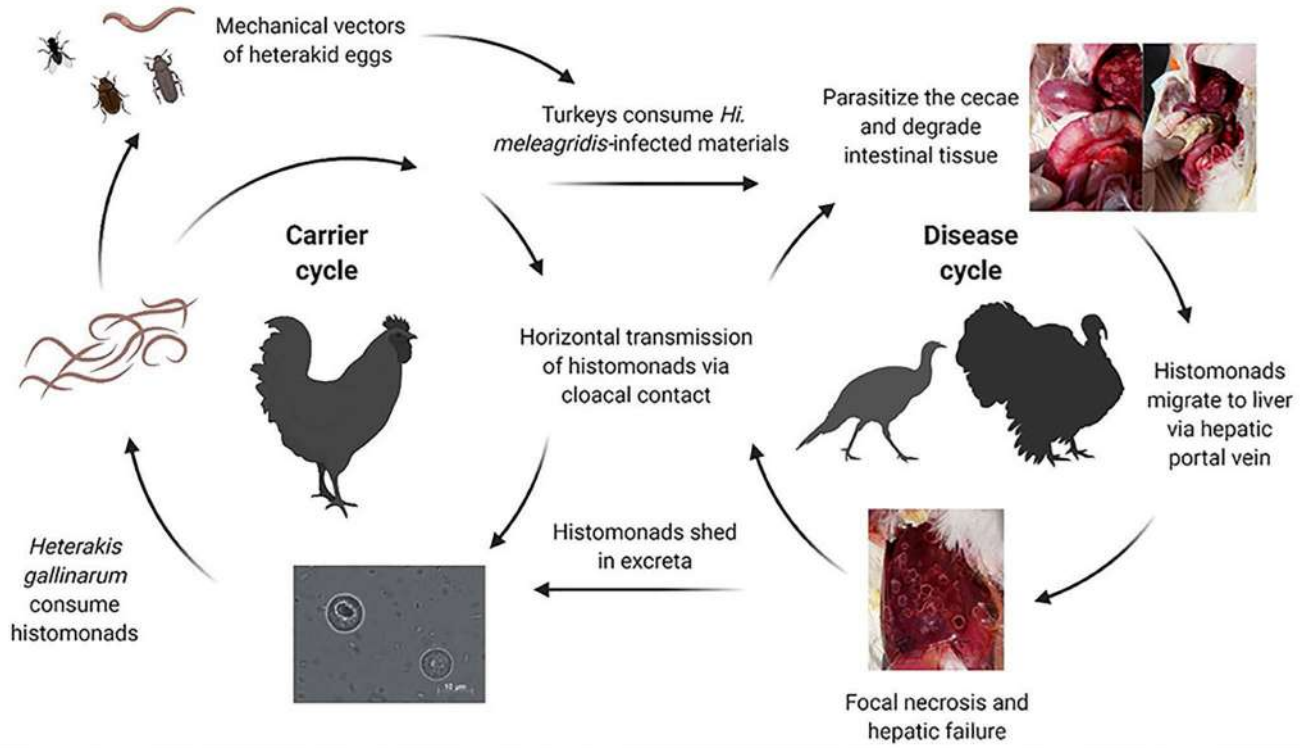

Figure 5. Complex transmission of *Histomonas meleagridis*, a venereal disease of nematodes. In the galliform host, infective eggs of *Heterakis gallinarum* are ingested incidentally as the bird eats earthworms or other soil-dwelling invertebrates from soil contaminated with feces from infected birds. The eggs hatch in the intestine and juvenile nematodes move to the cecae in the lower part of the gastrointestinal tract of the bird where they feed on cecal contents, grow, molt to adults, mate, and produce eggs. Histomonid protozoans living and reproducing in the cecae of the bird invade the nematode via the vulva and move through the ovijector and uterus up to the ovary of the female nematode hosts (*Heterakis gallinarum*) where they proliferate, utilizing the germinal zone of the ovary of the nematode as nutrients. As the protozoans increase their numerical density in the ovary of the nematode, some penetrate the developing oocytes and are encased in the newly formed eggs. *Histomonas meleagridis* can also invade the cloaca and vas deferens of male *Heterakis* and may act as a venereally-transmitted protozoan. In the bird host, the protozoan escapes when the egg hatches and establishes in the cecae of the intestine. Source of image: Beer et al., 2022. Created with BioRender.com. License: CC BY 4.0. | Source of caption: Adapted from Anderson, 2000. License: CC BY 4.0.

The Heterakoidea include some species that are associated with relictual groups of animals, including *Hatterianema hollandei* present in the tuatara *Sphenodon punctatus*. Although the species is not listed as threatened by the International Union for Conservation of Nature (IUCN; Yeates et al., 2012), it includes a unique group of animals that serves as the only known host for this species of heterakoid. Furthermore, their phylogenetic relationships appear to be blurred by the combination of characters shared with other heterakoids present in southern continents and the potential extinction of ancient lineages of scaled reptiles, birds, and lisamphibians that could have harbored them (Chabaud and Dollfus, 1966; Inglis and Harris, 1990; Inglis, 1991). The association of the parasite with its host and its geography is the result of the optimal factors that make infection possible, including the chance encounter and the compatibility among hosts (Combes, 1991). The use of different and novel hosts depends on the evolutionary distinctiveness of both parasites and hosts. Parasites would be able to hack the immune system of hosts that may be closer biologically to their original hosts (Park et al., 2018) although there is abundant evidence of the ability of parasites to infect widely disparate hosts due to deep phylogenetic historical signals (Brooks et al., 2019).

Conservation biologists have urged identification of clades with unique genetic diversity. This genetic diversity can be evaluated as to how rare the genetic information is in the members of the group. Between any 2 sister clades, the one of critical conservation importance would be the one that holds the rarest species and includes unique genetic information that would be lost with the extinction of the species that features it. Consequently, the most relictual distribution of a species of nematode in addition to a high specificity suggest

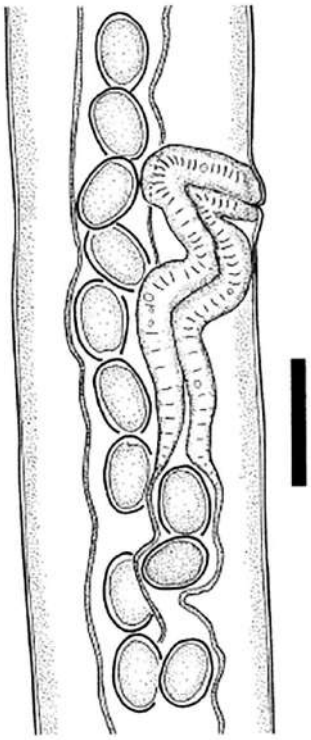

Figure 6. *Lauroia bolivari*. Lateral view of the ovijector with eggs. Scale bar = 100 mm. Source: Adapted from Jiménez-Ruiz and Gardner, 2003. License: CC BY 4.0.

that the species lineage of nematodes is particularly unique. Invoking the earlier example, *Hatterianema hollandei* would be a very important species worthy of extraordinary efforts at conservation (Yeates et al., 2012) since it holds unique information that summarizes an evolutionary lineage in which most of the descendants have become extinct. This evolutionary lineage includes another relict, *Kiwinema gracilicauda*, a parasite of kiwis. The conservation of these lineages will allow scientists to identify the important factors that regulated the interactions of biological associates, including parasites and mutualists of the earliest tetrapods (Boast et al., 2018).

Further evidence of the relevance of heterakoids in the fields of conservation and evolution is provided by the eggs of some heterakoids. In some cases, these structures have been preserved in coprolites that document the associations of recently extinct organisms (Sardella and Fugassa, 2009; Boast et al., 2018). The preservation of some of these eggs has enabled researchers to identify them based on the unique morphology of the eggs (Figure 6) and has enabled the extraction and amplification of small fragments of DNA that allow scientists to identify the egg as coming from a unique species with marked distinctiveness with species currently present in the area (Boast et al., 2018).

Acknowledgement

A portion of this section was adapted with permission from Roberts et al. (2014, p. 421–422). No byline was included for this contribution at the beginning of this section, so this serves as acknowledgement of the contribution by the authors of that borrowed section.

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Nemata

Oxyurida (Order): Pinworms

Haylee J. Weaver

Phylum Nemata

Order Oxyurida

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Chapter 52

Oxyurida (Order): Pinworms

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Introduction

The order Oxyurida, commonly known as pinworms or oxyurids, comprises almost 900 species of parasitic nematodes that inhabit the posterior gut of vertebrates and some arthropods. This diversity of hosts is unique to oxyurids, as no other nematode groups have so successfully parasitized both vertebrates and invertebrates, with species of oxyurids found in all classes of vertebrates and in arachnids, millipedes, insects (beetles, cockroaches, mole crickets, flies), and annelids (Adamson, 1994).

The current higher taxonomy and classification is not used consistently throughout the literature (see the section on systematics and taxonomy below), but the fine-scale taxonomy at the family level and lower is stable and consistent. Oxyurids are strictly parasitic but are microphagous, which distinguishes them from other groups of parasitic nematodes. The group (order Oxyurida, or also known as infraorder Oxyuridomorpha) comprises 3 superfamilies: Oxyuroidea, parasitizing vertebrates, and Coronostomatoidea and Thelastomatoidea, both parasitizing herbivorous arthropods.

Key Features of Oxyurids

Oxyurids are small nematodes that inhabit the posterior gut (cecum and large intestine) of vertebrates and arthropods. Oxyurids display **sexual dimorphism** where females are much larger than males, but both sexes are tapered at both ends with the tail more narrow than the head. They are characterized by having an **esophagus** with a large **terminal bulb**, and in males by having a **single spicule**, or **no spicules** at all (Carreno, 2014). They have 3 **lips** surrounding the **buccal aperture**, and several **cephalic papillae** and **amphids** around the **oral surface**. Males in some taxa have raised **mamelons** on their **ventral cuticle**; other features of the cuticle of both sexes include cervical, lateral, or pre-anal **alae**.

History

Because of its association with humans, *Enterobius vermicularis* (Linnaeus, 1758) was known of long before it was formally named, with the symptoms of enterobiasis described by the ancient Greeks (Moulé, 1911; Hugot et al., 1999). The parasite is considered to be one of the oldest human parasites, with a pre-hominid evolutionary origin (Iñiguez et al., 2003). The oldest record of *E. vermicularis* is from a coprolite dated to 7837 BCE, from what is now Utah, United States (Fry and Moore, 1969). Ancient DNA has been extracted from *E. vermicularis* eggs from coprolites dated between 4000 BCE to 900 CE from Chile and the United States (Iñiguez et al., 2003; 2006), and from 1985 BCE from Brazil (Lino et al., 2018). Evidence of *E. vermicularis* infection has also been found from Roman-occupied Egypt (30 BCE–395 CE) (Horne, 2002), and from Iran from 2500–1500 BCE (Paknazhad et al., 2016).

Despite having no bearing on human health, thelastomatoid oxyurids have been known about since the 1800s when *Cephalobellus cuspidatum* (Rudolphi, 1814) was described from a rhinoceros beetle larva (Carreno, 2014). As more species were described, several workers revised the taxonomy of the Thelastomatoidea, with the family and subfamily status of taxa changing over time (Carreno, 2014). The definitive monographs on the systematics of the Thelastomatoidea were published by Adamson and van Waerebeke (1992a; 1992b; 1992c) and updated by Carreno (2014).

Life History and Ecology

Like many groups of nematodes, oxyurids are monoxenous, and their development and mode of transmission is very similar in vertebrates and invertebrates (Anderson, 2000). In contrast to most other nematodes, however, oxyurids are haplodiploid, where males arise from unfertilized eggs but females form from fertilized eggs (Adamson, 1990). Haplodiploid development is not a common life history trait in nematodes (*Caenorhabditis elegans* notwithstanding) but is common in some groups of rotifers, mites, and some insects.

The life history of oxyurids is fairly similar across the groups that parasitize vertebrates and invertebrates. Females produce thick-shelled eggs that are usually flattened on one side and have an operculum (Anderson, 2000). Eggs are either deposited in an early stage of development with juveniles hatching after they have been passed in feces, or via females migrating to the anus of the host and laying eggs on the host perianal area (Anderson, 2000). The latter method is more common in the Oxyuridae and allows for autoinfection, where the grooming activities will facilitate transfer of eggs to the

mouth of the host (Morand and Hugot, 1998). Oxyurids have no free-living or extraintestinal stages, and the infective third-stage juveniles (J_3) hatch directly from eggs following ingestion by the new host and subsequently remain in the posterior gut for their whole life cycle (Adamson, 1994). This combination of traits, combined with the usually low effect on host health, means that oxyurids can have a highly aggregated distribution within host species (Grear and Hudson, 2011).

The biogeographic distribution of oxyurids is linked with those of their hosts. Coevolution and cospeciation, where the phylogeny of hosts mirrors that of the parasites, was thought to be common among oxyurids (for example, in primates; see Hugot, 1999). More recently, however, evidence for strict cospeciation of oxyurids within host taxa was not found, for example in rodents (Weaver et al., 2016) and in lizards (Mockett et al., 2017). This suggests that speciation of oxyurids is explained by elements of the Stockholm Paradigm, rather than via strict coevolution and cospeciation (see Box 1).

Animal Health/Effects on Hosts

Pinworm life cycles are direct, with autoinfection of hosts common, and do not include any extraintestinal migrations (such as those for hookworm juveniles). Further, pinworms feed on bacteria in the hindgut or cecum, rather than feeding on host tissue. Therefore, the effect on hosts is generally low, with little to no pathogenicity and the only main symptoms in mammals being itching in the perianal area where the females have migrated to lay eggs (Beveridge et al., 2015).

Systematics and Taxonomy

Note that, regardless of any purported establishment of higher-level classifications, the family-level taxonomy is stable and will be used here to avoid any confusion that can result from selective implementation of taxonomies at higher levels.

Formerly grouped as an order within the phylum Nematoda, the Oxyurida was downgraded to be reclassified as the Oxyuridomorpha, 1 of 5 infraorders within the suborder Spirurina (order Rhabditida) along with the Ascaridomorpha, Spiruromorpha, Rhigonematomorpha, and Gnathostomatomorpha (De Ley and Blaxter, 2002; Wijová et al., 2006; Nadler et al., 2007). This group is at times referred to as a member of Clade III nematodes (sensu Blaxter et al., 1998). The Oxyuridomorpha comprises 2 superfamilies, Oxyuroidea and Thelastomatoidea.

Summary of the Main Groups

Superfamily Oxyuroidea

Parasites of mammals, birds, reptiles, amphibians, and to a lesser extent, fish.

Box 1. The Stockholm Paradigm

The Stockholm Paradigm is a conceptual framework to understand host-parasite evolutionary relationships (see Hoberg and Brooks, 2015 for a detailed discussion.) It includes overviews of: 1) Ecological fitting; 2) the oscillation hypothesis; 3) the geographic mosaic theory of coevolution; and 4) the taxon pulse to explain the suites of parasite and host relationships over time and space. Ecological fitting is the idea that parasites can fit into niches. Switching to a new host is an extremely energy-intensive evolutionary activity for a parasite, so switching to a new host that has the same niche available as the old host (for example, the cecum or hindgut) is possible with relatively low evolutionary effort and thus can lead to speciation of parasites over time (Brooks et al., 2006). This can explain how some species can be found across a wide range of hosts, and/or distributions, and also how, over time, closely-related host species can harbor speciose parasite communities. Taxon pulses are bursts of colonization/radiation and subsequent speciation of taxa (Erwin, 1985), for example when land bridges were exposed during periods of glaciation, for example, for *Syphacia* spp. and their host rodents in Australia (Weaver et al., 2016).

Family Oxyuridae

The Oxyuridae is a large family of over 35 genera with a global distribution in a wide range of host mammals, for example, marsupials, rodents, primates, ungulates, and hyraxes (Figures 1–3). Infecting mostly wildlife, some species also affect domestic animals, such as pet rodents, rabbits, and horses. Notable species of this family include *Oxyuris equi*, the pinworm of domestic and wild horses, and *Enterobius vermicularis*, the common pinworm of humans. Neither species is especially pathogenic, but the method of gravid females laying eggs in the perianal region causes itching and irritation to hosts (Beveridge et al., 2015). *Syphacia muris* is a common parasite of wild and domestic black rats (*Rattus rattus*) and *Passalurus ambiguus* is a parasite of wild and domestic rabbits (Leporidae).

Family Heteroxynematidae

The 17 genera of the Heteroxynematidae are found in sciurid rodents and lagomorphs (mainly pikas) in Nearctic and Palearctic regions, and birds from the Americas, for example, sandgrouse and tinamou (Petter and Quentin, 1974). Those from birds are thought to be a recent host switch based on the

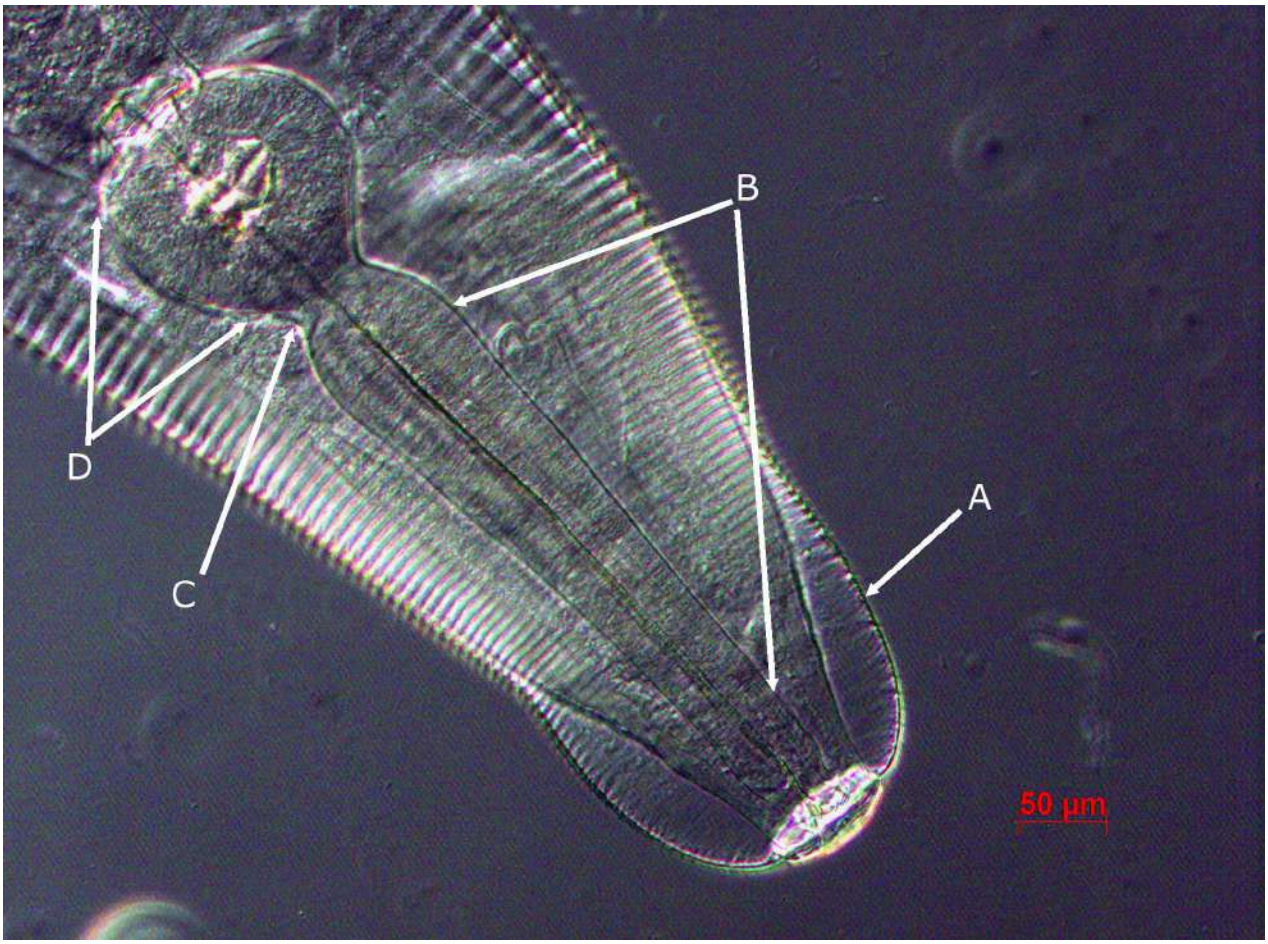

Figure 1. Anterior end of a species of *Syphacia* from rodents of the family Sciuridae (squirrels). Typical of pinworms of rodents, the inflated cuticle near the mouth can be seen (A), in addition, the esophagus expands posteriad (B), narrowing into an isthmus (C), and then expanding into a definite bulb (D). Source: S. L. Gardner, HWML. License: CC BY.

Figure 2. The anterior end of a species of *Passalurus* from the cecum of a rabbit (*Sylvilagus* sp.). The mouth is shown labeled (A), the corpus of the esophagus is marked by arrows (B), the bulb of the esophagus is shown at (C), and the intestine is shown at (D). Source: G. Drabik, HWML, 2016. License: CC BY.

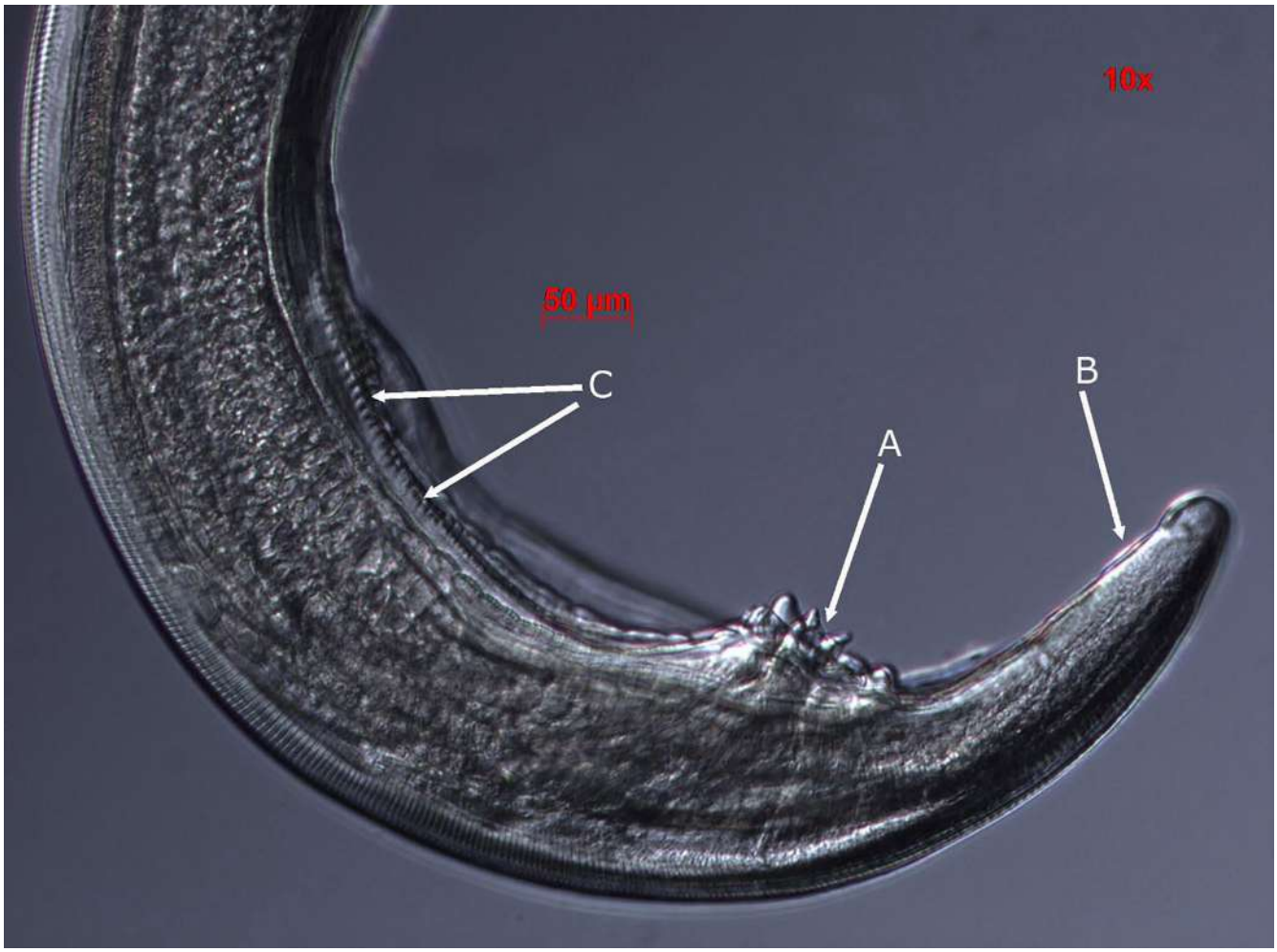

Figure 3. Posterior end of a male *Passalurus* pinworm of rabbit and hares (order Lagomorpha: family Leporidae). The tail (B) is the part of the animal posteriad to the cloaca. The cloaca is shown surrounded by small sensory papillae (A). In male nematodes the exit to the digestive system and the reproductive system share a common duct called the cloaca. Small annulations on the ventral part of the male in species of this genus are evident and are thought to assist the male in mating with the females in the gut of the host. Source: S. L. Gardner, HWML. License: CC BY.

majority of species being found in rodents and lagomorphs (Adamson, 1994). One genus, *Paleoxyuris*, was discovered from a 240-million year-old coprolite from a cynodont (primitive synapsid) (Hugot et al., 2014).

Family Pharyngodonidae

The Pharyngodonidae comprises 30 genera and are parasites of a wide range of vertebrate hosts, including reptiles, amphibians, mammals, and to a lesser extent, fish. One species of monotreme, the short-beaked echidna *Tachyglossus aculeatus* is host to *Parapharyngodon anomalus*, a genus otherwise only found in reptiles (Hobbs, 1996). The relationships between the 7 genera of pharyngodonids occurring in fish and the rest of the family are unresolved, whereas those parasitizing lizards and amphibians show clearer links (Adamson, 1994).

Superfamily Thelastomatoidea

Parasites of arthropods, particularly insects.

Family Thelastomatidae

The Thelastomatide is a large family of over 45 genera with a diverse range of invertebrate hosts (Adamson, 1994; Carreno, 2014). Genera are not limited to insects, with hosts including millipedes, an arachnid, an oligochaete, beetles, cockroaches, flies, and mole crickets (Jex et al., 2006).

Family Hystrignathidae

There are 35 genera in the Hystrignathidae and all are restricted to passalid beetles (Coleoptera: Passalidae) (Adamson, 1994; Carreno, 2014). The family is characterized by having cuticular rods to support the anterior part of the phar-

ynx, elongated eggs that have ornamentation on the shell surface, and at least 1 medial single papilla in males (Adamson and Van Waerebeke, 1992c).

Family Protrelloididae

Members of the 4 genera of Protrelloididae are found in cockroaches only, from North America and South America, India, Madagascar, and Australia (Adamson and Van Waerebeke, 1992b; Jex et al., 2006).

Family Pseudonymidae

The 5 genera of Pseudonymidae are parasites of water scavenger beetles (Coleoptera: Hydrophilidae), except for the genus *Jarryella*, which is found in scarabs (Coleoptera: Scarabeidae) (Adamson and Van Waerebeke, 1992b).

Family Travassosinematidae

Species of the 10 genera of the Travassosinematidae infect mostly mole crickets and are found in India, Madagascar, North America, and South America (Adamson and Van Waerebeke, 1992b).

Superfamily Coronostomatoidea

Superfamily Coronostomatoidea comprises parasites of millipedes and cockroaches.

Family Coronostomatidae

The family Coronostomatidae was erected by Kloss (1961) as part of the superfamily Thelastomatoidea but was moved into its own superfamily, Coronostomatoidea, by Poinar (1977). Both the family and superfamily were subsequently synonymized by Adamson and van Warebeke (1992a) without explanation but were resurrected by Phillips and colleagues (2016). The Coronostomatidae comprises a single genus, *Coronostoma*, with 7 species found in mostly millipedes from Brazil, Burkina Faso, Madagascar, and India, and from a species of cockroach in Australia (Phillips et al., 2016).

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Nemata

Spirurida (Order)

Valentin Radev

Phylum Nemata

Order Spirurida

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Chapter 53

Spirurida (Order)

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Introduction

The representatives of the order Spirurida are nematode parasites of fishes, amphibians, reptiles, and mammals. They are some of the most common parasites found in vertebrates. Morphologically and phylogenetically diverse, the order includes 2 suborders, with 12 superfamilies with a large number of families, subfamilies, species, and subspecies that often inhabit a unique site of localization in the host, such as the esophagus, stomach, body cavities, blood vessels, and so on.

Recently their study has involved modern methods, such as scanning electron microscopy, molecular biology, and other techniques. As a result, new conceptions about their classification and complex life cycles are available and are presented here.

Morphology and Locations within the Host

Spirurids are parasites having typical morphological features clearly distinguishing them from other nematodes. Their body is spindle-shaped. The front and back edges can be narrow or tapered. They possess an anterior extremity which is bilaterally symmetrical and they lack lateral, external labial papillae. In some members, the **sexual dimorphism** between males and females is very pronounced. Typically, the **cuticle** of the spiruridis has clear ornamentation. Their body surface may be transverse grooved, having different forms—spikes, teeth, edges, wart-like formations, wrinkles, and others. The **mouth opening** usually is surrounded by 2 lateral 3-section **lips**. In some cases, they possess additional dorsal and ventral lips. Some spirurids have a clearly-differentiated **buccal cavity** or **stoma** which leads into the **pharynx**, which can have different forms. The **esophagus** is divided into 2 parts: The anterior, which is muscular and shorter, and the posterior, which is glandular and longer. Males are without a genital bursa, but sometimes they have **tail-cuticular wings**. The **caudal papillae** are always ventral or ventrolateral in position. There is no pre-anal sucker. There are usually 2 **spicules**, different in shape and length from one another. Normally the right one is shorter and wider. The female **genital opening** is variable in its distance from the anterior end depending on the species (see Figures 1 and 2).

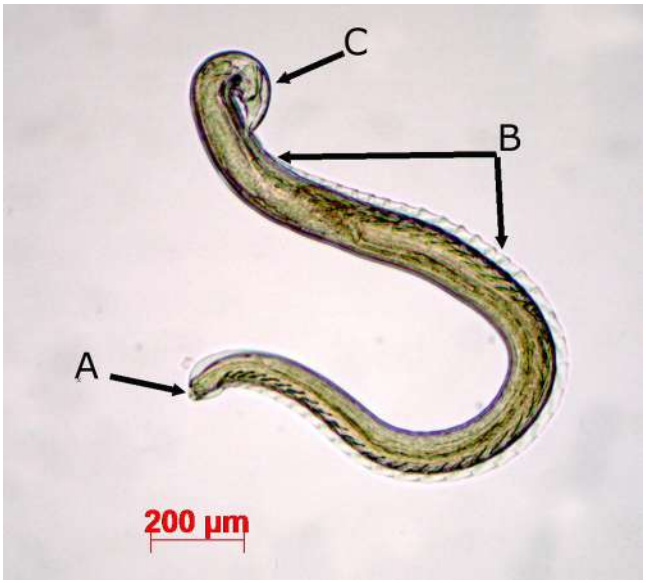

Figure 1. Whole body view of a species of *Pterygodermatites* from a bat. Showing: The mouth is at the anterior end (A), a double row of spines runs the length of body terminating just before the tail (B), and the tail (C). Source: K. Cajiao Mora, 2022. License: CC BY.

Figure 2. Anterior end of a species of spirurid, *Pterygodermatites*, from a bat of the genus *Myotis* collected in eastern Colombia. Small hooks of the cuticle can be seen around the mouth and thin spinose parts of the cuticle can be seen running posteriad. In this species, the spines occur down the body and terminate before the tail. Source: K. Cajiao Mora, 2022. License: CC BY.

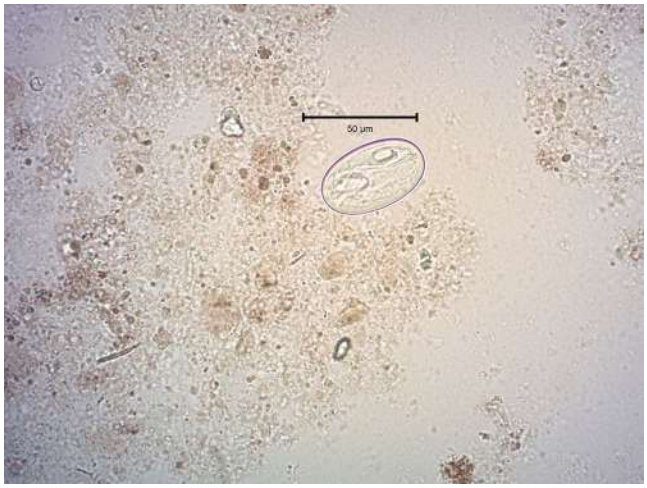

Figure 3. Spirurid egg from a short-eared owl *Asio flammeus*. Source: T. Pennycott, Edinburgh DataShare, https://datashare.ed.ac.uk/handle/10283/2139. License: CC BY.

Females lay eggs (Figure 3) with juveniles already formed. The juvenile stages, which are preinfective in the final or definitive host, develop entirely within an intermediate host, which may be crustaceans, beetles, coprophages, and other insects. Adults are parasites in the host's gastrointestinal tract, nasal cavity, blood vessels, eyes, and conjunctival sacs, under the skin, and in different tissues or body cavities of fishes, birds, and mammals.

Taxonomic Hierarchy with Descriptions

See Table 1 for an aggregated, selective implementation of the higher-level taxonomy for this group with hosts and sites of localization noted (see also Kanchev et al., 2016; Vassilev et al., 1986).

Table 1. Selected superfamilies of nematodes with hosts and sites of localization.

Superfamily	Intermediate hosts	Final hosts	Sites of localization	Sources
Acuarioidea	insects	birds, mammals	upper alimentary tract, stomach	Hodda, 2022; Anderson, 2000
Aproctoidea	eyes of small fish	birds	air sacs, nasal cavities, orbits, subcutaneous tissues of the head and neck	Hodda, 2022; Anderson and Bain, 1976; Dubinin, 1949
Camallanoidea	copepods, arthropods	marine, estuarine, and freshwater fishes	gut, deeper tissues, cavities	Ivashkin et al., 1971
Diplotriaenoidea	worms, such as *Diplotrema*, *Quadriplotriaena*	birds	air sacs, nasal cavities, subcutaneous tissues of the head and neck	Hodda, 2022; Anderson and Bain, 1976
Dracunculoidea	cyclopoid copepods	fishes, reptiles, birds, mammals, rarely in amphibians	under the skin	Hodda, 2022; Chabaud, 1975; Petter and Planelles, 1986
Filarioidea	tabanid fly, *Musca domestica*	all classes of vertebrates other than fishes (for example, horses, cattle)	body cavities, blood vessels, lymph vessels, connective tissues	Hodda, 2022; Soulsby, 1965; 1982; Anderson and Bain, 1976
Gnathostomatoidea	copepods	lower vertebrates, mammals	gastric mucosa	Hodda, 2022; Anderson, 2000
Habronematoidea	muscid dipterans	birds, mammals	proventriculus, stomach, causes cutaneous habronemiasis	Hodda, 2022; Anderson, 2000
Physalopteroidea	cockroaches	vertebrates, birds, reptiles,	lumen or wall of the stomach	De Lay and Blaxter, 2004; Cheng, 1973; Anderson, 2000
Rictularioidea	coprophagous insects	carnivores	free in the lumen or firmly attached to the mucosa of the intestine	Hodda, 2022; Witenberg, 1928
Spiruroidea	arthropods	vertebrates	lumen or the wall of the stomach	Hodda, 2022; Soulsby, 1981
Thelazioidea	ovoviviparous and oviparous, *Musca autumnalis*	birds, mammals (such as primates), fishes	eyeworms	Hodda, 2022; Anderson, 2000

Superfamily Acuarioidea

Acuarioidea tend to inhabit the upper alimentary tract or the muscles of the gizzard in birds, or may occur in the stomach of mammals, and are sometimes pathogenic. According to Anderson (2000), most adult acuarioids occur in the gizzard of birds, while a few species are found in both the proventriculus and in the posterior half of the esophagus. Most acuarioids occur in birds living in aquatic habitats and relatively few in birds associated with terrestrial habitats.

Acuarioids can move from one attachment site to another, leaving behind lesions devoid of worms. Cram (1931) reported openings through the gizzard lining associated with *Acuaria hamulosa*. Alicata (1938) found adult *A. hamulosa* mainly in tissues of the gizzard at its junction with the intestine. All acuarioids produce oval, smooth, thick-shelled eggs, each of which contains a small first-stage juvenile (J_1).

Hamann (1893) gave an account of how transmission probably occurs. Piana (1897) showed that *Dispharynx nasuta* of the proventriculus of gallinaceous and passerine birds developed in terrestrial isopods. Cram (1931; 1934), Cuvillier (1934), and Alicata (1938) conducted experiments to investigate the transmission and development of some acuarioids in terrestrial hosts and showed the importance of various insects and isopods as intermediate hosts. Garkavi (1956) investigated the development and transmission of *Streptocara crassicauda*.

Species of acuarioids in terrestrial hosts develop successfully in a great variety of arthropod intermediate hosts, including isopods, grasshoppers, beetles, and even diplopods. Acuarioids which parasitize aquatic hosts develop to the third stage in the haemocoel of aquatic crustaceans or in amphipods. Third-stage juveniles (J_3) vary considerably in morphology especially in the posterior quarter or fifth of the body, which is bent dorsally, and the tail is always armed with spines or tubercles. In other species the tail is unarmed and generally conical. Infections come after ingesting arthropods containing infective third-stage juveniles (J_3). In piscivorous birds, such as cormorants, transmission depends on frog and fish paratenic hosts (Anderson, 2000).

Superfamily Aproctoidea

Aproctoidea include parasites in air sacs, nasal cavities, subcutaneous tissues of the head and neck, and orbits of birds (Anderson and Bain, 1976). Their eggs are thick-shelled and include a fully developed first-stage juvenile (J_1). Little is known about the transmission of any of the species in the Aproctoidea (Anderson, 2000).

Superfamily Camallanoidea

Camallanoidea are parasites of the stomach and intestines of lower predaceous vertebrates (Chabaud, 1975) (see the chapter by Choudhury for a more in-depth summary of the Camallanoidea). Some of them occur in amphibians and reptiles, especially turtles (Baker, 1987), but also in marine, estuarine, and freshwater fishes (Ivashkin et al., 1971). One of the first demonstrations of heteroxeny in the Nemata was by Metchnikoff (1866) and Leuckart (1876) concerning the development of *Camallanus lacustris* of European fishes in copepods. Camallanoids are viviparous nematodes. Their intermediate hosts are crustaceans (Kupriyanova, 1954). Juveniles enter the haemocoel and develop into the third infective stage and they are armed with a few terminal spines. In the paratenic hosts (planktonivorous fishes) juveniles may grow to the fourth stage or become encapsulated in the tissues. Jackson and Tinsley (1998) found juveniles in aquatic toads (Pipidae) in Africa. The paratenic hosts move the juveniles in the food chain. The predator (piscivorous) definitive host can become infected by ingesting copepods or paratenic hosts with juveniles. Linstow (1909) described the juveniles of *Camallanus lacustris* in the isopod *Asellus aquaticus* and Fusco (1980) reported that some juveniles of *Spirocamallanus cricotus* developed successfully in white shrimp (*Penaeus setiferus*). Invasion of predaceous vertebrates is carried out after eating of intermediate or paratenic hosts which contain infective juveniles of camallanids (Anderson, 2000).

Superfamily Diplotriaenoidea

Diplotriaenoidea include spirurids in the air sacs of reptiles and birds (Anderson and Bain, 1976). Chabaud (1955) noted that eggs passed through the respiratory system and out via the feces. Anderson (1957) confirmed these observations experimentally. The female produces oval, smooth, thick-shelled eggs containing a fully developed first-stage juvenile (J_1). Eggs hatch in the gut of their intermediate hosts (grasshoppers and locusts). Their anterior end is surrounded by rows of spines and the tail tip is rounded and also encircled by a row of spines.

Superfamily Dracunculoidea

Dracunculoidea consist of Spiruridae species that occur in tissues and serous cavities mainly of fishes, reptiles, birds, mammals (Chabaud, 1975), and rarely amphibians (Petter and Planelles, 1986) (see the chapter by Choudhury for a more in-depth summary of the Dracunculoidea). According to Anderson (2000), after insemination, the female grows large numbers of first-stage juveniles (J_1). They must

be dispersed into the environment where an available copepod may be colonized; these serve as intermediate hosts. In many species, the fully gravid female must be immersed in fresh water, which causes her to burst, thus releasing the juveniles into the environment. The female elicits a skin lesion or migrates into the rectum and protrudes from the body of the host. In some species, juveniles released within the host make their way to the tissues, including the blood. Most dracunculoids occur in hosts which have contact with fresh water.

Superfamily Filarioidea

Filarioidea contain parasites of the tissues and tissue spaces of all classes of vertebrates other than fishes (Anderson and Bain, 1976) (see the chapter by Notarnicola for a more in-depth summary of the Filarioidea). They are all transmitted by haematophagous arthropods. Members of the Filariidae family cause skin lesions and release eggs and/or juveniles in the host. They attract arthropod vectors, mainly individuals in the Muscidae family. The cephalic structures are rather simple. Pseudolabia are absent, but in some groups there may be cuticular elevations or spines. The cephalic papillae are well developed. The buccal cavity usually is considerably reduced. Spicules are variable in length and dissimilar in morphology (Anderson, 2000). Anderson (1957) suggests that the specialized life cycles of onchocercids evolved from those of the orbit-inhabiting *Thelazia* and the subcutaneous filariids (*Filaria* and *Parafilaria*). Other authors have suggested a relationship between some onchocercids and habronematoids like *Draschia* and *Habronema* (Chandler et al., 1941; Bain, 1981).

Superfamily Gnathostomatoidea

Gnathostomatoidea constitute spirurid nematodes characterized by massive, complex pseudolabia, and often spinous cephalic inflations (Chabaud, 1975). They are parasites in gastric mucosa of turtles in eastern North America (Hedrick, 1935). Some species of *Gnathostoma* have been well studied because of their significance to human and animal health. Members of the Gnathostomatoidea separate eggs in an undeveloped state, embryonate to second-stage juveniles (J_2), and hatch in water. Intermediate hosts are copepods or insects of various crustaceans other than copepods (Anderson, 2000).

Superfamily Habronematoidea

Habronematoidea are nematodes with typical head structures. The pseudolabia are not large and median lips are present (Chabaud, 1975). It includes economically important and well-studied groups such as the tetramerids (including *Tetrameres* spp.) of the proventriculus of birds and noted for their peculiar sexual dimorphism, as well as the habronematids (including *Habronema*, *Draschia,* and *Parabronema*) which are transmitted by adult muscid dipterans to horses, certain ruminants, poultry, and other draft animals. They are localized in the stomach of horses and certain ruminants, including camels and elephants. Females, which occur in small tumors in the stomach wall, deposit oval, thin-shelled eggs. The latter usually hatch in the stomach releasing small, poorly differentiated juveniles with an anterior spine-like tooth. Juveniles pass out with the feces of the host. The superfamily also includes aberrant genera such as *Hedruris* (Anderson, 2000). In the United States, Ransom (1913) first discovered that the juveniles of horses developed in juveniles of muscid flies inhabiting nearby dung.

Superfamily Physalopteroidea

Physalopteroidea are parasites in the stomach and intestines of vertebrates. The mouth is encircled by large triangular lips having 1 or more teeth. A buccal capsule is absent. Males include a caudal alae. They usually meet ventrally in front of the cloaca and are supported by at least 4 papillae. The spicules are equal, subequal, or unequal. The female genital atrium is near then anterior or posterior half of body near the anus (Cheng, 1973).

Superfamily Rictularioidea

Rictularioidea consist of many species divided into several genera and subgenera (Quentin, 1969; Chabaud, 1975). They have no pseudolabia and have a denticulate, hexagonal oral opening and a sizeable buccal cavity with teeth. The presence of numerous large body spines is also diagnostic. The eggs are oval, with smooth, thick shells and each contains a fully developed first-stage juvenile (J_1). Eggs hatch in the gut of the insect intermediate host. These worms are parasites in the lumen of the intestine or firmly attached to the mucosa (ileum and in the region immediately posterior to its junction with the Malpighian tubules (Seureau, 1973). Witenberg (1928) fed young dogs the viscera of reptiles in which he had found rictularioid juveniles. The juvenile provokes the formation of a syncytium of epithelial cells which becomes surrounded by a fibrous capsule, which lies between the circular muscles and the epithelium of the ileum (Seureau, 1973).

Superfamily Spiruroidea

Spiruroidea include thelazioids, gnathostomatoids, habronematoids, rictularioids and physalopteroids (Chitwood and Chitwood, 1950). According to Chabaud (1975) the removal and elevation to superfamily status of several groups reduced Spiruroidea to 4 small families.

Spirurids are parasites in the stomach. They hatch thick-shelled eggs containing a fully differentiated first-stage juvenile (J_1) having a cephalic hook and rows of minute spines around the rather blunt anterior end. The tail of the first-stage juvenile (J_1) is often blunt and surrounded by a circlet of minute spines. **Paratenesis** is a common phenomenon in the transmission of spiruroids and the third-stage juveniles (J_3) of several species have been found in tissues of a variety of vertebrates which ingest infected insects, such as dung beetles. Third-stage juveniles (J_3) are generally large and possess some of the cephalic characteristics of adults. Their caudal extremities in some species possess terminal spines or tubercles, but in other species the terminal end is rounded and unornamented at the caudal extremity (Anderson, 2000).

Superfamily Thelazioidea

Thelazioidea consist of families united mainly on the basis of cephalic structures (Chabaud, 1975). The members of Thelazioidea are ovoviviparous and oviparous eyeworms of birds and mammals and the rhabdochonids (*Rhabdochona*) of fishes and primates. Intermediate hosts are different species of muscids.

Some Phylogenies and Molecular Characters

Within Spirurida, the superfamilies Habronematoidea and Thelazioidea are well established groups. Representatives of Cystidicolidae and Rhabdochonidae are widespread and show great diversity, especially in North America, but their phylogenetic relationships remain largely unexplored (Choudhury and Nadler, 2018). Choudhury and Nadler (2018) suggest that Hedruridae appears to be an early branching line of the spirurids.

Wu and colleagues (2008) explored the intra- and interspecific evolutionary variation among species of *Camallanus* collected from different fish species in various regions of China. Phylogenetic analyses of the nematodes suggested that there are 2 main clades, corresponding to different individuals of *C. cotti* and *C. hypophthalmichthys* from different fish species in various geographical locations, although the interior nodes of each clade received poor support.

Černotíková and colleagues (2011) have worked out the phylogenetic relationships of 38 orders, including many among the Spirurida (namely, Camallanidae, Cystidicolidae, Daniconematidae, Philometridae, Physalopteridae, Rhabdochonidae, and Skrjabillanidae) and some among the Ascaridida. The nematode species the authors examined are mostly parasites of marine and freshwater fishes from various locations in New Caledonia, as well as various locations in Africa, Asia, North America, South America, and Europe. Well supported trees allowing the study of phylogenetic relationships among some spirurine nematodes support the placement of Cucullanidae at the base of the suborder Spirurina, but the validity of the genera *Afrophilometra* and *Carranginema* is not supported. It is apparent that geographical isolation is not the cause of speciation in this parasite group and there is no evidence of coevolution with fish hosts (Černotíková et al., 2011).

Classification History

The specialized parasitology literature establishes multiple data which indicate the necessity for and the conduct of taxonomic revisions concerning the order Spirurida. Following are details of taxonomy, type species, and distribution of representatives from the order that were proposed by Gibbons (2010). They differ somewhat from previous views, for instance Chabaud (1975), Anderson (2000), and others, and Hodda's (2022) more recent treatment. The information is presented in a quasi tabular form with nominally narrative descriptions of the groups. Authority names are included and are not truncated. Some groups included here are covered additionally in other chapters within this book.

Order Spirurida Chitwood 1933

The genus *Spiroptera* Rudolphi, 1819 was created by Rudolphi (1819) and originally contained 30 species. The genus has been synonymized in part with *Acuaria* Bremser, 1811 and *Spirura* Blanchard, 1819, and many species assigned to this genus now extend to few genera, such as *Acuaria* Bremser, 1811, *Cosmocephalus* Molin, 1858, *Chevreuxia* Seurat, 1918, *Echinuria* Soloviev, 1912, *Habronema* Diesing, 1861, *Schistorophus* Railliet, 1916, *Sciadiocara* Skrjabin, 1916, *Seuratia* Skrjabin, 1916, *Spirura* Blanchard, 1819, and *Synhimantus* Railliet, Henry and Sisoff, 1912 (Yorke and Maplestone, 1926). The genus listed in Jones and Gibson (1987) is no longer considered valid.

The suborder **Camallanina** Chitwood, 1936 are parasites of the stomach and intestines of lower predaceous vertebrates (Chabaud, 1975). According to Gibbons (2010), Camallanina included the following superfamilies, families, subfamilies, genera, and subgenera: Superfamily **Camallanoidea** Travassos, 1920, with family **Camallanidae** and subfamily **Camallaninae** Railliet and Henry, 1915 constituting the next genera, subgenera, and type species: ***Camallanus*** Railliet and Henry, 1915 (= *Zeylanema* Yeh, 1960) (which is the type genus). These are parasites of fishes and amphibians (Chabaud, 1975), reptiles (Baker, 1987), and estuarine and freshwater fishes (Ivashkin et al., 1971). It includes subgenus ***Zeylanema*** (Yeh, 1960) Moravec and Scholz, 1991. The type species is *Camallanus (Zeylanema) anabantis* Pearse, 1933, which are parasites that live in the intestine of the freshwater

fish in the groups Anabantidae, Cyprinidae, Belontiidae, and Clariidae from India. ***Neocamallanus*** Ali, 1957, with type species *Neocamallanus singhi* Ali, 1957, are parasites that live in the intestinae of *Channa striata*, *Hampala dispar*, and *Xenetodon cancila* from Laos. ***Neoparacamallanus*** Bilqees and Akram, 1982, with the type species *Neoparacamallanus sweeti* (Moorthy, 1937) Bilqees and Akram, 1982, are parasites of freshwater fishes.

Another subfamily in Camallanidae is **Procamallaninae** Yeh, 1960. A list of the species in the subfamily has been presented by Petter (1979). The type genus is ***Procamallanus*** Baylis, 1923 and the type species is *Procamallanus laeviconchus* (Wedl, 1862) Railliet and Henry, 1915. They are parasites that live in the stomach and intestine of fishes and amphibians. The life cycle of *P. spiculogubernaculus*, a parasite of fishes, has been investigated by Sinha (1988). *Procamallanus* consist of several subgenera as follows: ***Denticamallanus*** Moravec and Thatcher, 1997, with type species *P. (Denticamallanus) dentatus* Moravec and Thatcher, 1997 are parasites that live in the intestine of characid fish *Bryconops alhurnoides,* from the Uburu River, Amazonas State, Brazil. ***Isospiculus*** Ali, 1957, including *P.* (*Spirocamallanus*) *hilarii* Vaz & Pereira, parasites that live in the intestines of *Acestrorhynchus microlepis* (unspecified), *Astyanax bimaculatus* (adult), *A. fasciatus* (adult), *A. parahybae* (adult), *Hoplias lacerdae* (adult), *H. malabaricus* (adult), *Oligosarcus macrolepis* (adult), *Rhamdia quelen* (adult), *Salminus hilarii* (adult), *Steindachnerina elegans* (adult and juvenile), *Trichomycterus piurae* (unspecified), all from Brazil (Luque et al., 2011). Moraveč and colleagues (2003) redescribed *P.* (*S.*) *fulvidraconis* from central China. ***Monospiculus*** Ali, 1957 is a genus with no designated type species. *?Procamallanus (Monospiculus) parasiluri* Fujita, 1927. ***Procamallanus*** (Baylis, 1923 genus) Ali, 1957 with no type species yet proven. *?Procamallanus (Procamallanus) laeviconchus* Baylis, 1923 is a spirurid species and are common parasites of African freshwater fishes. ***Punctocamallanus*** Moravec and Scholz, 1991, with type species *P. (Punctocamallanus) punctatus* Moravec and Scholz, 1991 are parasites that live in the stomach of freshwater fishes in Laos. ***Spirocamallanoides*** Moravec and Sey, 1988 have as a type species *P. (Spirocamallanoides) siluri* Osmanov, 1964. ***Spirocamallanus*** (Olsen, 1952 genus) Moravec and Sey, 1988, with type species *P. (Spirocamallanus) spiralis* (Baylis, 1923), are parasites that live in the intestine of fishes and amphibians. Other genera in the Procamallaninae are: ***Batrachocamallanus*** Jackson and Tinsley, 1995, with type species *Batrachocamallanus xenopodis* (Baylis, 1929) Jackson and Tinsley, 1995, are parasites of African amphibians, *Xenopus* spp. (Jackson and Tinsley, 1995). ***Malayocamallanus*** Jothy and Fernando, 1970, with type species *Malayocamallanus intermedius* Jothy and Fernando, 1970, are parasites in *Fluta alba,* in Malaysia (Jothy and Fernando, 1970). ***Onchocamallanus*** Petter, 1979 is a genus with type species *O. bagarii* (Karve and Naik, 1951) Petter, 1979. They are parasites that live in India in the intestine of *Bagarius bagarius* with an intermediate host of cyclopoid copepods *Mesocyclops leuckarti* and *M. crassus* (De and Maity, 1999). ***Platocamallanus*** Bilqees and Akram, 1982 with type species *P. mehrii* (Agrawal, 1930) Bilqees and Akram, 1982 are parasites of freshwater fishes.

Another subfamily in Camallanidae is **Paracamallaninae** Stromberg and Crites, 1974. Type genus is ***Paracamallanus*** Yorke and Maplestone, 1928 containing the subgenus ***Dentocamallanus*** Moravec and Scholz, 1991 with type species *P.* (*Dentocamallanus*) *sweeti* (Moorthy, 1937), which are parasites that live in teeth on the ribs of the buccal capsule of fishes.

Dracunculoidea is another superfamily in Camallanina. Representatives of this superfamily are parasites in tissues and serous cavities mainly of fishes, reptiles, birds, mammals (Chabaud, 1975), and sometimes in amphibians (Petter and Planelles, 1986). Intermediate hosts are copepods (Anderson, 2000). Dracunculoidea includes the genus ***Lockenloia*** Adamson and Caira, 1991, which is not assigned to a family, consists of parasites that live in the heart of sharks (*Ginglymostoma cirratum*), with type species *Lockenloia sanguineus* Adamson and Caira, 1991. According to Gibbons (2010), Dracunculoidea includes several families, subfamilies, genera, and subgenera, such as: **Dracunculidae** (Stiles, 1907 subfamily) Leiper, 1912 which are parasites of reptiles, birds, and mammals. The family includes several families, subfamilies, genera, and species, which, according to Gibbons (2010) are: ***Fuellebornius*** Leiper, 1926 with type species *F. medinensis* (Linnaeus, 1758) Leiper, 1926, which is a parasite of humans affecting the subcutaneous connective tissues, which then move to the surface of the skin, and provoke the formation of a blister, which bursts, causing the anterior end of the worm to be exposed.

Another family in Dracunculoidea is **Anguillicolidae** Yamaguti, 1935 which are parasites that live in the swimbladder of eels (Laetsch et al., 2012). This family has assigned to it the genus ***Anguillicola*** Yamaguti, 1935 with the subgenus ***Anguillicola*** (Yamaguti, 1935) Moravec and Taraschewski, 1988. The type species is *A. (Anguillicola) globiceps* Yamaguti, 1935. The final hosts are eels of the genus *Anguilla* and the intermediate hosts are planktonic copepods. Another genus in Anguillicolidae is ***Anguillicoloides*** Moravec and Taraschewski, 1988 (Moravec and Taraschewski, 1988) with type species *A. crassus* (Kuwahara, Niimi and Itagaki, 1974) Moravec and Taraschewski, 1988, which are parasites of the swimbladder of eels.

Another family in Dracunculoidea is **Skrjabillanidae** Shigin and Shigina, 1958. They are parasites generally that live in the peritoneal cavity of freshwater fishes. The occurrence of these nematodes in their final and intermediate host (*Argulus foliaceus*) in Hungary has been observed by Molnár and Szekely (1998). This family contains the subfamily **Skrjabillaninae** (Shigin and Shigina, 1958 family) Chabaud, 1965 with several genera, listed in the paragraphs below.

Kalmanmolnaria Sokolov, 2006 (= *Molnaria* Moravec, 1968) are parasites in the subcutaneous tissues of freshwater fishes (*Scardinius erythrophthalmus*) from Lake Balaton, Kis-Balaton, Fish Farms in Hungary. According to Anderson (2000), parasites of this genus can be found also in the serosa of the swimbladder, kidneys, and intestine, as well as on the mesentery of *S. erythrophthalmus* in CIS. Intermediate hosts are crustaceans.

Sinoichthyonema Wu, 1965 is a genus with type species *S. amuri* (Garkavi, 1972) Moravec, 1982. According to Zhokhov and Molodozhnikova (2008) *S. amuri* have been introduced into the Volga basin (Russia) occasionally during the process of introduction of fishes from the Amur River. This species has been also registered in Hungary by Molnár (1989). The systematic status of *S. itenopharyngodoni* Wu, 1973 was put forward by Moraveč (1982), including determining that this species is identical to *S. amuri*. Final hosts are *Rutilus rutilus* and *Scardinius erythrophthalmus*.

Garkavillanus Lomakin and Chernova, 1980 is a genus whose type species is *Garkavillanus amuri* (Garkavi, 1972) Lomakin and Chernova, 1980.

Another subfamily in Skrjabillanidae is **Esocineminae** Moravec, 2006 with type and only genus ***Esocinema*** Moravec, 1977 and with the type species *Esocinema bohemicum* Moravec, 1977, parasites that live under the serosa of the air bladder of pike *Esox lucius* in North Bohemia, Czechia.

Another family in Dracunculoidea is **Guyanemidae** Petter, 1975, which includes parasites that live in the peritoneal cavity and tissues of fish. This family includes the subfamilies, genera, and subgenera that are listed in the following paragraphs.

Several of the genera that are included in the subfamily **Guyaneminae** Petter, 1975 (Petter, 1975) include: ***Pseudodelphis*** Adamson and Roth, 1990, with the type species *P. oligocotti* Adamson and Roth, 1990. They are parasites that live in the peritoneal cavity and mesenteries surrounding the intestine of a marine fish species, *Oligocottus maculosus*, in coastal waters of British Columbia, Canada. Another genus is ***Histodytes*** Aragort et al., 2002, with the type species *H. microocellatus* Aragort et al., 2002, which are parasites in the gill, heart, kidney, spleen, and gonad tissues of the elasmobranch *Raja microocellata*. It was described based on material obtained from specimens from the continental shelf of the estuary of Muros y Noia, Spain (off the northwestern costs of the Iberian Peninsula) and is the only guyanemid genus described since the first was found on the European Atlantic coast (Aragort et al., 2002). ***Moravecia*** Ribu and Lester, 2004 are parasites that live in the gill filaments of green porcupine fish or may be found in the blood vessels and body cavity, with the type species *M. australiensis* Ribu and Lester, 2004. This genus was described based on materials obtained from *Tragulichthys jaculiferus* found in Moreton Bay, Queensland, Australia (Ribu and Lester, 2004). Another species of this genus is *Moravecia argentinensis* which was described by Braicovich and colleagues (2007) and are found in the blood vessels and body cavity of the Brazilian flathead, *Percophis brasiliensis*. This is the first species of the genus reported from South American waters.

Another subfamily in Guyanemidae is **Travassosneminae** Moravec, 2006 which according to Gibbons (2010) includes only 1 genus, ***Travassosnema*** De Araujo Costa, Mareira and De Oliveira, 1991, which has the type species *T. travassosi* De Araujo Costa, Moreira and De Oliveira, 1991. They are viviparous parasites that live in tissues behind the eyes of *Acestrorhynchus lacustris*, which may be found in the Tres Marias Reservoir, Mina Gerais State, Brazil. A subspecies, *T. t. paranaensis*, lives in the body cavity of the characid fish *Acestrorhynchus lacustris* from the Paraná River near Guaira in southern Brazil (Moraveč et al., 1993; Silva-Souza and Saraiva, 2002).

Another family of Dracunculoidea is **Philometridae** Baylis and Daubney, 1926, and includes the genus ***Afrophilometra*** Moravec, Charo-Karisa and Jirku, 2009. The type species is *A. hydrocyoni* (Fahmy, Mandour and El-Nafar, 1976) Moravec, Charo-Karisa and Jirku, 2009. The genus also includes species which parasitize *Hydrocynus forskahlii* from Lake Turkana, northwestern Kenya (Moraveč et al., 2009).

The subfamily **Philometrinae** (Baylis and Daubney, 1926) within the Philometridae includes the following genera: ***Icthyonema*** Diesing, 1861, with a type species that is not clearly determined, but it may be *I. fuscum* Diesing, 1861, parasites of the body cavity of marine fish (Gibbons, 2010). It also contains the genus ***Paraphilometroides*** Moravec and Shaharom-Harrison, 1989, with the type species *Paraphilometroides nemipteri* Moravec and Shaharom-Harrison, 1989. They are parasites found in the dorsal fin and operculum of the marine perciform fish *Nemipterus peronii,* from the coastal waters off Kuala Terengganu, Malaysia (Gibbons, 2010). Moraveč (2010) has imaged a gravid female of a paratype specimen of *P. nemipteri* using scanning electron microscopy after which he observed a unique cephalic structure, which clearly distinguishes *Paraphilometroides* from

other philometrids. Another genus is ***Margolisianum*** Blaylock and Overstreet, 1999, with the type species *M. bulbosum* Blaylock and Overstreet, 1999, which are found in the southern flounder *Paralichthys lethostigma* from Ocean Springs, Mississippi Sound, Mississippi, United States, and Galveston Bay, Texas, United States. During their maturation, these spirurids have a different localization. Immature females are parasites of the eye, while mature and gravid females can be found in the subcutaneous tissues of the mouth and head, and males may be found in the muscle adjacent to the dorsal fin just posterior to the head (Gibbons, 2010). Another genus is ***Dentiphilometra*** Moravec and Gui Tang Wang, 2002, with the type species *D. monopteri* Moravec and Gui Tang Wang, 2002. These are parasites found in the abdominal cavity of the ricefield eel, *Monopterus albus*, from Hubei Province in central China. This is the second philometrid species recorded from fishes of the Synbranchiformes (Moraveč and Wang, 2002). Another genus is ***Caranginema*** Moravec, Montoya-Mendoza and Salgado-Maldonado, 2008, with the type species *C. americanum* Moravec, Montoya-Mendoza and Salgado-Maldonado, 2008, which are parasites found in the subcutaneous tissue of the crevalle jack *Caranx hippos* from southern Gulf of Mexico. This is the seventh species of Philometrinae recorded from marine and brackish water fishes in Mexico (Moraveč et al., 2008).

Another subfamily in Philometridae is **Alineminae** Moravec, 2006, with the type genus ***Alinema*** Rasheed, 1963 (Gibbons, 2010).

Another subfamily in Philometridae is **Neophilometroidinae** Moravec, Salgado-Maldonado and Aguilar-Aguilar, 2002, with the type genus ***Neophilometroides*** Moravec, Salgado-Maldonado and Aguilar-Aguilar, 2002, whose type species is *N. caudatus* (Moravec, Schulz and Vivas-Rodriguez, 1995) Moravec, Salgado-Maldonado and Aguilar-Aguilar, 2002. These are parasites that live in the swimbladder of Neotropical freshwater catfish and the pimelodid catfish, *Rhamdia guatemalensis* from the Papaloapan River in Tlacotalpan, State of Veracruz, Mexico (Moraveč et al., 2002).

Another subfamily in Philometridae is **Phlyctainophorinae** (Roman, 1965) (Gibbons, 2010), including the genus ***Phlyctainophora*** Steiner, 1921. The type species is *P. lamnae* Steiner, 1921, which are parasites that live in the subcutaneous tissue of *Lamna nasus* from the North Atlantic Ocean. Jones and Delahunt (1995) found the same parasite species in tumor-like lesions on the tail fin and which provoke a chronic inflammatory response in the host, the dogfish *Squalus acanthias*. This is the first record for a member of the genus established in New Zealand and the first record of *Phlyctainophora* adults from the Southern Hemisphere. Another species in this genus is *P. squali*. Dwight, and Murrady, 1969 obtained from *Squalls acanthias* in eastern Pacific Ocean off Los Angeles, California, United States, at a depth of 200 m (Dwight and Murrady, 1969).

The family **Micropleuridae** (Baylis and Daubney, 1926) Travassos, 1960 in Dracunculoidea includes the subfamily **Micropleurinae** Baylis and Daubney, 1926, which are parasites found in fish, amphibians, and reptiles, and includes the following genera (Gibbons, 2010): ***Protenema*** Petter and Planelles, 1986, with the type species *P. longispicula* Petter and Planelles, 1986, which are parasites in the amphibians, *Necturus maculosus* (Proteidae), found in the lakes of Minnesota, United States. ***Granulinema*** Moravec and Little, 1988 with type species *G. carcharini* Moravec and Little, 1988, which are parasites of the bull shark, *Carcharhinus leucas,* found in Lake Borgne, Louisiana, United States. The site of localization in the host is unknown (probably the abdominal cavity). Another species in this genus is *G. simile* Moravec and Little, 1988, for which its localization is also unknown (Moraveč and Little, 1988). ***Kamegainema*** Hasegawa, Doi, Araki and Miyata, 2000, with the type species *K. cingulum* (Linstow, 1902) Hasegawa, Doi, Araki and Miyata, 2000, which are parasites that live in the subcutaneous tissue of amphibians (Hasegawa et al., 2000).

Another family in Dracunculoidea is **Daniconematidae** Moravec and Køie, 1987, which includes viviparous parasites of fish. The type genus is ***Daniconema*** Moravec and Køie, 1987 with the type species *D. anguillae* Moravec and Køie, 1987, which are parasites that live under the serosa of the swimbladder and intestine of eels, *Anguilla anguilla,* found in Lake Esrum, northern Zealand, Denmark. A new family Daniconematidae was established to accommodate it (Moraveč and Køie, 1987). According to Gibbons (2010), another genus in Daniconematidae is ***Mexiconema*** Moravec, Vidal and Salgado-Maldonado, 1992. The type species of this genus is *M. cichlasomae* Moravec, Vidal and Salgado-Maldonado, 1992, parasites that live in the abdominal cavity or viscera, or (rarely) the skin of cichlids, *Cichlasoma* spp., in the coastal lagoons of Celestun, North Yucatán, Mexico. They are parasites that live in the mesentery, swimbladder, liver, spleen, kidney, intestinal lumen, serosal cover of the intestine, or (rarely) in the skin of *Cichlasoma* spp. and other hosts, such as *C. helleri, C. motaguense*, and *C. pearsei* (Moravec, Vidal and Maldonado, 1992). Other habitats in Campeche, Mexico are El Vapor (a freshwater lagoon adjacent to Terminos Lagoon), Palizada, Santa Gertrudis, El Cayo (a saltwater portion within Terminos Lagoon), Pargos, and Rio Champoton. Habitats in Quintana Roo, Mexico include Rio Lagartos (a coastal lagoon) and Noh Bек (a lake). El Vapor, Palizada, Santa Gertrudis, Rio Lagartos, and Noh Bек are truly freshwater localities; all the remaining sites are saltwater or

marine localities (Moraveč et al., 1992). Another genus is ***Syngnathinema*** Moravec, Spangenberg and Frasca, 2001. The type species for this genus is *S. californiense* Moravec, Spangenberg and Frasca, 2001, which are parasites that live in the vascular system of the Bay pipefish, *Syngnathus leptorhynchus,* in California, United States. Based on histological studies, the parasites have been found also in other locations, such as in the circulatory system including the sinus venosus, atrium, and renal and hepatic veins.

Another family in Dracunculoidea is **Lucionematidae** Moravec, Molnar and Szekely, 1998, which are viviparous parasites of fish, with the type genus ***Lucionema*** Moravec, Molnar and Szekely, 1998, and with the type species *L. balatonense* Moravec, Molnar and Szekely, 1998. These are parasites that live in the swimbladder of the European pikeperch, *Stizostedion lucioperca* from Lake Balaton in Hungary (Moraveč et al., 1998).

Suborder Spirurina

Moraveč (2007) reviewed the spirurines of fish belonging to approximately 300 species in 4 superfamilies, namely Gnathostomatoidea, Habronematoidea, Physalopteroidea, and Thelazioidea. He has suggested that the classification and taxonomy of species of this suborder in fish requires reevaluation using new techniques, such as scanning electron microscopy and molecular biology (Moraveč, 2007).

In the superfamily **Acuarioidea**, Gibbons (2010) listed 1 family, namely, **Acuariidae** (Railliet, Henry and Sisoff, 1912), with 2 subfamilies: **Acuariinae** and **Schistorophinae**. Also according to Gibbons (2010), **Acquariidae** consists of 3 genera, namely: ***Deliria*** Vicente, Pinto and Noronha, 1980, parasites that live in the stomach of birds. *Pitangus sulphuratus* is found in Rio de Janeiro State, Brazil, of which the type species is *D. gomesae* Vincente, Pinto and Noronha, 1980. ***Paracuaria*** Rao, 1951 are parasites that live in the submucosa of crop in seabirds or the stomach of insectivorous mammals, with the type species being *Pa. adutica* (Creplin, 1946). ***Pseudoaviculariella*** Gupta and Kazim, 1978, are parasites that live in the gizzard of the cattle egret *Egretta garzetta* from Lucknow, India, with the type species *Ps. srivastavai* Gupta and Kazim, 1978. The family Acuariidae consists of 2 subfamilies. One is the **Acuariinae** Railliet, Henry and Sisoff, 1912, which includes the following 10 genera:

1) ***Antechiniella*** Quentin and Beveridge, 1986, parasites that live in Australian marsupials with the type species *A. suffodiax* (Beveridge and Barker, 1975) Quentin and Beveridge, 1986
2) ***Chandleronema*** Little and Ali, 1980, parasites that live in the stomach of raccoons, *Procyon lotor,* and muskrats from the United States and include the type species *C. longigutturata* (Chandler, 1942) Little and Ali, 1980
3) ***Cordonema*** Schmidt and Kuntz, 1972, parasites of birds with the type species *C. venusta* Schmidt and Kuntz, 1972
4) ***Molinacuaria*** Wong and Lankester, 1985, parasites that live under the gizzard lining of birds of the species *Dendragapus obscurus fuliginosus*, *Gallinula chloropus indica*, and *Alcippe brunnea brunnea* from Vancouver Island (Canada), China, and Taiwan, respectively. The type species is *M. bendelli* (Adams and Gibson, 1969) Wong and Lankester, 1985
5) ***Syncuaria*** Gilbert, 1927, parasites that live in the gizzard of birds (grebe, storks, and cormorants) with the type species *S. ciconiae* Gilbert, 1927
6) ***Tikusnema*** Hasegawa, Shiraishi and Rochman, 1992, parasites that live in the stomach and small intestine of the ricefield rat, *Rattus argentiventer,* in Indonesia, with the type species *T. javaense* Hasegawa, Shiraishi and Rochman, 1992
7) ***Voguracuaria*** Wong and Anderson, 1993, parasites that live in the esophagus of the whimbrel *Numenius phaeopus phaeopus* in Vogur, Iceland, with the type species *V. lankesteri* Wong and Anderson, 1993
8) ***Voguracuaria*** Wong and Anderson, 1993, parasites that live in the esophagus of the whimbrel, *Numenius phaeopus phaeopus,* in Vogur, Iceland, with the type species *V. lankesteri* Wong and Anderson, 1993
9) ***Willmottia*** Mawson, 1982, parasites of birds, *Malurus cyaneus*, from Tasmania, with the type species *W. australis* Mawson, 1982
10) ***Xenocordott*** Mawson, 1982, parasites that live in the gizzard of Australian birds, *Phylidonyris novaehollandiae* and *Gymnorhina tibicen*, with the type species *X. patonae* Mawson, 1982

The subfamily in Acquariidae is **Schistorophinae** Travassos, 1918, which according to Gibbons (2010) consists of 3 genera as follows: ***Quasithelazia*** Maplestone, 1932, ***Schistogendra*** Chabaud and Rousselot, 1956, and ***Sobolevicephalus*** Parukhin, 1964, parasites that live under the gizzard of birds, with the type species *So. chalycyonis* Parukhin, 1964. This genus was listed by Anderson and colleagues (2009) as synonym of *Hadjelia* Seurat, 1916.

The superfamily **Filarioidea**, according to Gibbons (2010), contains the genus ***Avifilaris*** Saunders, 1955, parasites that live in the blood of *Rhodothraupis, Passerina, Pitangus*, and *Empidonax*, with the type species *A. fringillidarum* Saunders, 1955. The family **Filariidae** (Weinland,

1858) Cobbold, 1879 represents a collective group for agamic forms named "***Agamofilaria***" Stiles, 1907. According to Gibbons (2010), Filariidae include a subfamily (**Filariinae** Weinland, 1858) and several genera, including: ***Cystofilaria*** Skrjabin and Shikhobalova, 1948, of which the adults may be found in cysts under the muscular layer of the esophagus in dogs and for which the type species is *C. balkanica* Skrjabin and Schikhobalova, 1948. ***Paracanthocheilonema*** Vladimirov, 1959 in Buliginskaya, Vladimirov and Markov, 1959 are parasites in *Rhombomys opimus, Meriones meridianus*, and *M. erytbrourus* found in the Kashkadarinsk region of Uzbekistan and whose type species is *P. vite* (Krepkogorskaya, 1933) Vladimirov, 1959 in Buliginskaya, Vladimirov and Markov, 1959.

Another family in the Filarioidea is family **Onchocercidae** (Leiper, 1911) which contains the subfamily **Onchocercinae** Leiper, 1911 and several genera (Gibbons, 2010), as follows: ***Bisbalia*** Bain and Guerrero, 2003, which are parasites found in the membranous pocket in the pleural cavity of *Heteromys anomalus* (Rodentia: Geomyoidea) in northern Venezuela and whose type species is *B. vossi* Bain and Guerrero, 2003. ***Cherylia*** Bain, Petit, Jacquet-Viallet and Houin, 1985, parasites of the ventral subcutaneous and perimuscular tissues of the South American marsupial, *Metachirops opossum,* found in French Guiana with the type species *Cherylia guyanensis* Bain, Petit, Jacques-Viallet and Houin, 1985. ***Cercopithifilaria*** (Eberhard, 1980 subgenus), parasites found in primates, ruminants, carnivores, marsupials, and monotremes. *Cercopithifilaria* are transmitted by ticks and the type species is *Cercopithifilaria kenyensis* Eberhard, 1980. ***Chabfilaria*** Bain, Purnomo and Dedet, 1983, parasites of Xenarthra in French Guiana and Guyana, with the type species *Chabfilaria jonathani* Bain, Purnomo and Dedet, 1983. ***Cruorifilaria*** Eberhard, Morales and Orihel, 1976, parasites that live in the renal and pulmonary blood vessels, and (rarely) the coronary vessels of the capybara *Hydrochoerus hydrochaeris* in Colombia with the type species *Cruorifilaria tuberocauda* Eberhard, Morales and Orihel, 1976. ***Dasypafilaria*** (Eberhard, 1982 subgenus), parasites that live in the omentum of Dasypodidae (including the 9-banded armadillo *Dasypus novemcinctus*) found in southern Louisiana, United States, with the type species *Dasypafilaria averyi* Eberhard, 1982. ***Josefilaria*** Moorhouse, Bain and Wolf, 1979, parasites of the ghost bat *Macroderma gigas* found in Australia, with the type species *Josefilaria mackerrasae* Moorhouse, Bain and Wolf, 1979. ***Loxodontofilaria*** Berghe and Gillain, 1939, parasites of elephants in Africa and Burma, Caprinae and Bovidae in Japan, and hippopotamus in Africa, with the type species *Loxodontofilaria loxodontis* Berghe and Gillain, 1939. ***Mansonella*** Faust, 1929, parasites that develop in the subcutaneous tissues of their hosts, and may be found in the Caribbean region, Central America, South America, and Africa, with the type species *Mansonella ozzardi* (Manson, 1897) Faust, 1929. According to Gibbons (2010), there are 6 subgenera as follows ***Cutifilaria*** (Bain and Schulz-Key, 1974 genus) Uni, Bain and Takaoka, 2004, parasites in Cervidae in Europe and Japan, with the type species *Mansonella (Cutifilaria) wenki* (Bain and Schulz-Key, 1974). ***Esslingeria*** (Chabaud and Bain, 1976) Eberhard and Orihel, 1984, parasites of humans, African anthropoid apes, and South American rodents, with the type species *Mansonella (Esslingeria) perstans* (Manson, 1891) Eberhard and Orihel, 1984. ***Mansonella*** (Faust, 1929) Eberhard and Orihel, 1984, parasites of humans, rodents, and carnivores, with the type species *Mansonella (Mansonella) ozzardi* (Manson, 1897) Faust, 1929. ***Sandnema*** (Chabaud and Bain, 1976) Eberhard and Orihel, 1984, parasites of Asian primates and insectivores, with the type species *Mansonella (Sandnema) digitata* (Chandler, 1929) Eberhard and Orihel, 1984. ***Tetrapetalonema*** (Faust, 1935) Eberhard and Orihel, 1984, parasites of platyrrhine primates, with the type species *Mansonella (Tetrapetalonema) marmosetae* (Faust, 1935) Eberhard and Orihel, 1984. ***Tupainema*** Eberhard and Orihel, 1984, parasites of tree shrews in Southeast Asia, with the type species *Mansonella (Tupainema) dunni* (Mullin and Orihel, 1972) Eberhard and Orihel, 1984. Other onchocercid genera according to the author are: ***Molossinema*** Georgi, Georgi, Jiang and Frongillo, 1987, parasites of the cerebral ventricular system of the bat *Molossus ater* in Trinidad, with the type species *Molossinema wimsatti* Georgi, Georgi, Jiang and Frongillo, 1987. ***Strianema*** Eberhard, Orihel and Campo-Aasen, 1993, parasites that live in the subcutaneous tissues of Venezuelan armadillos, *Dasypus* spp., with the type species *Strianema venezuelensis* Eberhard, Orihel and Campo-Aasen, 1993. ***Struthiofilaria*** Noda and Nagata, 1976, parasites which live in the body cavity of the ostrich *Sruthio camelus* found in in Misaki Park Zoo, Osaka Prefecture, Japan, with the type species *Struthiofilaria megalocephala* Noda and Nagata, 1976. ***Yatesia*** Bain, Baker and Chabaud, 1982, parasites that live in the skeletal muscle fascia of capybara *Hydrochoerus hydrochaeris* in Colombia, with the type species *Yatesia hydrochoerus* (Yates, 1980) Bain, Baker and Chabaud, 1982. Another subfamily within the Onchocercidae is **Waltonellinae** Bain and Prod'hon, 1974. According to Gibbons (2010) this subfamily includes several genera, as follows: ***Edesonfilaria*** Yeh, 1960. One of the species in this genus is *E. malayensis* which live in the subserosal connective tissues of the abdominal and thoracic cavities of cynomolgus monkeys (*Macaca fascicularis*) from Indonesia (Nonoyama et al. (1984). ***Foleyella*** Seurat, 1917, parasites that live in the subcutane-

ous and intermuscular connective tissues and body cavities in chameleonid reptiles, with the type species *Foleyella candezei* (Fraipont, 1882) Seurat, 1917. ***Foleyellides*** Caballero, 1935, parasites of anuran amphibians, mainly Ranidae, with the type species *Foleyellides striatus* (Ochoterena and Caballero, 1932) Caballero, 1935. ***Loaina*** Eberhard and Orihel, 1984, parasites of North American rabbits, with the type species *Loaina uniformis* (Price, 1957) Eberhard and Orihel, 1984. ***Ochoterenella*** Caballero, 1944, parasites that live in the body cavity of anuran amphibians, mainly Neotropical Bufonidae, with the type species *Ochoterenella digiticauda* Caballero, 1944. ***Paramadochotera*** Esslinger, 1986, parasites of *Mantidactylus redimitus,* a racophorid frog in Madagascar, with the type species *Paramadochotera guibei* (Bain and Prod'hon, 1974) Esslinger, 1986 Dirofilariinae Sandground, 1921. ***Pelecitus*** Railliet and Henry, 1910, parasites that live in the tendons, muscles, and (rarely) wings of birds and mammals, with the type species *Pelecitus helicinus* (Molin, 1860).

Another subfamily in Onchocercidae is **Splendidofilariinae** Chabaud and Choquet, 1953 which, according to Gibbons (2010), includes the following genera: ***Splendidofilaria*** Skrjabin, 1923, with 4 subgenera, as follows: ***Amfilaria*** Lopez Caballero and Jimenez Millan, 1979, with the type species *Splendidofilaria (Avifilaria) mavis* (Leiper, 1909) Anderson, 1961. ***Arteriofilaria*** Lopez Caballero and Jimenez Millan, 1979, with the type species *Splendidofilaria* (*Arteriofilaria*) *algonquinensis* (Anderson, 1955) Anderson, 1961. ***Soninella*** Lopez Caballero and Jimenez Millan, 1979, with the type species *Splendidofilaria* (*Soninella*) verrucosa Oschmarin, 1950 and ***Splendidofilaria*** (Skrjabin, 1923 genus) Lopez Caballero and Jimenez Millan, 1979, with the type species *Splendidofilaria* (*Splendidofilaria*) *pawloski* Skrjabin, 1923. Other genera in the Splendidofilariinae are: ***Andersonfilaria*** Bartlett and Bain, 1987, parasites that live in the fossa of the dorsal wall of the pelvic girdle of the common waxbill *Estrilda astrild* (Passeriformes) in Africa, with the type species *Andersonfilaria africanus* Bartlett and Bain, 1987. ***Dessetfilaria*** Bartlett and Bain, 1987, parasites that live in the capsule of the outer wall of the aorta in the heart of toucans in French Guiana and Brazil, with the type species *Dessetfilaria guianensis* Bartlett and Bain, 1987. ***Rumenfilaria*** Lankester and Snider, 1982, parasites in the subserosal connective tissue between the folds of the ruminal wall of moose *Alces alces* from northwestern Ontario, Canada, with the type species *Rumenfilaria andersoni* Lankester and Snider, 1982. ***Serofilaria*** Wu and Yun, 1979 (in Wu et al., 1979), parasites that live in the lymphatic vessels of the serous membrane covering the internal organs of pigs in China, with the type species *Serofilaria suis* Wu and Yun, 1979 (in Wu et al*.,* 1979). ***Splendidofilarioides*** Texeira de Freitas and Nicanor Ibafiez, 1968, parasites of the birds *Mimus longicaudatus* in Peru, with the type species *Splendidofilarioides pachacuteci* Texeira de Freitas and Nicanor Ibanez, 1968. ***Eulimdana*** Founikoff, 1934, parasites of birds, with the type species *Eulimdana clava* (Wedl, 1856). The last subfamily in Onchocercidae is **Lemdaninae** Lopez-Neyra, 1956q, which contains 2 genera, namely, ***Lemdana*** Seurat, 1917, parasites that live in the subcutaneous connective tissue of the head, neck in the vicinity of the trachea, the esophagus, and crop of birds, with the type species *Lemdana marthae* Seurat, 1917. ***Makifilaria*** Krishnasamy, Singh and Iyamperumal, 1981, parasites that live in the peritoneal cavity of the island flying fox *Pteropus hypomelanus* found in Pulau Langkawi, Malaysia, with the type species *Makifilaria inderi* Krishnasamy, Singh and Iyamperumal, 1981.

In the superfamily **Aproctoidea**, Gibbons (2010) listed the following taxa: The family **Aproctidae** (Yorke and Maplestone, 1926 subfamily) Skrjabin and Shikhobalova, 1945, with 1 genus, ***Hovorkonema*** Jurasek, 1977, parasites that live in the stomach of the Carpathian wild boar *Sus scrofa atilla* in Lucenec, Slovakia, with the type species *Hovorkonema gastrofilana* Jurasek, 1977. The subfamily **Aproctinae** Yorke and Maplestone, 1926, with the genus ***Desmidocercella*** Yorke and Maplestone, 1926 and the type species *Desmidocercella (Desmidocercella) numidica* (Seurat, 1920), including the subgenus ***Skrjabinocercella*** Gushanskaya, 1953, with the type species *Desmidocercella (Skrjabinocercella) incognita* Solonitzin, 1932. Furthermore, Gibbons (2010) listed in Aproctidae 4 other genera, as follows: ***Lissonema*** Linstow, 1903, parasites that live in the abdominal cavity of *Otus sunia* from eastern Asia, with the type species *Lissonema rotunda* Linstow 1903. ***Parasaurositus*** Gupta and Johri, 1989, parasites that live in the intrahepatic spaces of the Indian soft shell turtle *Aspideretes gangeticus* found in India, with the type species *Parasaurositus yamagutii* Gupta and Johri, 1989. ***Pseudodiomedenema*** Gupta and Johri, 1988, parasites of the pleural cavity of hoopoe *Upupa epops* found in Lucknow, India, with the type species *Pseudodiomedenema cameroni* Gupta and Johri, 1988 and ***Squatnoftlaria*** Schmerling, 1925.

According to Gibbons (2010), the superfamily **Diplotriaenoidea** includes the family **Diplotriaenidae** (Skjrabin, 1916 subfamily) Anderson, 1958 and the superfamily **Diplotriaeninae** Skrjabin, 1916, with 2 genera, namely: ***Spinodiplotriaena*** Kalyankar and Pallawadar, 1989, parasites that live in the body cavity of the common mynah bird. *Acridotheres tristis* in India, with the type species *Spinodiplotriaena urmilii* Kalyankar and Pallawadar, 1989. ***Versternema*** Bain, Chabaud and Burger, 1992, parasites that live in the body cavity of the ostrich *Struthio camelus* in Botswana, with the type species *Vesternema struthionis* Bain, Chabaud and Burger, 1992.

According to Gibbons (2010), the superfamily **Gnathostomatoidea** comprises the family **Gnathostomatidae** Railliet, 1895, and the subfamily **Ancyracanthinae** Yorke and Maplestone, 1926, with 2 genera, namely: ***Elaphocephalus*** Molin, 1860, parasites that live in the feet of birds *Psittacus macao* with the type species *Elaphocephalus octocornutus* Molin, 1860. The other genus is ***Metaleptus*** Machida, Ogawa and Okiyama, 1982. *Metaleptus rabuka*, parasites that live in the stomach of *Mustelus griseus*, and *M. manazo*, which have been recorded by Moraveč and Nagasawa (2000) in the north Pacific Ocean off Honshu, Japan.

In the superfamily **Habronematoidea**, Gibbons (2010) lists the family **Habronematidae** (Chitwood and Wehr, 1932 subfamily) Ivaschkin, 1961, the subfamily **Habronematinae** Chitwood and Wehr, 1932, and the genus ***Dermofilaria*** Rivolta, 1884, parasites of equids and bovines, with the type species *Dermofilaria irritans* Rivolta, 1884. Furthermore, Habronematidae includes the subfamily **Histiocephalinae** Gendre, 1922, with the genus ***Sobolevicephalus*** Parukhin, 1964 having as the type species *Sobolevicephalus chalcyonis* Parukhin, 1964. It also includes the family **Tetrameridae** Travassos, 1914, with the subfamily **Tetramerinae** (Travassos, 1914), which contains the following genera: ***Acanthophorus*** von Linstow, 1876, which has been accepted as a synonym of *Tetrameres*. ***Ascarophis*** van Beneden, 1871, parasites that live in the gastrointestinal tract of marine fish, with the type species *Ascarophis morrhuae* Beneden, 1871 (Gibbons, 2010). Intermediate hosts are decapods (*Enalus gaimardi*, *Eupagurus pubescens*, *Hetairus polaris*, *Pagurus pubescens*, *Pandalus borealis*, and *Spirontocaris spinus*) from the Bering Sea (Uspenskaya, 1953; 1954), lobster (*Homarus americana*) in North America (Uzmann, 1967), crab, *Carcinus maenas*, from off the coast of Brittany in France (Petter, 1970), crustaceans (*Anisogammarus kygi*, *A. ochotensis*, *A. tiuschovi*, *Idothea ochotensis*, and *Pagurus middendorffii*) from the littoral zone of Big Shantar Island in the Okhotsk Sea (Tsimbalyuk et al., 1970), shore crabs (*Hemigrapsus oregonensis*), porcelain crabs (*Pachycheles rudis*) in California, United States (Poinar and Kuris, 1975), and *Callianassa californiensis*, *Pagurus samuelis*, *P. granosimanus*, *Pachycheles pubescens*, and *Pugettia producta* (Poinar and Thomas, 1976). Moraveč et al. (1995) described *Ascarophis mexicana* from the stomach of *Epinephelus morio* and *E. adscensionis* from the Gulf of Mexico and southeastern Mexico in the states of Yucatán and Veracruz. According to the authors, *Ascarophis mexicana* is the second *Ascarophis* species known to parasitize fishes of the genus *Epinephelus* (Moravec et al., 1995). ***Caballeronema*** Margolis, 1977, parasites that live in the alimentary canal of the marine fish*, Scorpaenichthys marmoratus,* found off the Pacific coast of Canada, with the type species *Caballeronema wardlei* (Smedley, 1934) Margolis, 1977. ***Capillospirura*** Skrjabin, 1924, parasites of the digestive tract of sturgeons, with the type species *Capillospirura ovotrichuria* Skrjabin, 1924 (Gibbons, 2010). Based on the characteristics of the cephalic structure of specimens from Old World sturgeons, *Capillospirura* Skrjabin, 1924 (Nematoda: Cystidicolidae) has been redefined.

Three species have been assigned by Appy and Dadswell (1978) to *Capillospirura*, specifically: *C. ovotrichuria* Skrjabin, 1924 and *C. argumentosa* (Skrjabina, 1966) (= *Ascarophis argumentosus*) from Old World sturgeons and *C. pseudoargumentosa* (= *Caballeronema pseudoargumentosus*) from a New World sturgeon, as has been suggested by Appy and Anderson (1982). ***Comephoronema*** Layman, 1933, parasites that live in the alimentary tract of freshwater fish, with the type species *Comephoronema werestschagini* Layman, 1933. Pereira and colleagues (1993) have described *Comephoronema multipapillatum* from the anterior intestine and cecum of the squirrelfish, *Holocentrus adscensionis*. According to the authors (Pereira et al., 2014), this is the fifth nominal species of *Comephoronema* and the first nematode registered in *H. adscensionis* and the first species of the genus in the Neotropical part of the Atlantic Ocean. ***Crenatobronema*** Solov'eva, 1987, parasites in fish from the Pacific Ocean, with the type species *Crenatobronema guentheri* (Baylis, 1929) Solov'eva, 1987. In his review concerning to the suborder Spirurina, Moraveč (2007) considers this genus "inadequately known." ***Cystidicoloides*** Skinker, 1931, parasites of South American freshwater fish, with the type species *Cystidicoloides fischeri* (Travassos, Artigas and Pereira, 1928) Skinker, 1931.

Moraveč et al. (2008) have redescribed *Cystidicoloides fischeri* (Travassos, Artigas and Pereira, 1928) noting the localization in the stomach of *Pygocentrus piraya* and *Serrasalmus brandtii* from Três Marias Reservoir, Upper São Francisco River, Minas Gerais state, Brazil. Based on morphological features, the authors (Moraveč et al., 2008) accomplished several taxonomic transformations, such as: *Heliconema izecksohni* Fabio, 1982 is transferred to *Cystidicoloides* as *C. izecksohni* (Fabio, 1982). *Cystidicoloides uniseriata* Valovaya and Valter, 1988 is considered a species inquirenda. It has been proposed as a newly erected genus, *Salmonema*, with the type species *S. ephemeridarum*. *Cystidicoloides prevosti* (Choquette, 1951) has been transferred to *Salmonema* as *S. prevosti* (Choquette, 1951). *Sterliadochona savini* Skryabin, 1948 and *Sterliadochona* Skryabin, 1948 are considered as species and genus inquirenda, respectively. ***Echinurioides*** Thwaite, 1926 are parasites of the spurwinged goose, *Plectropterus* sp., in northern Nigeria, with the type species *Echinurioides plectropteri* Thwaite, 1926 (Gibbons,

2010). Skrjabin and Sobolev (1963) list *Echinurioides* as a synonym of *Tetrameres* Creplin, 1846. Alexander and McLaughlin (1997) report the type species as *Tetrameres plectropteri* (Thwaite, 1926), with host *Plectropterus gambensis* in Nigeria. ***Gubernaculomeres*** Oshmarin and Parukhin, 1963 are parasites that live in the proventriculus of *Astur gentilis* and *Aquila clanga*, with the type species *Gubernaculomeres tubocloacis* (Oshmarin, 1956) Oshmarin and Parukhin, 1963 (Gibbons, 2010). ***Moravecnema*** Justine, Cassone and Petter, 2002 is considered to be a parasite of the deep sea hydrothermal fish *Pachycara thermophilum* from the Mid-Atlantic Ridge, with the type species *Moravecnema segonzaci* Justine, Cassone and Petter, 2002 (Gibbons, 2010). This is the first species of parasitic nematode described from a fish endemic to hydrothermal deep sea vents.

The genus ***Prospinitectus*** Petter, 1979 are parasites that live in the intestine of the fish, *Euthynnus affirtis,* off Kuala Lumpur, Malaysia and in the China Sea, with the type species *Prospinitectus mollis* (Mameav, 1968) Petter, 1979 (Gibbons, 2010). The genus ***Pseudascarophis*** Ko, Margolis and Machida, 1985 are parasites that live in stomach of the fish, *Kyphosus cinerascens,* from off the southeastern coast of Japan, with the type species *Pseudascarophis kyphosi* Ko, Margolis and Machida, 1985 (Gibbons, 2010).

Pereira and colleagues (2013) described *Pseudascarophis brasiliensis* found in the stomach of *Kyphosus sectatrix* from off Rio de Janeiro, southeastern Brazil. The genus ***Salmonema*** Moravec, Santos, Brasil-Sato, 2008 are parasites that live in the digestive tract of freshwater fish, with the type species *Salmonema ephemeridarum* (Linstow, 1872) Moravec, Santos, Brasil-Sato, 2008 (Gibbons, 2010). ***Similascarophis*** Munoz, Gonzalez and George-Nascimento, 2004 are parasites of the digestive tract of marine fish off the Chilean coast, with the type species *Similascarophis maulensis* Munoz, Gonzalez and George-Nascimento, 2004 (Gibbons, 2010). Also included is the genus ***Sterliadochona*** Skrjabin, 1948.

A number of genera were discovered in the mid-1900s, namely, *Cristitectus* Petter, 1970, *Salvelinema* Trofimenko, 1962, *Ctenascarophis* Mamaev, 1968 and 1967 Petter, 1969. Rasheed (1965) and Moraveč (1967) have synonymized *Sterliadochona* Skrjabin, 1946 with *Cystidicoloides.* Characters used by Maggenti and Paxman (1971) to re-establish 2 genera have no generic value for nematode parasites of vertebrates (Anderson et al., 2009). The genus ***Tetrameres*** Creplin, 1846 is remarkable for the fact that the mature female is almost spherical in shape, blood-red in color, and lies embedded in the proventricular glands of birds. There are many species in this genus, among which are: *T. americana* Cram, 1927, which occurs in the proventriculus of fowl and turkeys. The final hosts of *T. americana* are the grasshoppers *Scyllina cyanipes* in Puerto Rico and *Melanoplus femurrubrum* and *M. differentialis* in mainland United States, and have been recorded elsewhere from the United States and in South Africa. Intermediate hosts are *M. femurrubrum, M. differentialis*, and *Blatella germanzca. Tetrameres fissispina* (Diesing, 1861) occurs in the duck, pigeon, fowl, turkey, and wild aquatic birds, and has a wide distribution. Intermediate hosts for *T. fissispina* are the water crustacean *Daphnia pulex* and *Gammarus pulex*. *Tetrameres crami* Swales, 1933 occurs in domestic and wild ducks in North America. Its intermediate hosts are the amphipods *G. fasciatus* and *Hvalella knickerbockeri*. *Tetrameres confusa* Travassos, 1919 occurs in the proventriculus of fowl pigeon and other birds in Brazil. Its intermediate hosts are probably similar to those for *T. fissispina*. *Tetrameres mohtedai* Bahlerao and Rao, 1944 occurs in fowl in India and Southeast Asia. Its intermediate hosts are cockroaches and grasshoppers, such as *Spathosternum prasznferum* and *Oxya nitidula*. *Tetrameres pattersoni* (Cram, 1933) occurs in quail, and the intermediate hosts are grasshoppers and cockroaches (Soulsby, 1982). *Tetrameres cardinalis* Quentin and Barre, 1976 has been found in the northern cardinal (*Cardinalis cardinalis* (syn. *Richmondia cardinalis*) in Mexico; its development occurs in *Locusta migratoria*. The intermediate hosts for *T. pattersoni* Cram, 1933 are *Chortophaga viridifasciata* and *Melanoplus femurrubrum* and its final host is *Colinus virginianus* (Anderson, 2000). According to Junker and Boomker (2007), the genus *Tetrameres* also includes *T. coccinea* (Seurat, 1914) Travassos, 1914 from the *Phoenicopterus ruber*, *Bubulcus ibis*, and *Platalea leucorodia* Linnaeus, 1758. *Tetrameres lhuillieri* (Seurat, 1918) is found in *Alectoris graeca* (Meisner, 1804) and *Columba oenas* Linnaeus, 1758 from Algeria. *Tetrameres nouveli* (Seurat, 1914) Travassos, 1914 is found in the black winged stilt, *Himantopus himantopus* (Linnaeus, 1758) in Algeria and Nigeria. *Tetrameres plectropteri* Thwaite 1926 is found in *Plectropterus gambensis*. Both *T. paradisea* Ortlepp, 1932 and *T. prozeskyi* (Ortlepp, 1964) have been described from South African hosts. *Tetrameres paradisea* has been recovered from *Anthropoides paradisea* (Lichtenstein, 1793). *Tetrameres prozeskyi* occurs in *Tockus erythrorhynchus* and *T. leucomelas*. The authors described that and *T. numida* Junker and Boomker, 2007 in *Numida meleagris* from Musina (Messina), Limpopo Province, South Africa.

The superfamily **Physalopteroidea** includes the family **Physalopteridae** (Railliet, 1893 subfamily) Leiper, 1908. According to Gibbons (2010), parasites of the alimentary canal (as well as the esophageal, gastric, or aortic walls) of the selachian *Chlamydoselachus anguineus* from the Pacific coast of central Honshu, Japan, with the type species *Metaleptus rabuka* Machida, Ogawa and Okiyama, 1982.

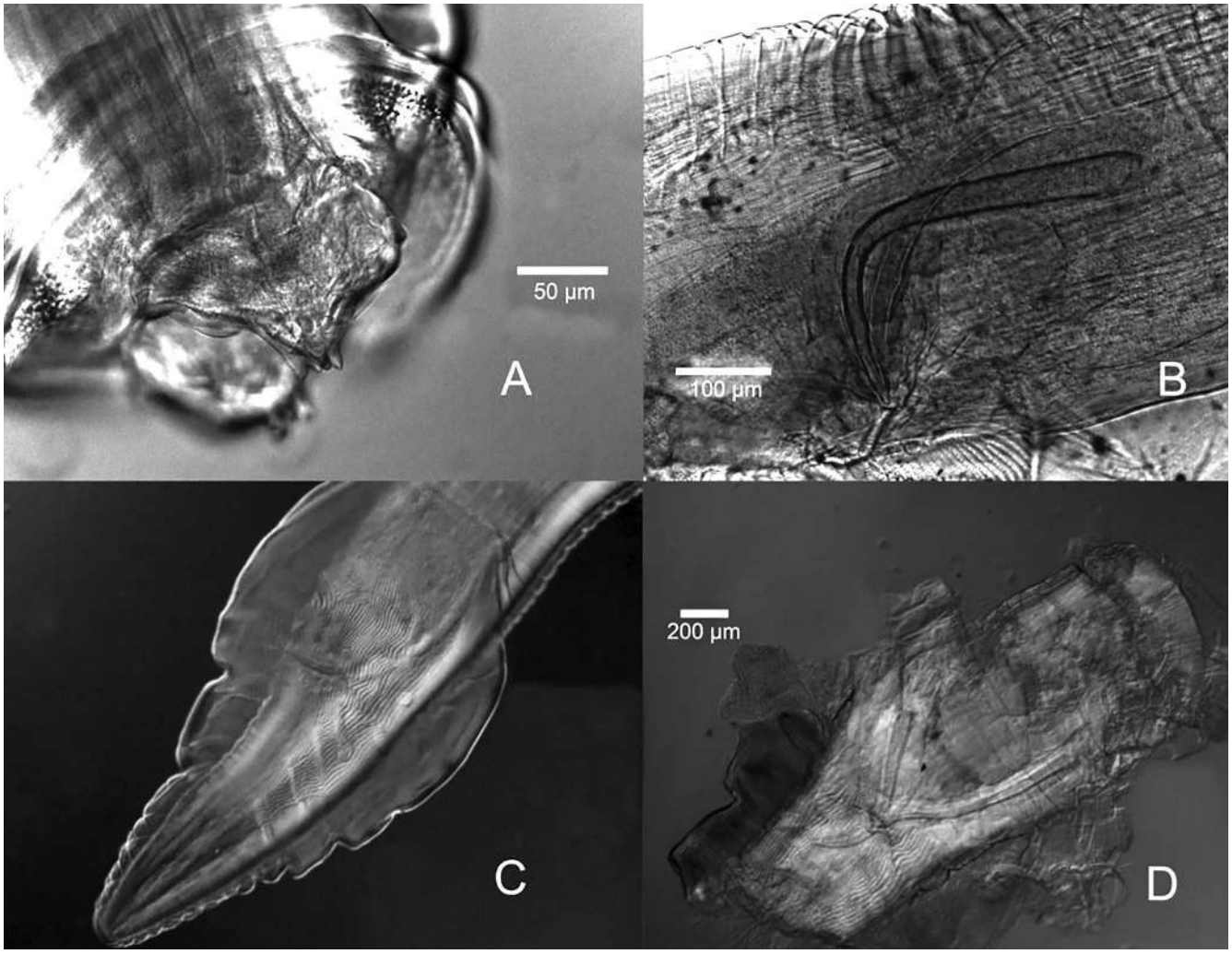

Figure 4. A) Anterior end of *Physaloptera rara* (Nemata: Spirurida: Physalopteridae) from a domestic dog obtained from Iowa, United States. Note the 2 large lips each with 3 small anteriorly-directed teeth (which is typical of *Physaloptera* spp.); B) lateroventral view of the cloacal area showing spicules of *P. rara* from a bobcat in Nebraska, United States; C) ventral view of rays and associated velum of posterior end of *P. rara* from Iowa, United States. Note that the scale bar is the same for both C and D; D) ventral view of a dissected specimen of *P. rara*. Note that this is the same individual as is shown intact in figure C. Source: S. L. Gardner, HWML. License: CC BY.

The subfamily **Physalopterinae** Railliet, 1893 includes the genus ***Kreisiella*** Jones, 1985. According to Gibbons (2010), members of this genus live in the stomach of the Australian lizard *Egernia inornate,* with the type species *Kreisiella chrysocampa* Jones, 1985. Goldberg and colleagues (2008) report finding *Kreisiella chrysocampa* in *Emoia* (Scincidae) from Papua New Guinea. The type species for ***Leptosoma*** Travassos, 1920 is *L. leptosoma* (Gervais, 1848), the adult worms of which live in the stomach or intestine of mammals, birds, reptiles, and amphibians. The genus ***Paraphysaloptera*** Gupta and Kazim, 1979 are parasites of the gizzard lining of the hoopoe *Upupa epops*, with the type species *Paraphysaloptera alii* (Gupta and Kazim, 1978) Gupta and Kazim, 1979. According to Martín-Vivaldi and colleagues (2014), other phisalopterid species in the same final host are *P. indica*, found in the intestine (Gupta and Johri, 1985) and *P. alii*, found in the gizzard (Gupta and Kazim, 1979). Widmer (1970) experimentally infected cats using third-stage *Physaloptera* juveniles (J_3) from the rattlesnake *Crotalus viridis*.

The genus *Paraphysaloptera* possesses 2 subgenera. One subgenus is ***Chlamydonema*** (Hegt, 1910 genus) Gupta and Johri, 1987, with the type species *Physaloptera* (*C.*) *praeputiale* (Linstow, 1888) Travassos, 1917. They are parasites that live in the stomach of *Canis latrans*, *Felis catus domesticus*, *F. pardus*, and *C. familiaris* and are found in Asia, Africa, Europe, North America, and South America. Linstow (1888) described *P. praeputialis* from a wild cat (*F. catus*) from Brazil. Later, Walton (1927) assigned this specimen to the group Mammal.

This is probably the first record of *P. praeputialis* in North America. In the stomach of lynx (*Lynx rufus texensis*) and ocelot (*F. pardalis*) from Mexico the same species has been reported also by Caballero y Caballero and Peregrina (1938).

The other subgenus of *Paraphysaloptera* is ***Physaloptera*** (Rudolphi, 1819 genus) Gupta and John, 1987, with the type genus *Physaloptera* Rudolphi, 1819. *Physaloptera* are common nematodes found in the stomach and muscles of mammals (such as dogs, cats, and humans), reptiles, amphibians, and birds. Physalopterids attach to the walls of the duodenum and stomach (Naem and Asadi, 2013) and are known to have pathological consequences such as catarrhal gastritis, gastrointestinal upset, erosion of the mucosa, ulcers, and vomiting (Soulsby, 1965).

Physaloptera spp. have a complicated life cycle. They have numerous definitive hosts. Intermediate hosts are arthropods, specifically, ground beetles (*Harpalus* spp.) and crickets (*Achetaassimilis* spp.) (Widmer, 1967). Aberrant infections occur at times, and there are possibly second intermediate hosts or paratenic hosts. For example, *Physaloptera* spp. juveniles have been found within the tissues of wild northern bobwhite quail *Colinus virginianus* and it is suspected that quail may serve as paratenic or secondary hosts of these parasites (Kalyanasundaram et al., 2018). Widmer (1970) identified all rodents as potential paratenic hosts for physalopterids. Olsen (1980) used juveniles from rattlesnakes to infect cats. Baughn and Bliznick (1954) found physalopterids in cats in New York, United States. Ackert (1936) and Ackert and Furumoto (1949) found *Physaloptera* spp. in cats in Kansas, United States. In particular, Shoop and colleagues (1991) reported *P. rara* from cats in Arkansas, United States. Marchiondo and Sawyer (1978) recovered *P.* (*Physaloptera*) *clausa* Rudolphi, 1819 specimens from cats in Utah, United States. Using scanning electron microscopy, Chen and colleagues (2017) studied *P. clausa* obtained from the Amur hedgehog *Erinaceus amurensis* in China. Supplementary data on morphological and morphometric characters have been obtained through these additional studies which allows more accurate identification of these species.

Another genus in the Physalopterinae is ***Skrjabinoptera*** Shulz, 1927, which is found in reptiles. According to Anderson (2000), *S. phrynosoma* (Ortlepp, 1922) is a common stomach worm of reptiles that live in Texas, United States, as well as horned toads *Phrynosoma cornutum*. According to Lee (1957), the intermediate hosts are the ants *Pogonomyrmex barbatus* var. *molefaciens*.

Another subfamily in Physalopteridae is **Proleptinae** (Schulz, 1927), including the genus ***Neoleptus*** Ubelaker and Dailey, 1975. According to Specian and colleagues (1975), *Neoleptus* spp. are parasites found in the fish *Heterodontus philippi* and *Mustelus antarticus*, with the type species *Neoleptus australis* (Johnston and Mawson, 1943) Specian, Ubelaker and Dailey, 1975.

According to Gibbons (2010), another subfamily in Physalopteridae is **Mirzalopterinae** Wason and Johnson, 1977, with the type genus ***Mirzaloptera*** Wason and Johnson, 1977. They are parasites that live in the stomach of the bat *Rhinopoma microphyllum* in Jodhpur, India. The type species is *Mirzaloptera barbari* Watson and Johnson, 1977.

Another family in Physalopteroidea is **Rictulariidae** (Hall, 1915 subfamily) Railliet, 1916, and which contains 2 genera. One genus is ***Quentius*** Chabaud and Bain, 1981, which are parasites that live in the duodenum and small intestine of Neotropical marsupials (*Marmosa* spp.) in Cali, Colombia (Chabaud and Bain, 1981). The type species is *Q. kozeki* Chabaud and Bain, 1981. The other genus is ***Shamimana*** Gupta and Masoodi, 1990, which includes parasites that live in the intestine of the marine fish *Plotosus arab* off the Trivandrum coast near Kerala, India. The type species is *Shamimana durdanae* Gupta and Masoodi, 1990.

The superfamily **Spiruroidea** combines the family **Spiruridae** Oerley, 1885, which, according to Gibbons (2010), contains 4 genera.

1) ***Gastronodus*** Singh, 1934, parasites that live in nodules on the stomach wall of the muskrat *Crocidura coerulea* in Hyderabad State, India. The type species is *Gastronodus strasseni* Singh, 1934
2) ***Dollfusnema*** Caballero, 1974, parasites that live in the intestine of the marine fish *Paralabrax clathratus* from Mexico. The type species is *Dollfusnema piscicola* Caballero, 1974
3) ***Isospirura*** Sood and Parshad, 1972, parasites that live in the stomach of *Millardia meltada, Mus musculus bactrianes*, and *Mus booduga* in Ludhiana, India. The type species is *Isospirura meltadi* Sood and Parshad, 1972
4) ***Paracymea*** Gupta and Jaiswal, 1987, parasites that live in the intestine of the birds *Anser indicus* in the Prince of Wales Zoological Gardens, Lucknow, India. The type species is *Paracymea yamagutii* Gupta and Jaiswal, 1987.

Another family in this superfamily is **Gongylonematidae** (Hall, 1916 subfamily) Sobolev, 1949, which, according to Gibbons (2010), contains several genera. ***Gongylonema*** Molin, 1857 embeds in the mucosa and submucosa of the anterior region of the gut of birds and mammals. Usually, the final hosts are sheep and goats, and sometimes also horses, cattle, swine, poultry, dogs, cats, and numerous other wild and domestic mammals and birds. As such, according to

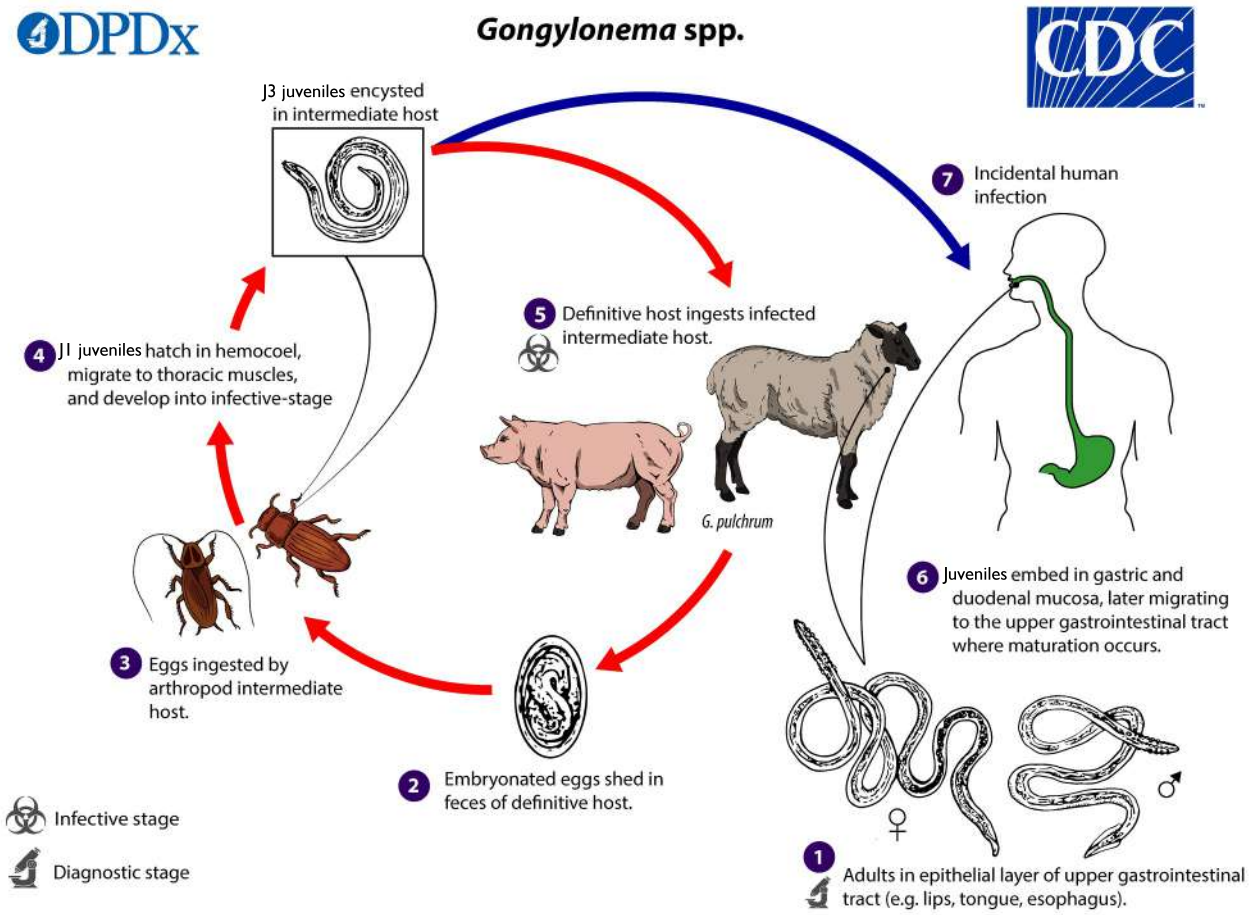

Figure 5. *Gongylonema* is a genus of spirurid nematodes which includes the veterinary parasite *G. pulchrum* (also called the gullet worm or stitch worm) along with several other parasites of mammals and birds. Incidental human infections with *Gongylonema* are rare, and species-level identifications are difficult and seldom confirmed. The life cycle diagram of *Gongylonema* spp. shows: Adult *Gongylonema* inhabit the upper gastrointestinal tract of the definitive host in sites such as the mouth, esophagus, rumen, and stomach (1). The long, thin adults are found in shallow tunnels in the squamous epithelial surfaces of these tissues; the female produces thick-shelled, embryonated eggs containing first-stage (J_1) juveniles. Expelled eggs are released from the tunnels during epithelial desquamation and are carried down the gastrointestinal tract and shed in the feces (2). Intermediate host insects become infected after ingesting eggs in host feces (3). Juveniles develop in the hemocoel of the intermediate host, eventually becoming encapsulated as infective third-stage (J_3) juveniles in the thoracic muscles (4). Suitable definitive hosts become infected after ingesting infected intermediate hosts (5). Juveniles are released in the stomach, which embed in the gastric or duodenal mucosa, and eventually migrate to the upper gastrointestinal tract after 2–3 months (6). Migration of juveniles often creates characteristic zig-zag or sinusoidal tracks in the affected epithelial tissues. Maturation is completed in the upper gastrointestinal tract. Human infections occur following the ingestion of intermediate host arthropods (7), either intentionally or accidentally, in contaminated food or water. In these cases, worms have been found in the mucosal tissues of the lips, cheek, tongue, tonsils, gums, and occasionally esophagus. A few cases of spurious egg passage have been documented, which may be due to the inadvertent consumption of adult *Gongylonema* in certain types of meat (for example, chicken gizzards or pork tongue). Source: Adapted from United States Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, 2019. Public domain.

Soulsby (1982), some gongylonemids can affect the health of humans and domestic animals, for example, *G. pulchrum* Molin, 1857, which can be found in most parts of the world. This parasite species occurs in sheep, goats, cattle, pigs, zebu, buffalo, and (less frequently) horses, camels, donkeys, and wild boar. It may also develop in humans, particularly in the oral epithelium, but also subcutaneously (see Figure 5). The site of localization in non-human animals is the esophagus where *G. pulchrum* embeds in a zigzag pattern in the mucosa or submucosa. In ruminants, it may also appear in the rumen. The intermediate hosts are coprophagous beetles of the genera *Aphodius*, *Onthophagus*, *Blaps*, *Caccobius*, and others (over 70 species). Migrating juveniles root in the wall of the gastroesophageal region. They excyst in the stomach and then

migrate anteriorly to the oral cavity and finally reach the wall of the esophagus. The species *G. verrucosum* (Giles, 1892) may be present in the rumen of sheep, goat, cattle, deer, and zebu in India, the United States, and South Africa. *Gonglyonema monnigi* Baylis, 1926 develops in the rumen of sheep and goats in South Africa. *Gonglyonema ingluvicola* Ransom, 1904 and *G. crami* Smit, 1927 occur in fowl in North America, India, the Philippines, Taiwan, Europe, and Australia. *Gonglyonema sumani* Bhalerao, 1933 occurs in the crop of domestic fowl in Uttar Pradesh State, India. The cockroach *Blatella germanica* may be infected with this worm. *Gongylonema verrucosum* embeds in the epithelium, causing just a slight chronic inflammatory reaction with hypertrophy and cornification, but *G. ingluvicola* may burrow into the crop and cause severe lesions in heavy infections.

Bickova and colleagues (2017) report some gongylonematid species that occur in Belarus, such as: *Gongylonema neoplasticum* (Fibiger et Ditlevsen), which occurs in the European water vole *Arvicola amphibius*, forest dormouse *Dryomys nitedula*, and common dormouse *Muscardinus avellanarius*, all from the Brest and Gomel regions (Luninety District). *Gongylonema sorici* Fain, 1955 is found in the common shrew *Sorex araneus* from NP "Belovezhskaya Pushcha" in Belarus. Kinsella and colleagues (2016) describe *G. archboldi* found in tunnels in the gastric mucosa of the cotton rat *Sigmodon hispidus* from Highlands County, Florida, United States. Measurements are also given for specimens from the cotton mice *Peromyscus gossypinus*, oldfield mice *Pe. polionotus*, Florida mice *Podomys floridanus*, and golden mice *Ochrotomys nuttalli* from the same locality. Additional specimens have been collected from the cotton rat and the rice rat *Oryzomys palustris* from Berry Island, San Patricio County, Texas, United States.

Chlamydoprocta Chandler, 1954 are parasites of the skunk *Mephitis mephitis* in Minnesota, United States. The type species is *Chlamydoprocta itascensis* Chandler, 1954, with a subgenus ***Progongylonema*** Hernandez-Rodriguez and Gutierrez-Palomino, 1992. They are parasites that live in the mucosa under the tongue of *Pica pica, Garrulus glandarius, Cyanopica cyanus*, and *Corvus monedula* (Passeriformes, Corvidae) in Córdoba Province, southern Spain. The type species is *Gongylonema (Progongylonema) pacoi* Hernandez-Rodriguez and Gutierrez- Palomino, 1992.

Gibbons (2010) described other genera in Gongylonematidae, which are listed below. ***Mastigonema*** Dailey and Perrin, 1973 are oviparous parasites of the forestomach of Cetacea, *Stenella graffmani* and *S. longirostris,* that are found in the eastern tropical Pacific Ocean. The type species is *M. stellae* Dailey and Perrin, 1973. ***Mazzia*** Khalil and Vogelsang, 1932 are parasites found in dasypodid mammals in Argentina, with the type species *M. mazzia* Khalil and Vogelsang, 1932. ***Paraspiralatus*** Gibbons, Nicholls, Bailey and Samour, 2004 includes a recently discovered species*, P. sakeri*, which was found in the stomach of a wild-caught, female saker falcon in Saudi Arabia (Gibbons et al., 2004). It has been accepted as a type species for the genus *Paraspiralatus*.

Chabaud and colleagues (1983) described *Mazzia bialata*, a parasite of dasypodid mammals (such as *Chaetophractus villosus*) from Buenos Aires, Argentina. According to the authors (Chabaud et al., 1983), this genus is morphologically more specialized than other Neotropical genera that parasitize paleoendemic mammals. Other genera described by this group of researchers includes ***Spirobakerus*** Chabaud and Bain, 1981, which are parasites of the cricetid *Zygodontomys brevicauda* of Colombia. The type species is *Spirobakerus weitzeli* Chabaud and Bain, 1981. Another genus in this group is ***Spirosprattus*** Smales, 2004, parasites found in cysts in the stomach wall of Australian rodents, such as the Cape York rat *Rattus leucopus* (family Muridae). The type species is *Spirosprattus scyphiformis* Smales, 2004.

According to Gibbons (2010), the superfamily **Thelazioidea** contains 3 families, as listed and described here.

Thelaziidae Skrjabin, 1915 includes the genus ***Thelazo*** Pearse, 1933. According to Pearse (1933), *Thelazo* is erected for *T. glossogobii* described from the final host, the tank goby *Glossogobius giurus*. The diagnosis is based on the work of Pearse (1933) who placed the genus in the Thelaziidae, of which the type species is *T. glossogobii* Pearse, 1933, which may be found in marine and brackish waters from the Red Sea, East Africa, South Asia, the Indian Ocean, China, Australia, and the islands of the Pacific Ocean.

The subfamily **Thelaziinae** (Skrjabin, 1915 family) Baylis and Daubney, 1926 contains the genus ***Thelazia*** Bosc, 1819. Members of the genus, such as *T. rhodesi* and *T. skrjabini*, are parasites of the orbits (including under the lids, conjunctiva, and nictitating membrane, and in the lachrymal glands and ducts) of birds and mammals. Other species, such as *T. callipaeda*, are said to develop in the fat body (Anderson, 2000). According to Soulsby (1982), *T. rhodesii* (Desmarest, 1828) occurs primarily in cattle, sheep, goats, and buffaloes, and its habitat is cosmopolitan. *Thelazia gulosa* Railliet and Henry, 1910 appears in cattle in most parts of the world. *Thelazia alfortensis* Railliet and Henry, 1910 occurs in cattle in Europe. *Thelazia lacrymalis* (Gurlt, 1831) develops in the horse in most parts of the world. *Thelazia skrjabini* Ershov, 1928 is found in cattle in Europe, Asia, and North America. *Thelazia callipaeda* Railliet and Henry, 1910 lives under the nictitating membrane of the dog in East Asia and has been reported from rabbits and humans. *Thelazia californiensis* Price, 1930 occurs in sheep, deer, cats, dogs, and humans in the United

States. *Thelazia leesei* Railliet and Henry, 1910 has been reported from dromedary camels in the former Soviet Union and elsewhere in Asia. *Thelazia rhodesi*'s intermediate hosts are *Musca larvipara*, *M. convexifrons*, and *M. amica*. *Musca oseris* transmits *T. lachrymalis* in regions delineated by the former Soviet Union, while *M. autumnalis* appears to be an important vector in the United States (Soulsby, 1982).

According to Gibbons (2010), the genus *Thelazia* consists of 3 subgenera, listed below. ***Isothela*** Railliet, 1925 are viviparous parasites occurring in birds. ***Pericyema*** Railliet, 1925 are ovoviviparous parasites in mammals, with the type species *T.* (*P.*) *callipaeda* Railliet and Henry, 1910. ***Thelazia*** (Bosc, 1819 genus) are viviparous parasites that occur in mammals, with the type species *T.* (*T.*) *rhodesi* (Desmarest, 1827) Railliet and Henry, 1910.

Another family in Thelazioidea is **Rhabdochonidae** (Travassos, Artigas and Pereira, 1928 subfamily) Skrjabin, 1946, parasites that live in the gallbladder of freshwater fish, and is allocated into 6 genera, as listed here. The first is ***Beaninema*** Caspeta-Mandujano, Moravec and Salgado-Maldonado, 2001. Caspeta-Mandujano and colleagues (2001) re-erected this genus and described a new species and new genus. The members of this genus are parasites in the gallbladder of the freshwater fish *Cichlasoma hearli* from the Santiago River, Tepic, Nayarít, Mexico, with the type species *Beaninema nayaritense* Caspeta-Mandujano, Moravec and Salgado-Maldonado, 2001. ***Fellicola*** Petter and Køie, 1993 are parasites that live in the gallbladder of the marine fish *Coryphaenoides rupestris* (a ray-finned fish) from the North Atlantic off the Faroe Islands (Petter and Køie, 1993). According to Petter and Køie (1993), the new genus is close to the genera *Johnstonmawsonia*, *Vasorhabdochona*, and *Pancreatonema* but differs from these genera in having longitudinal thickenings in the anterior dilated part of the pharynx. The type species is *F. longispiculus* Petter and Køie, 1993 (Gibbons, 2010). ***Megachona*** Mejía-Madrid and Pérez-Ponce de León, 2007 was described by Mejía-Madrid and Pérez-Ponce de León (2007) and identified the species *M. chamelensis* from the intestinal cecae of the blue striped chub *Sectator ocyurus* (Kyphosidae, Perciformes) from Chamela Bay, Mexico. According to the authors (Mejía-Madrid and Pérez-Ponce de León, 2007), *Megachona* most closely resembles *Beaninema* Caspeta-Mandujano, Moravec, and Salgado-Maldonado, 2001, F. Petter and Køie, 1993, and *Rhabdochona* Railliet, 1916. The type species is *M. chamelensis* Mejía-Madrid and Pérez-Ponce de León, 2007. The reconstruction of this genus ***Rhabdochona*** Railliet, 1916 was suggested by Moraveč (1975). The members of this group of spirurids are parasites that live in the intestine of fish and they possess 2 subgenera: ***Afrochona*** Puylaert, 1973, which are parasites that live in the intestine of the fish *Aphyosemion cameronensis* in Olounou, Cameroon (Gibbons, 2010). The type species is *A.* (*A.*) *camerounensis* Puylaert, 1973. The other subgenus is ***Globochonoides*** Moravec, 1975. According to Gibbons (2010), they are parasites that live in the intestine of freshwater fishes. The type species is *Rhabdochona* (*G.*) *coronacauda* Belouss, 1965.

Two new species of rhabdochonid nematodes that live in the intestines of freshwater fishes in Chiang Mai Province, northern Thailand were recorded by Moraveč and Yooyen (2011). One of them, *Rhabdochona* (*R.*) *pseudomysti*, is from the catfish *Pseudomystus siamensis* (Regan) (Bagridae, Siluriformes) from Fang Brook, a tributary of the Kok River in the Mekong River basin, Fang District, Thailand. The other is *R.* (*Globochona*) *thaiensis* from the cyprinid *Mystacoleucus marginatus* (Valenciennes) (Cyprinidae, Cypriniformes) in the Ping River in the Chao Phraya River basin, Muang District, Thailand. In accordance with the authors (Moraveč and Yooyen, 2011), these are the first nominal species of *Rhabdochona* reported from Thailand.

Moraveč and Kanda (2012) discovered another new species of nematode, namely, *R.* (*G.*) *rasborae* (Rhabdochonidae), from the intestine of the freshwater cyprinid fish, sidestripe rasbora *Rasbora paviana* from Tirant in the Bangbaimai Subdistrict, Muang District, Surat Thani Province, southern Thailand. According to the authors (Moraveč and Kanda, 2012), this is the third nominal species of *Rhabdochona* Railliet, 1916, and the second species of the subgenus *Globochona* reported from fishes in Thailand. One of the next 2 genera in Rhabdochonidae is ***Johnstonmawsonoides*** Machida, 1975, which are parasites that live in the intestine of the marine teleost fishes *Nemichthys scolopaceus* in Suruga Bay, Japan, with the type species *J. nemichthyos* Machida, 1975. Among the known helminths of meso- and bathypelagic fishes of Norfolk Submarine Canyon, in the western North Atlantic, Gartner and Zwerner (1989) reported nematodes which have been determined to be *Johnstonemawasonia* spp. Another genus in Rhabdochonidae is ***Neoascarophis*** Machida, 1976, parasites that live in the intestine of the marine teleost fishes *Coelorhynchus multispinulosus* and *Bathygadus garretti* in Suruga Bay, Japan, with the type species *Neoascaropbis yarihige* Machida, 1976.

A subfamily of the Rhabdochonidae is **Prosungulonematinae** Skrjabin, Sobolev and Ivashkin, 1967, with the type genus ***Prosungulonema*** Roitman, 1963. It was presented by Chabaud (1975) as a synonym of Rhabdochonidae. Later, Caspeta-Mandujano and colleagues (2001) did not list the genus as valid in the family Rhabdochonidae. McVicar and Gibson (1975) supported the validity of the genus *Prosun-*

gulonema. The members of *Prosungulonema* Roitman, 1963 are parasites of freshwater teleost fishes, with the type species *P. siniperca* (Dogiel and Akhmerov, 1959). According to Chabaud (1975), another genus in Prosungulonematinae is ***Pancreatonema*** McVicar and Gibson, 1975. A new genus and species of nematode, *P. torriensis*, from the pancreatic duct of *Raja naevus* from off the coast of Aberdeen in northwest Scotland has been described and aspects of its biology were discussed by McVicar and Gibson (1975). The type species is *P. torriensis* McVicar and Gibson, 1975.

Another family in the Spiruroidea is **Pneumospiruridae** Wu and Hu, 1938, containing genus ***Pneumospirura*** Wu and Hu, 1938. The type species is *P. hainanensis* Wu and Hu, 1938?. They are parasites of birds and mammals, including some carnivores. Pence and Stone (1977) described a new species, *P. bassarisci*, from the ringtail *Bassariscus astutus* and redescribed 2 species from the bobcat *Felis rufus* in North America. The genus includes the species *P. hainanensis*, *P. capsulata*, and *P. bassarisci*, with site of localization the bronchioles of carnivorous mammals. Wertheim and Giladi (1977) described *P. rodentium* as a lung parasite of *Gerbillus dasyurus* and *Meriones crassus*. Two other species include *P. capsulata*, parasites in the common badger, and *P. rodentium*, found in the lungs of gerbils and birds (Wertheim and Giladi, 1977).

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Nemata

Camallanina (Suborder): Guinea Worm and Related Nematodes

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Phylum Nemata

Suborder Camallanina

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Chapter 54

Camallanina (Suborder): Guinea Worm and Related Nematodes

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Introduction

Including the infamous species *Dracunculus medinensis*, the famed guinea worm of humans that has been known since antiquity, this group contains nematodes that live as adults in the gastrointestinal tracts, body cavities, or tissues of their vertebrate hosts (Anderson, 2000; Moraveč, 2006). A common, if not universal, feature of this order is that the embryos develop through ovoviviparity and hatch in utero. In many species, the body of the gravid female ruptures, often upon contact with water, to release these newborn juveniles. The life cycle is aquatic and involves crustacean first intermediate hosts; as such, not surprisingly, the vast majority of these nematodes parasitize fishes, although *D. medinensis* infects humans and other terrestrial mammals.

The group has been classified within the conventional order Camallanida but is now better recognized as a natural (monophyletic) suborder Camallanina (Černotíková et al., 2011). There appear to be no unique morphological features that distinguish the camallinidans as a whole, but each of the 2 superfamilies (see below) have their own typical morphology and biology. Among them, not just *Dracunculus medinensis*, but also several other species cause pathology and disease.

Systematics, Taxonomy, and Phylogenetics

The order Camallanida (or suborder Camallanina) now comprises 2 superfamilies: **Camallanoidea** with 1 family, Camallanidae, and **Dracunculoidea** with the families Daniconematidae, Dracunculidae, Guyanemidae, Lucionematidae, Micropleuridae, Philometridae, Philonemidae, Skrjabillanidae, and Tetanonematidae (Moraveč, 2006; Nadler et al., 2007; Černotíková et al., 2011) (Figure 1). Molecular phylogenetic studies have confirmed that many of these camallanids form a natural group, but the relationships of the lesser-known families, namely, Guyanemidae, Lucionematidae, and Tetanonematidae, remain untested (Wijová et al., 2005; 2006; Nadler et al., 2007; Choudhury and Nadler, 2018). Phylogenetic studies have also shown that Anguillicolidae, comprising nematodes of the swimbladder of anguillid eels, belongs with the Gnathostomoidea (Wijová et al., 2006).

Superfamily Camallanoidea

Family Camallanidae

As mentioned above, camallanids are mainly parasites of the gastrointestinal tract of fishes but also parasitize turtles, amphibians, and occasionally aquatic snakes.

Morphology

The camallanids are easily recognizable by their distinctive thick-walled **buccal capsule** that may be ridged on its inner surface, a rounded or slit-like **mouth opening**, mostly without lips, and an **esophagus** with anterior muscular and posterior glandular regions. Females possess a **vulva** near the mid-body. Males are often considerably smaller than females and have caudal **alae** with pedunculate **papillae**. The slender first stage juveniles (J_1) have typically attenuated **tails** that in some species have digitate ends (Moraveč and Justine, 2006). The third stage juveniles (J_3) of many species have characteristic **spike-like processes** at the tip of their tails (see Figures 2 and 3).

Distribution

Camallanids are widely distributed in freshwater as well as marine environments. The genus *Camallanus*, in particular, has a worldwide distribution with numerous species in freshwater and marine fishes. The freshwater bodies of the Neotropical region have a rich diversity of camallanids in fishes, mainly in the genus *Procamallanus* (Moraveč, 2009). Baker (1987) counted approximately 150 species of camallanids worldwide, of which 40 species were in hosts other than fishes (turtles being the most common among these).

Life cycles

Gravid females presumably release juveniles (J_1) into the intestinal tract of their definitive hosts, but it is not uncommon to see females hanging out of the anus of the fish hosts, in which case, the juveniles are shed directly into the surrounding water. Gravid females of the North American species, *Camallanus oxycephalus*, rupture to shed their juveniles. These shed juveniles are active and attract the attention of copepods that ingest them as food. The juveniles burrow through the copepod gut and enter the copepod's hoemocoel

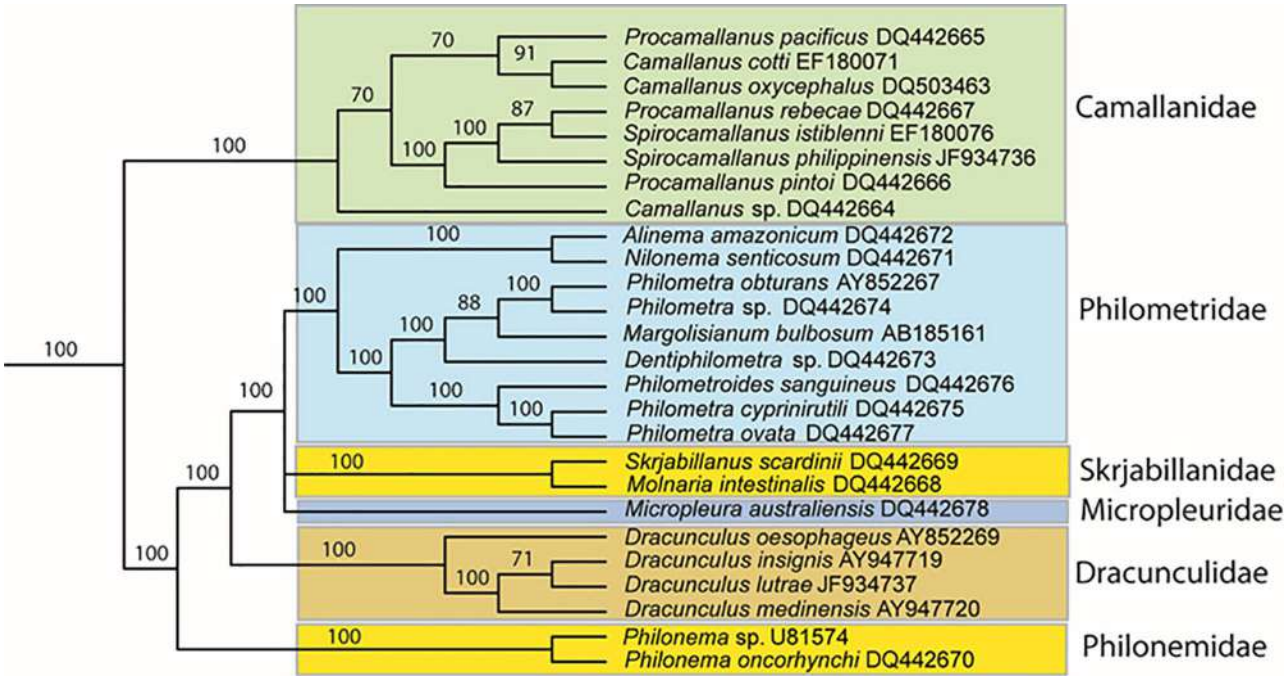

Figure 1. Part of the phylogeny of endoparasitic nematodes based on Bayesian analysis, showing interrelationships of some of the families in suborder Camallanina. Numbers refer to Bayesian Posterior Probability values. Values higher than 90 indicate strong support. Source: Adapted from Choudhury and Nadler, 2018. License: CC BY-NC-SA 4.0.

Figure 2. *Camallanus ancylodirus* from quillback (*Carpiodes cyprinus*: family Catstomidae). Source: A. Choudhury. License: CC BY-NC-SA 4.0.

Figure 3. *Procamallanus* sp. from a tetra (*Bryconamericus scleroparius*: family Characidae); bc = buccal capsule, es = esophagus. Source: A. Choudhury. License: CC BY-NC-SA 4.0.

(fluid filled body cavity); there they develop to a stouter J_3 stage. The J_3 stage is easily identified as a camallanid because it already has the distinctive buccal capsule of the family and its tail has the characteristic terminal spike-like processes. When copepods infected by developed J_3 juveniles are ingested by the definitive hosts, the juveniles develop into J_4s and then adult worms in the host's gastrointestinal tract. However, it is common for camallanids, especially those species with piscivorous definitive hosts, to use another host in their life cycles, most commonly a paratenic host. Such a paratenic host is typically a smaller fish that is also often prey for the piscivorous definitive host. The J_3 juveniles from the ingested copepods consumed by the paratenic host persist in the gut or remain encapsulated in visceral organs. When the correct definitive host consumes an infected paratenic host, the J_3 juveniles develop to adulthood. In some cases, the J_3 juveniles may even develop to the J_4 stage in a much smaller paratenic fish host, but because further development (to the J_4) occurs, such a host may now be arguably considered a true second intermediate host rather than a paratenic host. In these ways, many camallanids bridge an ecological gap in the food web, to reach their piscivorous definitive hosts that would not normally consume copepods directly.

The life cycles of the common European species *Camallanus lacustris* and the equally common North American species *C. oxycephalus* illustrate the principles and phenomena discussed in the foregoing section. Both species use paratenic hosts, and in addition, *C. lacustris* can also re-establish as adults in larger predatory fish hosts (**post-cyclic hosts**) (Moraveč, 1994). Partial or full life cycles are also known in species of *Neocamallanus*, *Paracamallanus*, and *Procamallanus* (including *Spirocamallanus*) (Moraveč, 1998; Anderson, 2000).

Pathology and disease

Camallanids are capable of causing considerable pathology as adults (Meguid and Eure, 1996; Dick and Choudhury, 1995). For example, *Camallanus oxycephalus* can cause rectal prolapse accompanied by the destruction of the gut epithelium, hyperplasia of underlying tissue, inflammation, vascularization, and infiltration by various leucocytes and fibroblasts (Dick and Choudhury, 1995). *Procamallanus spiculogubernaculus* causes blood loss in the stinging catfish, *Heteropneustes fossilis*, in India (Sinha and Sinha, 1988). The highly successful invasive species *C. cotti* causes visible swelling and inflammation of the anus in poeciliids (for example, guppies) accompanied by hemorrhaging, edema and extensive rectal tissue erosion; it has become the main nematode parasite of poeciliids in aquaculture (Rigby et al., 1987; Menezes et al., 2006; Moraveč and Justine, 2006).

Superfamily Dracunculoidea

The dracunculoids are unique in that, unlike most other nematodes, they occupy the various cavities of the body other than the gut, as well as some tissues.

The anatomy of dracunculoids is also peculiar in several ways. Unlike their camallanoid relatives, species of all but 1 order of dracunculoids have no buccal capsule; only species in Skjrabillanidae have a relatively small buccal capsule. The lack of a buccal capsule gives the unique appearance of a muscular esophagus opening directly to a mouth, especially because these worms also lack lips. The vulva and anus of mature females of several species are often atrophied (Moraveč, 2006).

The dracunculoids include some of the smallest parasitic nematodes, such as the ~ 1 mm-long adults of *Lucionema* spp. as well as some of the longest, such as *Dracunculus medinensis* and some philometrids, that can exceed 1 m in length (Moraveč, 2006). The males of many species are markedly smaller than the females.

Like their camallanoid cousins, dracunculoids use copepods as first, and often the only, intermediate hosts. They are distributed worldwide and the vast majority of species are parasites of fishes; only species in Dracunculidae are exclusively parasites of tetrapods, including humans.

In fact, the superfamily includes one of the most high-profile nematodes of humans, the guinea worm, *Dracunculus medinensis*, which is discussed in more detail below. Moraveč (2006) has provided a thorough review of the morphology and biology of this group, including a key to species. Life cycles have also been reviewed by Anderson (2000).

Family Dracunculidae

The family contains 2 genera, *Avioserpens* and *Dracunculus*. The 4 known species of *Avioserpens* are all parasites of aquatic birds. *Dracunculus* comprises 11 species, of which 6 are in snakes in Asia, Africa, Madagascar, and Australia, as well as 1 found in turtles, and 4 in mammals (including *D. medinensis* of humans) (Moraveč, 2006; Jones and Mulder, 2007).

Dracunculus medinensis

This is the famed guinea worm afflicting humans, a nematode known since antiquity. The ancient Egyptians, Israelites, Vedic Indians, Persians, Arabs, Greeks, and Romans all seemed to have been aware of this parasite; ancient texts of several of these cultures mention the disease. The fiery serpent Moses speaks of in the Old Testament may refer to the guinea worm because the parasite was common in the Levant. Calcified remains of the guinea worm were found in an Egyptian mummy, and their extraction—by coiling the female worm on a small twig—was described by classical historians. The common emblem in the medical profession, the caduceus, which depicts 2 snakes wound around a staff, may have been inspired by the guinea worm. Linnaeus (1758), in his famous Systema Naturae, gave the worm its first scientific name, *Gordius medinensis*, but taxonomists later placed the worm in its current genus, *Dracunculus*. Muller (1971) and Moraveč (2006) provide excellent accounts of the history of this worm's association with humans.

Morphology

The females of *Dracunculus medinensis* are best known and most commonly encountered. Their slender, cylindrical bodies reach lengths of 80 cm in humans. The anterior end is blunt with a small mouth opening surrounded by raised papillae. The tail is curved and conical with a tiny blunt mucron at the tip. The intestine is collapsed. The uterus is voluminous and filled with up to 3 million J_1 juveniles at a time. The vulva is not functional. In stark contrast, the males are slender worms only 3 mm-long, at most. They have a similarly rounded anterior with a tiny mouth opening surrounded by small papillae. They have a conical pointed tail. The males are hardly ever seen by humans. Elements of the morphology of *D. medinensis* are shown in Figure 4.

Distribution

The original range of the guinea worm formed a broad belt across Sub-Saharan Africa, north across the Levant and the Arabian Peninsula, and through Iran into India. However, due to sustained eradication programs, the parasite is now localized only in a few areas in Africa and India.

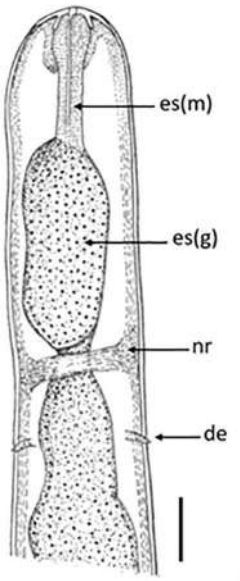

Figure 4. Anterior end of adult male guinea worm *Dracunculus medinensis*; es(m) = muscular esophagus, es(g) = glandular esophagus, nr = nerve ring, de = deirids. Source: Adapted from Moorthy, 1937. License: CC BY-NC-SA 4.0.

Figure 5. Aleksei Pavlovich Fedchenko (1844–1873), Russian naturalist and explorer. Source: Brockhaus and Efron Encyclopedic Dictionary (Энциклопедический словарь Брокгауза и Ефрона). https://upload.wikimedia.org/wikipedia/commons/8/85/Alexei_Fedchenko.jpg. Public domain.

Life cycles

Humans become exposed to the infective J_3 stage in copepods—the first and only intermediate hosts—by drinking unfiltered water contaminated by infected copepods. After a long—10 months or more—period of development in the human body, the gravid female reaches the connective tissue under the skin and begins initiating a localized blister, commonly on the upper part of the foot. The blister grows and eventually bursts; a small portion of the gravid female's body near its anterior end protrudes from the open blister and ruptures on contact with water. The J_1 juveniles are thus shed into the surrounding water, ready to infect copepods. Once that portion of the worm is spent, it dries and the female worm moves a fresh portion of her body into the opening so she can shed more juveniles. The mechanical extraction of the worm from human tissue is facilitated by its behavior (see section below). Eventually, the female sheds all her juveniles and then dies. A variety of cyclopoid copepods in several genera can be hosts of the J_3 juveniles. Eberhard and colleagues (2016) have shown that frogs can serve as paratenic hosts. Dogs can serve as reservoir hosts. As mentioned above, Anderson (2000) and Moraveč (2006) provide thorough reviews of the life cycle.

Experimental life cycle studies in cats, dogs, and rhesus monkeys (see review by Anderson, 2000) suggest that infective J_3 juveniles burrow through the duodenum and undertake a month-long journey, first migrating to the mesentery, and then to the abdominal and chest muscles, where they mature. This is also where copulation and fertilization occur in the following 2.5 months. Males die in the musculature. In the 4–6 months following fertilization, females migrate to the extremities, become gravid and initiate the characteristic blisters that prepare them to shed their juveniles (Anderson, 2000). Twenty-seven-year old Russian biologist Aleksei Fedchenko (Figure 5) was the first to describe the life cycle; his work was published in 1871. A number of parasitologists, notably Moorthy, Onabamiro, and Muller (for example, see Muller, 1968; 1971, and references therein) added important details to Fedchenko's pioneering work.

Disease, pathology, and treatment

The disease caused by these organisms is called dracunculiasis, or sometimes dracunculosis. In the older literature it has also been called draconitis. The localized blister caused by the adult females, most commonly on the legs and feet, causes a burning sensation and is painful. The blister is itself an acute inflammatory response to a relatively small number of juveniles released under the skin by a localized rupturing of the female worm when it is ready to shed its juveniles. Neutrophils, eosinophils, lymphocytes, and macrophages infiltrate the area and are part of the blister fluid. Once the blis-

ter bursts, and the female begins shedding juveniles to the outside, the open blister turns into an inflamed ulcer that becomes larger with time and affects the surrounding skin, especially while the worm is being mechanically extracted from the opening over a period of weeks. During this time, there is a risk of secondary infection, which may lead to septicemia, gangrene, and death. Once the worm is extracted, the lesion usually heals quickly. If the infection/ulcer is near joints, secondary complications such as arthritis and fibrous ankyloses can occur and the effects can be crippling. Infections of other areas of the body cause pathology and complications in those locations, such as inflammation of the scrotum and testes, or general cellulitis.

Chemotherapy, surgery, and mechanical extraction have been used to treat guinea worm infections. The drug metronidazole is often used in dracunculiasis; it does not kill the worm but makes it easier to remove, presumably because the drug acts as an anti-inflammatory agent. The age-old, crude, but effective technique of wrapping the spent portion of the worm on a short, slender twig or stick and extracting it manually is still common. Cool water is applied to the open blister so that the worm ruptures and sheds its eggs; this induces the worm to move a fresh portion of its body into the opening and the spent portion of the body can be wound around the twig (see Figure 6). The process is repeated over days or weeks until the entire worm is extracted. Usually only a few centimeters of the worm can be extracted at a time and the afflicted person must take care not to break the worm in the process. Large numbers of juveniles released under the skin by a ruptured worm can cause severe allergic and inflammatory responses and even anaphylactic shock.

Figure 6. Image of adult female guinea worm being extracted from the leg of an infected person by a health care worker in Africa. Source: World Health Organization. Informed consent as per WHO protocols. License: Cf. WHO terms of acceptable uses (non-commercial, educational).

Guinea Worm Eradication Program

The guinea worm provides a remarkable example of how philanthropy, education, and tireless volunteering may converge to improve public health outcomes since this human parasite has been nearly eradicated in Africa (Tayeh et al., 2017). In 1981, the World Health Organization (WHO) initiated the guinea worm eradication program in Africa as a key desired outcome of its overall strategy to improve drinking water supply and sanitation. Five years later, in 1986, the Carter Center began its partnership with WHO and UNICEF (also known as the United Nations Children's Fund) to lead philanthropic efforts to eradicate the disease (see information from the WHO on eradicating dracunculiasis at https://www.who.int/activities/eradicating-dracunculiasis.) As a result of the coordinated network of field volunteers, health workers, various WHO and UNICEF branches, the United States Centers for Disease Control and Prevention (CDC), and the Carter Center, the number of cases dropped from 3.5 million in 1986 to fewer than 2 dozen per year today. A WHO situation report from January 2022 noted that there were a total of 27 human cases of dracunculiasis in Africa reported in 2020, from Chad, Ethiopia, Angola, Mali, and South Sudan. In addition to these human cases, it was reported that 1,520 infected dogs were identified in Africa, and 71 cats and 4 baboons were also reported to be infected during this same period. The persistence of the guinea worm in reservoir hosts like dogs, even in countries where new cases of dracunculiasis are no longer reported (for example, Mali and Ethiopia), illustrates the need for vigilance and continued control measures to prevent the re-emergence of the disease in humans.

There is an acclaimed documentary on guinea worm, How to Slay a Dragon, which first aired on Al Jazeera on November 19, 2014 as part of its series Lifelines: The Quest for Global Health. It features the biology of the parasite, the efforts of health care workers, and the philanthropy led by former United States President Jimmy Carter. It is available for viewing at https://www.aljazeera.com/program/lifelines/2014/11/19/how-to-slay-a-dragon.

***Dracunculus* in Wildlife**

Two North American species, *Dracunculus insignis* in raccoons and *D. lutrae* in otters, and the South American species *D. fulleborni* in opossums, are examples of species of *Dracunculus* that parasitize terrestrial wild mammals. The life cycle of *D. insignis* is very similar to that of the human guinea worm, *D. medinensis*, which occurs as follows: The first intermediate host is a copepod and juveniles ingested with infected copepods in contaminated water migrate and mature in the mammal host; the females reach the skin and release their juveniles to the external environment by an open blis-

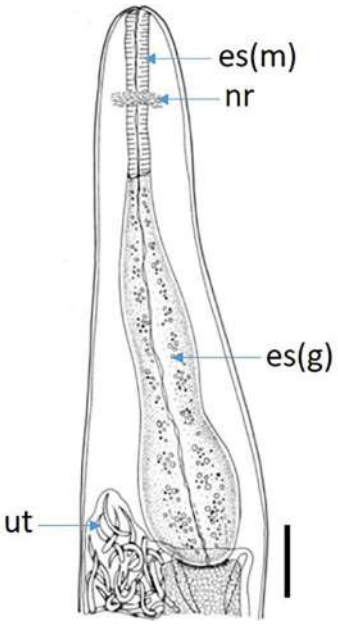

Figure 7. *Philonema agubernaculum*, female, anterior end; es(m) = muscular esophagus, nr = nerve ring, es(g) = glandular esophagus, ut = uterus. Scale bar = 100 µm. Source: Arai and Smith, 2016. License: CC BY 3.0.

ter or abscess on the skin of the animal's lower leg. Inflammation of the blister area occurs in wild and experimentally infected animals. One may assume that the life cycles of the other species are similar. As with *D. medinensis* in Africa, dogs can serve as reservoir hosts for *D. insignis* in North America across a considerable range (Cleveland et al., 2018).

Family Philometridae

Philometrids are widely distributed parasites of fishes (Hoffman, 1999; Moraveč and de Buron, 2013). Like their dracunculid relatives, the gravid females of many species are large, packed with juveniles, and reside under the skin, often of the extremities such as the fins (for example, *Philomeroides huronensis*), cheeks (*P. nodulosa*), or even in nodules in the eye socket (*P. fulvidraconi*). The female releases its juveniles when its body makes contact with water through a rupturing of the host's skin. Most species have copepod intermediate hosts. Several freshwater and marine species cause pathologies (see Choudhury and Cole, 2011).

Family Philonemidae

Philonemids are typically parasites of the body cavity of salmonid fishes. *Philonema agubernaculum* and *P. oncorhynchi* (Figures 7 and 8, respectively) are common species in salmonids. Gravid females release their juveniles in the body cavity of their fish hosts. The juveniles are carried into the water along with the eggs and milt of spawning fish. Copepods are the first intermediate hosts. Smaller prey fish such as smelt can be paratenic hosts. Infection with *Philonema* spp. may cause visceral adhesions in infected fish (see Choudhury and Cole, 2011).

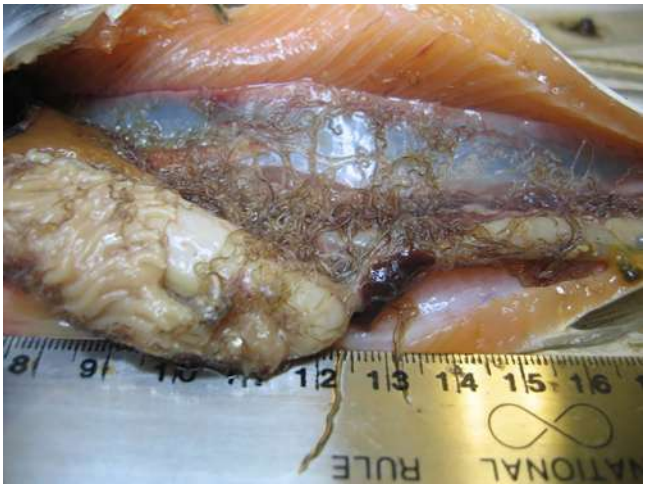

Figure 8. *Philonema oncorhynchi* from a Pacific salmon (*Oncorhynchus* sp.). Source: A. Choudhury. License: CC BY-NC-SA 4.0.

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55

Nemata

Filarioidea (Superfamily)

Juliana Notarnicola

Phylum Nemata

Superfamily Filarioidea

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2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 55

Filarioidea (Superfamily)

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Introduction

Filarioid nematodes are parasites of all classes of vertebrates except fish. Most of the species are parasites of wild animals, however some of them parasitize humans and domestic animals, triggering diseases. The majority of these filarioids are included in the family Onchocercidae. They are tissue-dwelling nematodes, with an indirect life cycle including hematophagous arthropods. Adults have been found in almost all tissues of their hosts; however, they prefer a particular location depending on the species. Usually, they are found parasitizing the body cavity, lymphatic vessels, nodules under the skin, or the right ventricle of the heart. They are viviparous, therefore first-stage juveniles (J_1), also known as microfilariae, are ingested by arthropods from the blood or skin of the definitive host. Later, the J_1s develop into J_2s in different organs of the arthropod, such as the Malpighian tubules in mosquitoes or the muscle cells in ticks, until they molt into J_3s, which migrate near the mouthparts of the vector to be transmitted to a new vertebrate host.

The filarioids have developed unique and highly evolved biological features compared with their parasitic spirurid ancestors. Adults are confined to the internal body of their hosts and have adapted their life cycle to transmission with a motile embryo, the microfilaria, which is accessible to hematophagous arthropods. There are 2 groups of filarioids, one of which is included in the family Filariidae, which includes nematodes that produce skin lesions. In this family, females inhabit the subcutaneous tissue and make a hole in the skin to deposit the eggs and/or juveniles which attract the arthropod vector, such as individuals in the family Muscidae. In contrast, nematodes in the family Onchocercidae have evolved to inhabit a more internal position of adult worms in the body of the hosts. Females deposit their embryos in the connective tissue drained by the initial lymphatic vessels, and the vermiform shape of the embryo or microfilaria allows individuals to reach the peripheral cutaneous lymphatic or blood vessels, thus becoming readily accessible to the vector animal. Microfilaria-like juveniles are an evolved character within the Onchocercidae. In this group, vectors may belong to a variety of arthropods, like biting midges, blackflies, fleas, mosquitoes, lice, mites, and ticks, creating lesions or perforating the skin to suck the infected lymph or blood.

Morphology

Compared to other nematodes, the morphology of filarioids is simple. They are long and slender worms, with **sensing structures** at the anterior extremity which are poorly developed. Their length can be variable from 5 mm to more than 50 cm, with males being smaller than females. Males display a posterior region that is coiled or J-shaped. The anterior extremity of filarioids possesses 2 rings of **papillae**; the internal ring with 4 **labial papillae**, and the external ring with 4 **cephalic papillae** usually located around the **oral opening**. Between them there are 2 lateral **amphids** (Figure 1). However, some species have a smaller number of head papillae. The number and arrangement of the papillae are important characters when identifying members of the species. Filarioids display a small **buccal capsule**. The capsule is constituted of 4 segments: An anterior segment that is transparent and corresponds to the **invaginated cuticle**, and 3 **cuticularized segments** that are more or less developed, depending on the genus. The buccal capsule is sometimes absent in some filarioids, such as specimens in the genus *Mansonella* (Figure 2). The stoma rests on the **esophagus**, which is long and occasionally differentiated in an anterior muscular portion and a posterior glandular

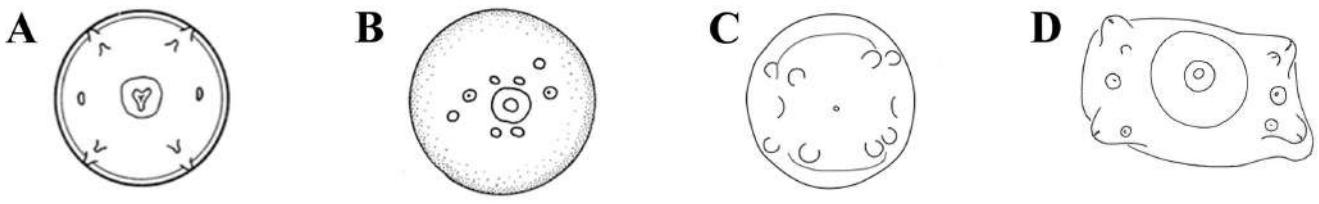

Figure 1. Apical views of some filarioids showing the arrangement of sensitive structures. A) *Brugia beaveri*. B) *Litomosoides odiale*. C) *Mansonela (Tupainema) dunni*. D) *Dipetalonema yatesi*. Sources: A) Adapted from Ash and Little, 1964; B) adapted from Notarnicola and Navone, 2002; C) adapted from Bain et al., 2015; D) adapted from Notarnicola et al., 2007.

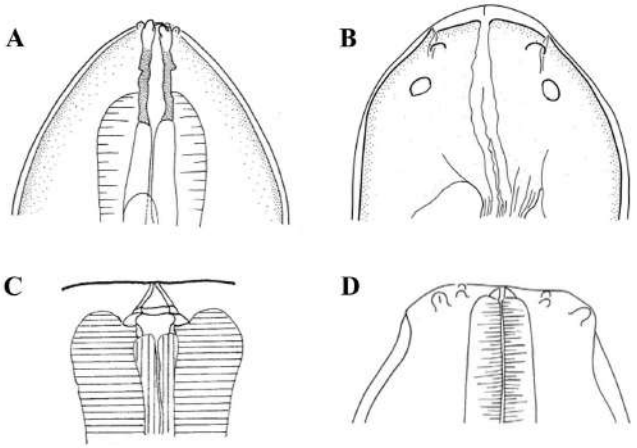

Figure 2. Anterior extremities of some filarioids showing the buccal capsule and the papillae. A) *Litomosoides odilae*, buccal capsule tubular; B) *Mansonella (Mansonella) interstutium*, buccal capsule absent; C) *Litomosa filaria*, buccal capsule constituted by 4 cuticularized segments;D) *Dipetalonema yatesi*, buccal capsule minute. Sources: A) Adapted from Notarnicola and Navone, 2002; B) adapted from Bain et al., 2015; C) adapted from Bain et al., 1966; D) adapted from Notarnicola et al., 2007. License: CC BY-NC-SA 4.0.

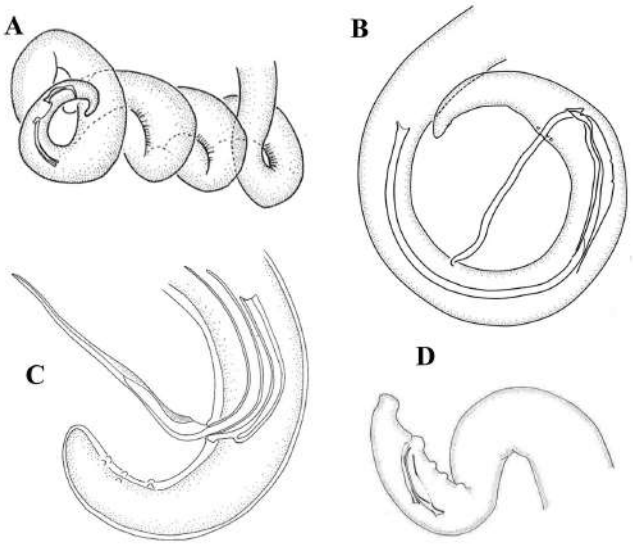

Figure 3. Male posterior ends showing the spicules. A) Posterior extremity of *Litomosoides odilae* showing the coiled region with area rugosa; B) Posterior extremity of *Mansonella (Pseudolitomosa) musasabi* showing the spicules in lateral view. Left spicule longer and different from right; C) Posterior extremity of *Litomosoides salazari* in lateral view; D) *Piratuboides huambensis* posterior region lateral view, with spicules few dissimilar but unequal. Sources: A) Adapted from Notarnicola and Navone, 2002; B) adapted from Bain et al., 2015; C) adapted from Notarnicola et al., 2010; D) adapted from Petit et al., 1983. License: CC BY-NC-SA 4.0.

portion. Neither the esophagus nor the **intestine** present diverticula. The intestine ends in a **cloaca** in males and in an **anus** in females. Filarioids display a **nerve ring** located anteriorly at the level of the esophagus.

The reproductive system in filarioids is amphidelphic. The male has 2 **testes**, 1 which is anterior and usually visible at the level of the esophagus-intestine junction, and the other that is posterior and visible near the **tail**. This continues with a duct which passes posteriorly without convolutions and opens into the cloaca. The cloaca possesses a **spicular pouch** where the **spicules** and the **gubernaculum** lie. The spicules in filarioids are unequal (meaning different in shape), and dissimilar (meaning different in size). The right spicule usually is shorter than the left spicule (Figure 3). The gubernaculum is sometimes present in some Onchocercinae species. Males also have cloacal papillae placed anterior, around, and/or posterior to the cloacal opening. In some species, there is a cuticle structure called the **area rugosa** all along the median ventral line generally extended into the posterior coiled region of the male. The area rugosa is constituted by **transversal ridges** in *Litomosoides* (Figure 4) or *Dipetalonema*, or by tiny **cuticular bosses**, as in *Litomosa* (Figure 5). Both structures, the papillae and the area rugosa, serve to attach the female during copulation (Figure 6). The length and shape of the spicules, as well as the number of cloacal papillae and the presence or absence of the area rugosa, are characters that help in the identification of the different genera and species.

The reproductive system in the females is convoluted. The anterior **ovary** is located near the level of the esophagus-intestine junction and continues backward in an **oviduct** and the **uterus**. In gravid females, eggs can be observed all along the uterus in different stages of development, such as, in the proximal portion, oval **eggs** containing **blastomera**; in the median portion, oval eggs containing the J_1; and in the distal portion, the extended microfilariae. The uterus continues to be situated within a long muscular **ovijector** and the **vagina**, which in some filarioids is a simple muscular tube (such as in specimens of *Ochoterenella* spp.) while in others it is more complex, differentiated into a **vagina vera** and a **vagina uterine**, as in *Dipetalonema* (Figure 7). The vagina is opened to a vulva at the anterior region, generally at the level of the esophagus or just posterior to it.

Microfilariae can be sheathed or unsheathed or not in the egg membrane, respectively. Females release thousands of microfilariae that migrate to the bloodstream, such as in *Dipetalonema*, or to the skin, such as in *Onchocerca*. Microfilariae are slender and fusiform; the anterior end is rounded usually with a hook, and the posterior end is pointed or blunt. Its length varies from 50 μm to more than 400 μm, depending on the species. The **sheath** is tightly applied to the body.

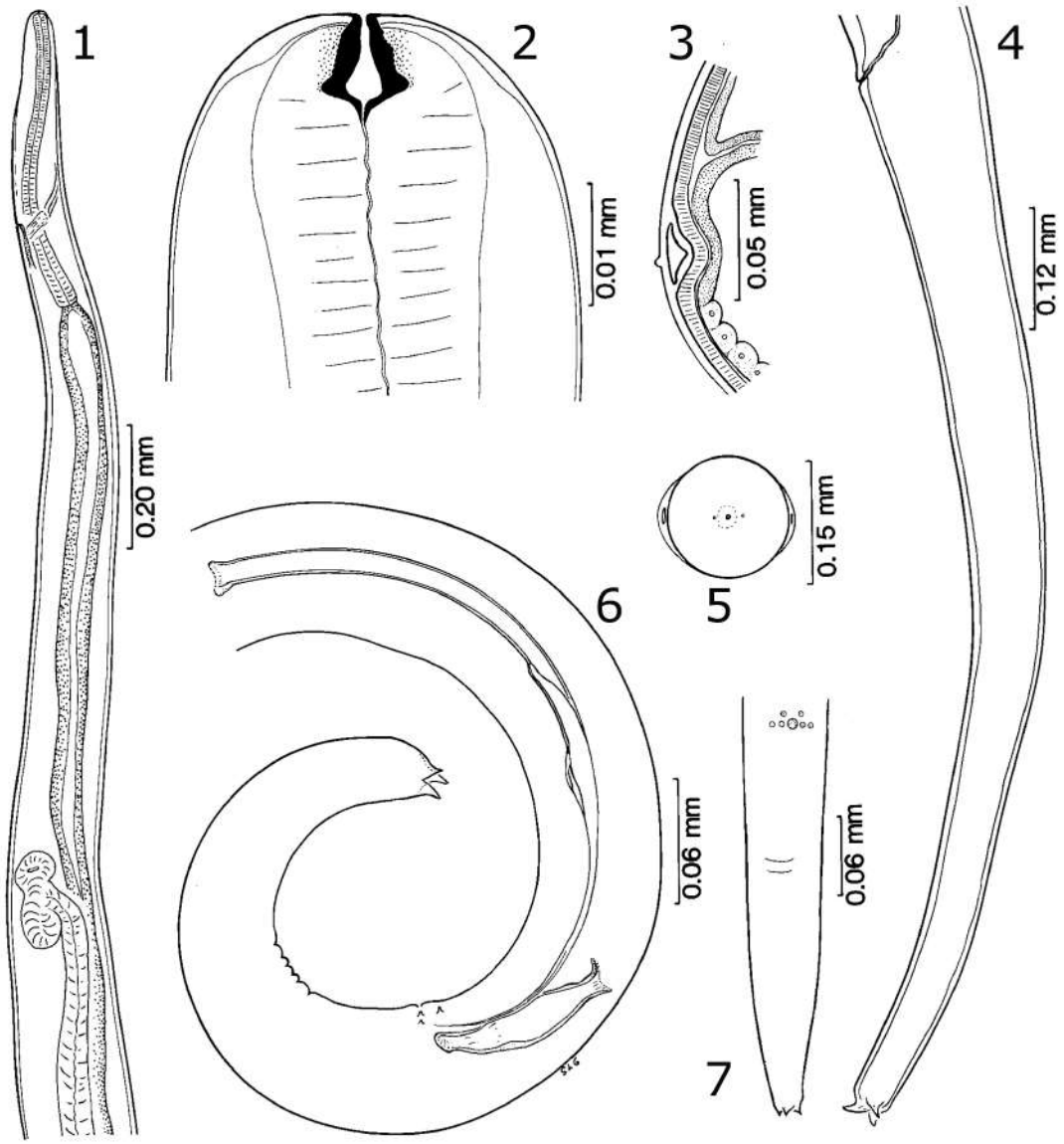

Figure 4. General morphological characters of a filarioid nematode of the genus *Litomosoides* that were obtained from pocket gophers collected in Weld County, Colorado, United States. Plate of *Litomosoides westi*: 1) Anterior end of female showing nerve ring, excretory pore, and vulva; 2) anterior end of female showing degree of development of stoma; 3) cross section of female showing lateral internal cuticular ridge; 4) posterior end of female showing species specific tail with three terminal points; 5) en face view of female; 6) posterior end of male showing coiled aspect, morphologically dissimilar spicules, and small cloacal papillae; 7) posterior end of male showing ventral view. Source: S. L. Gardner, HWML. License: CC BY.

When present, they could be visible at the anterior or posterior ends and appear as a delicate membrane. The internal anatomy of the microfilaria is unique, distinguished by several **internal nuclei** and **primordial organs** (Figure 8). From the anterior to the posterior end, it is possible differentiate the **nerve ring**, the **excretory vesicle** and the **excretory cell**, the inner body composed of few cells, a large stained **G1 cell**, and a row of 3 large, **stained cells** (R2–R4) similar to G1, connected to a clear area called the **anal vesicle** (Figure 8). The function of the G1 cell is unknown, but R2–R4 cells develop in the **rectum** and part of the reproductive system. It has been suggested that the inner body serves as a food reserve (Bain, 1972; McLaren, 1972; Anderson, 2000). The disposition and number of nuclei at the tip of the tail, plus the

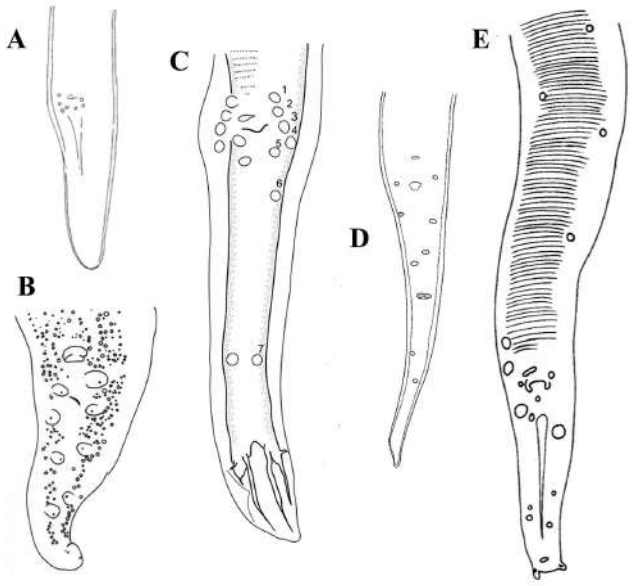

Figure 5. Different arrangements of male cloacal papillae, ventral views. A) *Litomosa goodmani* with a group of pericloacal papillae; B) *Ochoterenella esslingeri* with one precloacal papilla, 4 pairs of large symmetric postcloacal papillae, and ventral ornamentation made of irregular smaller bosses; C) *Mansonella (Mansonella) llewellyni*, precloacal *area rugosa* constituted by a row of small longitudinal ridges, caudal alae present, caudal papillae grouped near the cloaca, phasmids well developed at tip tail; D) *Litomosoides oxymycteri* with an attenuated tail, 1 precloacal papilla, one pair of ad-cloacal, and 4 pairs of asymmetric postcloacal papillae; E) *Orihelia anticlava* presenting precloacal *area rugosa* constituted by a row of small longitudinal ridges, 6 unpaired precloacal papillae, and several asymmetric postcloacal papillae, some larger than others, caudal lappets at tip tail. Sources: A) Adapted from Martin et al., 2006; B) adapted from Souza Lima et al., 2012; C) adapted from Bain et al., 2015; D) adapted from Notarnicola et al., 2000; E) adapted from Notarnicola and Navone, 2003. License: CC BY-NC-SA 4.0.

presence or absence of a sheath, are systematic characters of importance, mostly in species that parasitize humans.

One major characteristic of the Onchocercidae is the periodicity of the microfilariae, which refers to them flooding into the peripheral circulation at certain times of the day or night and disappearing from them at other times. The movement of the microfilariae appears to be associated with physiological changes of the host, as well as with the activity of the vector animals. The other notable characteristic is the longevity of the microfilariae, since they can live circulating in blood for several months after the adults have died. Both features are adaptations of microfilariae that allow them to be transmitted efficiently to the vectors.

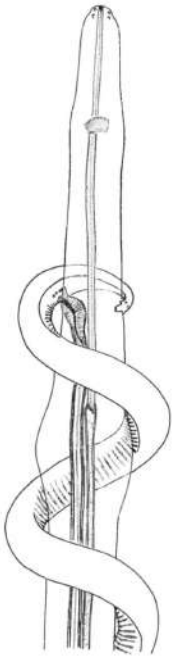

Figure 6. Schema of coupled male and female filarioids. Male cloaca positioned just opposite the female vulva during copulation. The area rugosa and cloacal papillae help for the attachment to female. Source: Adapted from Bain and Chabaud, 1988. License: CC BY-NC-SA 4.0.

Taxonomy of Suborder Filariata

See Anderson (2000) for a good reference to many of the topics following as well as Hodda (2022) for classification also for many life cycles and other important data on treatment and pathology see the web pages of the United States Centers for Disease Control and Prevention (2021).

Family Filariidae

This family includes filarioids parasitizing the subcutaneous tissues of certain mammals. Adults are small to medium-sized, and females possess a vulva located anterior to the nerve ring or near the oral opening, which facilitates the release eggs or juveniles in the skin (Figure 9). Adults and juveniles are located near one another. The family is composed of 2 subfamilies with only 5 genera (Table 1). Filarioids in this family are known to produce diseases clinically characterized by the occurrence of bleeding spots on the surface of the skin, or dermatitis.

Individuals of *Filaria taxideae* (in the subfamily Filarinae), for example, produce inguinal lesions in the skin of American badgers. Females are found in nodules containing embryonated eggs and few first juvenile stages. It is known that females live in the muscle fascia embedded in the dermis of their host and migrate to the epidermis evoking an

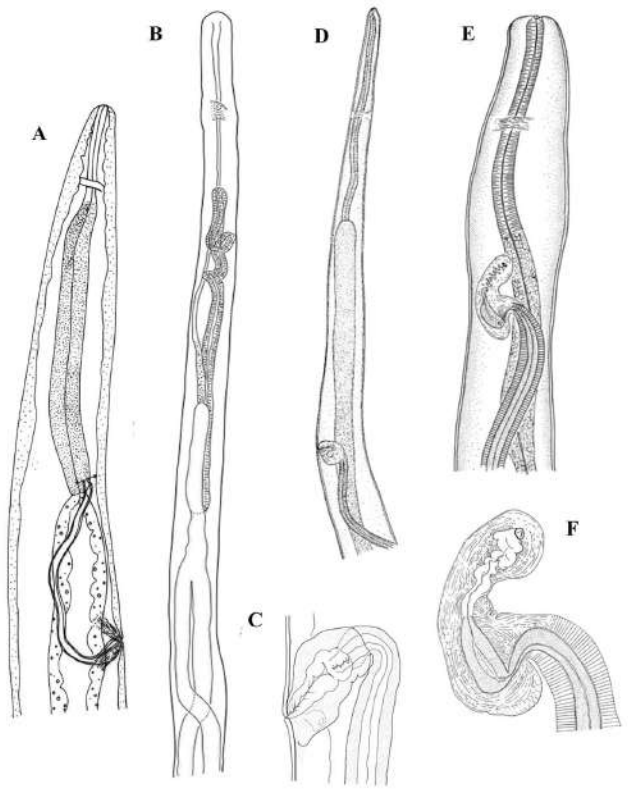

Figure 7. Anterior extremities showing the esophagus, position of the vulva, and shape of the vagina. A) *Ochoterenella esslingeri* possesses an esophagus divided in a short anterior muscular portion and a long posterior portion, the vagina is a simple muscular tube that opens posterior to the esophageal-intestinal junction; B, C) *Mansonella (Mansonella) ozzardi*. B) Anterior end showing a long fibrous esophagus with the vulva at mid-length of the esophagus; C) Vagina uterina simple; D) *Litomosoides oxymycteri* has an esophagus divided and vulva posterior to the esophageal-intestinal junction, vagina globular; E, F) *Dipetalonema robini*. E) Anterior end showing a divided esophagus, vulva at the level of the esophagus near the muscular-glandular division; F) Detail of the vagina, vagina vera conforming a chamber and vagina uterina muscular with a sinuous tube. Sources: A) Adapted from Souza Lima et al., 2012; B, C) adapted from Bain et al., 2015; D) adapted from Notarnicola et al., 2000; E, F) adapted from Vanderhoeven et al., 2017. License: CC BY-NC-SA 4.0.

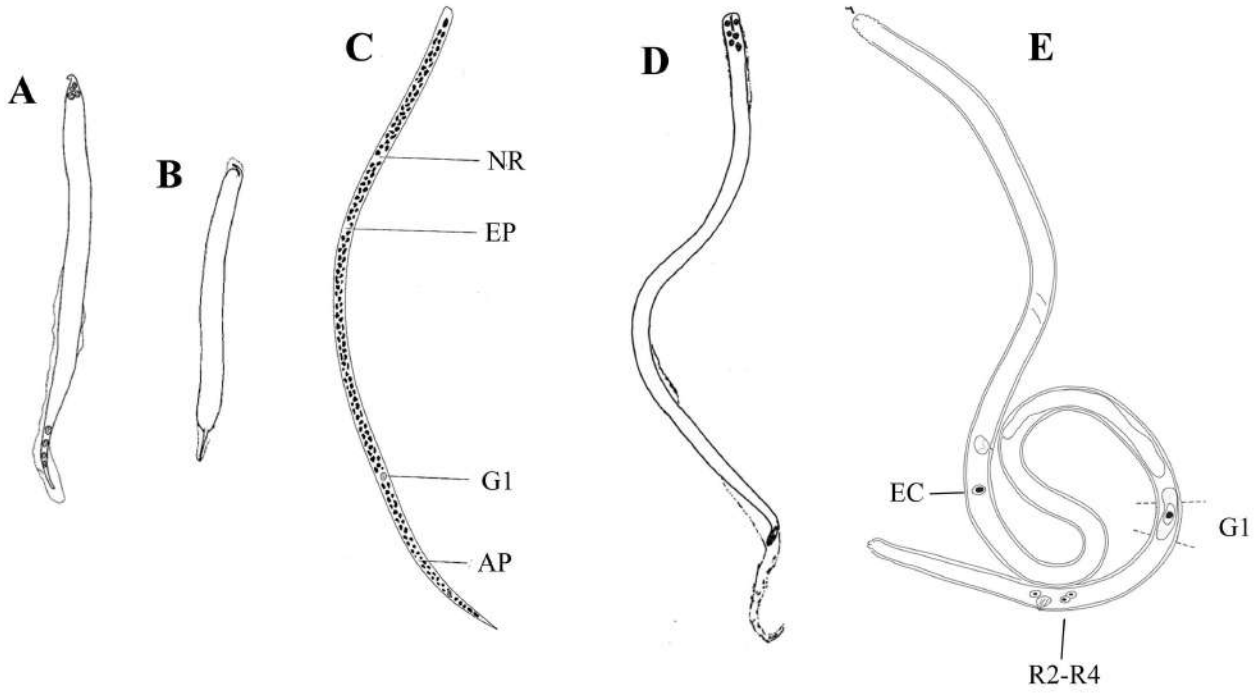

Figure 8. Different shapes of microfilariae. A) Uterine microfilaria of *Litomosoides oxymycteri*, sheath visible at tail; B) Uterine microfilaria of *Litomosoides solari* possessing a tail abruptly attenuated to a sharp point, sheath visible only at tip tail; C) Skin microfilaria of *Onchocerca lienalis*, unsheathed, tip tail with five nuclei aligned in a line; D) Uterine microfilaria of *Ochoterenella esslingeri*, sheathed; E) Blood microfilaria of *Mansonella (Tetrapetalonema) colombiensis*, unsheathed. NR: nerve ring; EP: excretory pore; G1: G1 cell; AP: anal pore; EC: excretory cell; R2-R4: R cells. Sources: A) Adapted from Notarnicola et al., 2000; B) adapted from Guerrero et al., 2002; C) adapted from Eberhard, 1979; D) adapted from Souza Lima et al., 2012; E) adapted from Bain et al., 2015. License: CC BY-NC-SA 4.0.

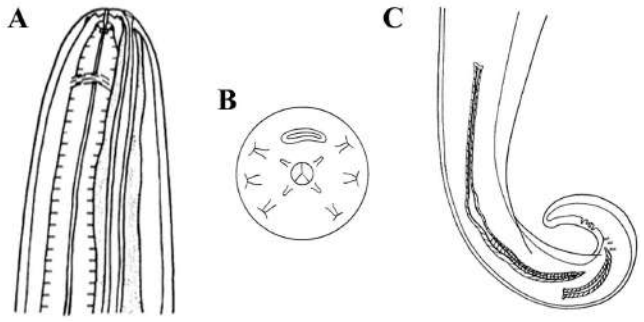

Figure 9. *Filaria taxideae*. A) Female anterior extremity showing the anterior position of the vulva; B) Female apical view, vulva located dorsal to the oral opening, 4 inner labial papillae and 4 external cephalic papillae, 2 lateral amphids; C) Male tail with dissimilar and unequal spicules. Source: Adapted from Keppner, 1969. License: CC BY-NC-SA 4.0.

inflammatory response due to the presence of both adults and eggs (Keppner, 1971). Similarly, species of *Parafilaria* (also in the subfamily Filariinae) have been described from the subcutaneous tissue on the upper parts of the body of horses, cattle, and water buffalo from Eurasia, Africa, and South America. In contrast to *Filaria taxideae*, females of *Parafilaria* migrate from the dermis and settle with their anterior ends immediately below the epidermis where they release embryonated eggs. The females pierce the skin of the nodule, causing bleeding which attracts the dipteran intermediate hosts (Figure 10). The first stage juvenile (J_1) is unsheathed and is carried in blood flowing from the skin. Second stage juveniles (J_2) are found in the body cavity and the fat body of *Musca* and *Haematobia* fly species, and the third stage juveniles (J_3) are found near the mouthparts (Anderson, 2000). Species of *Stephanofilaria* cause dermatitis, such as *S. stilesi* which causes dermatitis along the ventral midline, between the brisket and navel of cattle. In this species, adults are located in the dermis, just beneath the epidermis. The microfilariae are 50 µm-long and enclosed in a spherical sheath.

The development of the parasites coincides with the activity of the vector, that is, during the spring and summer. During these seasons, flies bite parasitized hosts, which become infected in 10 to 15 days, although the prepatent period for these filarioids is variable. Adults grow in 5 to 7 months post infection, depending on the species, and in 8 to 9 months the scratches characteristic of infection appear on the skin of cattle. The bloody spots are distributed in the hump, at the level of the neck, shoulders, withers, back, and rump, depending on the species. In *Parafilaria bovicola*, a parasite from cattle in Europe and Africa, the lesions tend to bleed when exposed to sunlight (Nevill, 1979).

Table 1. Genera included in the family Filariidae.

Subfamily	Genus	Hosts
Stephanofilariinae	*Stephanofilaria*	Parasites of Bovidae
Filariinae	*Filaria*	Parasites of carnivores and rodents
	Suifilaria	Parasites of Suidae
	Parafilaria	Parasites of ruminants and equids
	Pseudofilaria	Parasites of antelope

These worms are not overtly pathogenic; afflicted hosts do not become sick and consequently show no particular clinical symptoms. However, lacerated skin can become infected secondarily with bacteria, fly juveniles, and other pathogens. Moreover, it is known that symbiotic cleaning birds, like oxpecker or cattle tyrant, are also attracted by the spots due to the presence of insects and ticks, making the spots larger.

Several countries in Europe and Asia are endemic for these filarioids. Although the disease is not lethal, the nodules are painful and irritating, and slaughtered carcasses containing the worm are downgraded during inspection. From the point of view of conservation, these parasitoses are important. For example, in the 1960s, and more recently in 2012, there was a filariosis outbreak associated with *Stephanofilaria dinniki* (in the subfamily Stephanofilarinae) in threatened species of white and black rhinoceroses in Meru National Park in Kenya (Round, 1964; Mutinda et al., 2012) (Figure 10).

The therapy recommended for these parasitoses against the adult worms is ivermectin in different doses according to the host (such as cattle or horses), as well as high doses of levamisole and fenbendazole. It is also recommended to control flies and ticks to reduce the entry points of infective juveniles.

Family Onchocercidae

The Onchocercidae includes a diverse group of nematodes with more than 80 genera split among 8 subfamilies. The adult worms are small- to medium-sized. Females possess a vulva situated in the anterior region at the level of the esophagus, although occasionally may be found in the equatorial region. Males have a posterior extremity coiled with or without caudal alae. Microfilariae inhabit the skin, lymph, or blood. Unlike the Filaridae, adults live far away from the juvenile stages, inhabiting the body cavity, heart, skin, muscles, eyes, lymphatic system, and other regions of the host's body.

One of the 8 subfamilies, the Oswaldofilariinae, is confined to reptiles, another 2, the Waltoneliinae and Icosieliinae, to amphibians, while the Splendidofilariinae and the Lemdaniinae are parasites of reptiles, birds, and mammals. The Setariinae are confined to large mammals. Due to their great

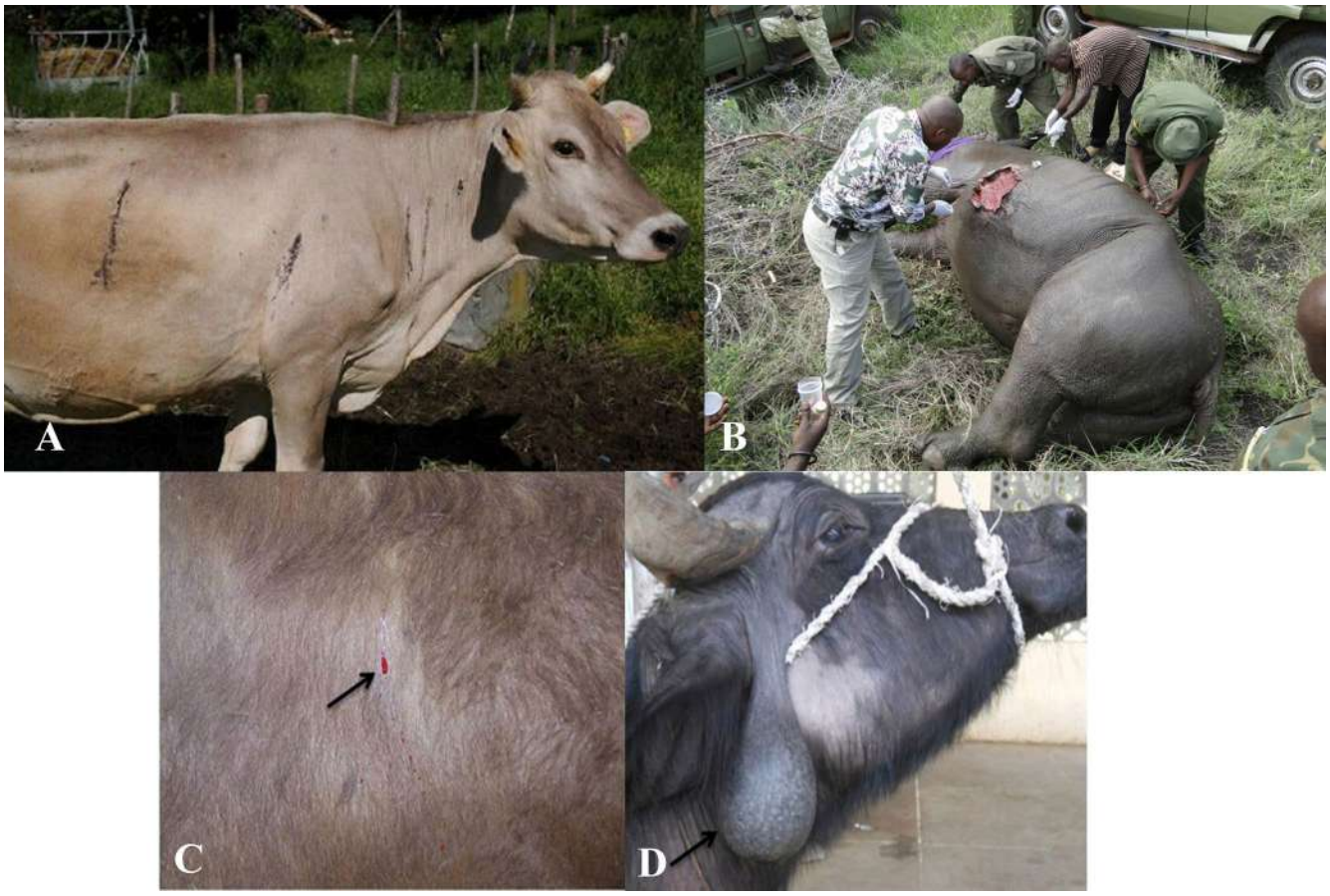

Figure 10. A) Adult female Buffalo from India with a growth below right ear with multifocal bleeding points over the skin; B) Bleeding spot in adult female Buffalo from India caused by *Parafilaria bovicola*; C) Bleeding spot from rhinoceros in Kenya caused by *Stephanofilaria dinniki*. Sources: A, B) Chandratre et al., 2017; B) Mutinda et al 2012. Licenses: A, B) CC BY-NC-SA; C) CC BY 4.0.

diversity and the numerous diseases they cause, the most important subfamilies are the Onchocercinae and Dirofilariinae. Both subfamilies are mainly parasites of mammals, although a few genera occur in birds and reptiles.

Subfamily Oswaldofilariinae

This subfamily includes filarioids that parasitize lacertilians and crocodiles. The location of adults is variable, being found in the connective tissue, heart, aorta, mesentery, intestinal wall, or body cavity. They are transmitted by mosquitoes. Members of the Oswaldofilariinae are distinguished by a vulva located in the middle or posterior region of the body, an esophagus that is well developed and divided, no caudal alae, sometimes large caudal papillae forming a subterminal group, and spicules that are often stout, unequal, and dissimilar from one another (Figure 11).

The subfamily is composed of 7 genera all parasitizing lizards, with the exception of *Oswaldofilaria*, which has 3 species in crocodiles. *Oswaldofilaria* is the most diverse genus with 13 species distributed in Australia, Africa, and South America (notably, a Gondwanian distribution). *Befilaria* comprises 3 species, 1 in the Neotropical region and 2 in the Ethiopian region; *Piratuboides* is present in South America and Australia only, with 1 and 2 species, respectively. The remaining genera have a restricted distribution. *Piratuba* includes 7 species in the Neotropics, *Conispiculum* includes 2 species, *Gonofilaria* has only 1 species occurring in India, and a single species in *Solafilaria* is found in lizards from Madagascar.

As mentioned above, it has been demonstrated that these filarioids are non-pathogenic to their hosts. A survey carried out in 110 *Tropidurus torquatus* from Brazil revealed that adult filarioids of *Oswaldofilaria chabaudi* were found 35% in the body cavity and 65% in the muscular aponeuroses of which 58% were found in the thighs and 7% at the base of the tail (Pereira et al., 2010). Microfilariae circulate in the blood. Experimental development in different species of *Oswaldofilaria* involved mosquitoes of the genera *Aedes*, *Culex*, and *Anopheles*. Juvenile stages were found in the adipose tissue or muscles of the dipterans.

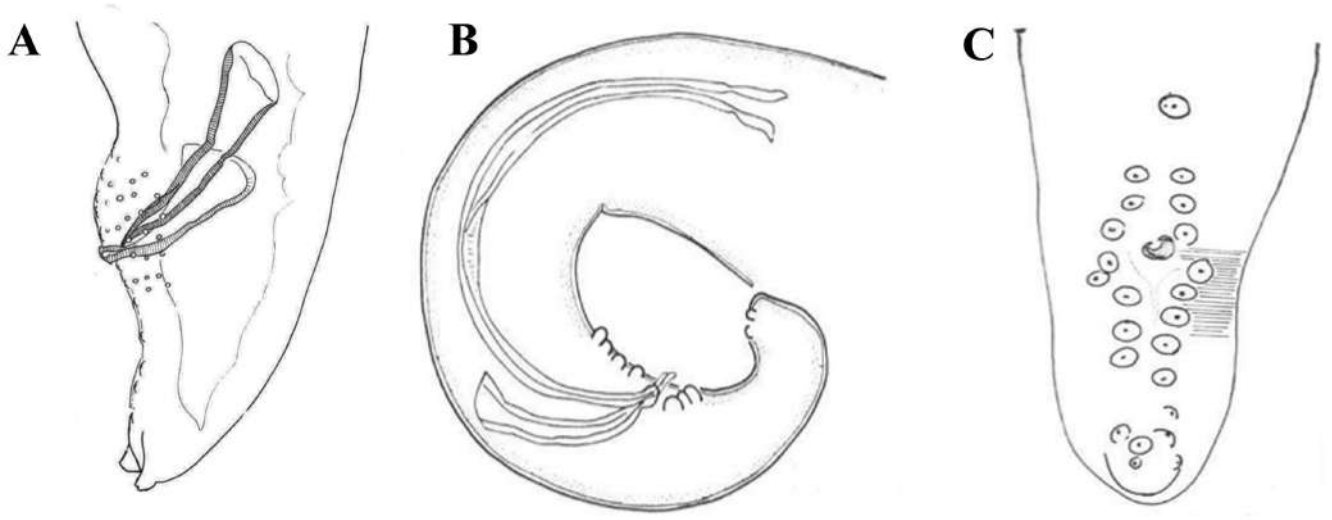

Figure 11. Posterior extremity of males from Oswaldofilariinae. A) *Conispiculum ramachandrani*, lateral view; left spicule slightly longer than right, unequal; numerous minute papillae around the cloaca and lappets at tip tail; B) *Oswaldofilaria versterae*, lateral view, showing pre and postcloacal papillae; left spicule long dissimilar and unequal than right; C) *Befilaria puertoricensis*, ventral view showing 2 lines of cloacal papillae. Sources: A, B) Adapted from Bain et al., 1982; C) adapted from Bain and Chaniotis, 1975. License: CC BY-NC-SA 4.0.

Subfamily Icosiellinae

This subfamily includes a single genus, *Icosiella*, including 9 species parasitizing the subcutaneous aponeurosis of amphibians from Palearctic, Occidental, and Australian realms. Adult worms are short with the posterior end of the body conical and blunt in females and protuberant in males. The buccal capsule is absent, 2 median cephalic spines are present, the esophagus is divided into a short anterior muscular and a long posterior glandular portion, and the anus is subterminal (Figure 12). Nine species were described from subcutaneous tissues from frogs of the family Ranidae, mainly in the genus *Rana*. Vectors in the life cycle of *I. neglecta* were shown to include *Forcipomyia* (biting midges in the family Ceratopogonidae) and *Sycorax* (in the family Psychodidae); these were observed to feed on the head of frogs. Second-stage juveniles (J_2) were detected in the muscles of the flies (Desportes, 1941; 1942).

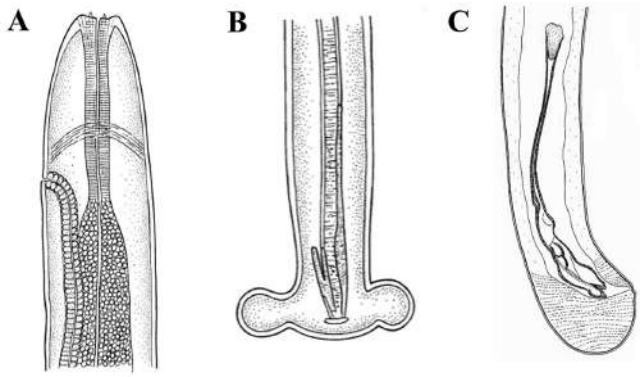

Figure 12. Examples of Icosiellinae species. A, B) *Icosiella turgeocauda*; A) Anterior end of female showing the muscular and glandular esophagus, and the muscular vagina; B) Posterior end of male, ventral view showing two lateral swollen, and dissimilar and unequal spicules; C) Male posterior end of *I. intani,* lateral view showing the spicules. Sources: A, B) Adapted from Bursey et al., 2003; C) adapted from Purnomo and Bangs, 1996. License: CC BY-NC-SA 4.0.

Subfamily Waltonelliinae

Members of this subfamily are parasites from the body cavity and mesentery of frogs and toads included in the families Bufonidae, Leptodactilidae, Racophonidae, and Ranidae. Adults are characterized by the presence of large cephalic papillae, lateral and caudal alae, and thin and dissimilar spicules (Figure 13). They are distinguished from members of the Icosiellinae by their long tail and the absence of cephalic spines.

There are 5 genera: *Waltonella*, *Ochoterenella*, *Madochotera*, *Foleyellides*, and *Paramadochotera*. Currently, *Ochoterenella* is the only genus reported in Central America and South America. The life cycle is only known for some species of *Waltonella*. They are transmitted by mosquitoes of the family Culicidae allowing microfilariae to develop in the body cavity (in *W. brachyoptera*), in the muscles (in *W. ranae*), or in the fat body (in *W. flexicauda*). Second- and third-stage juveniles (J_2 and J_3) may be found in mosquitoes 15 days post-infection and during the prepatent period in frogs in approximately 7 to 8 months.

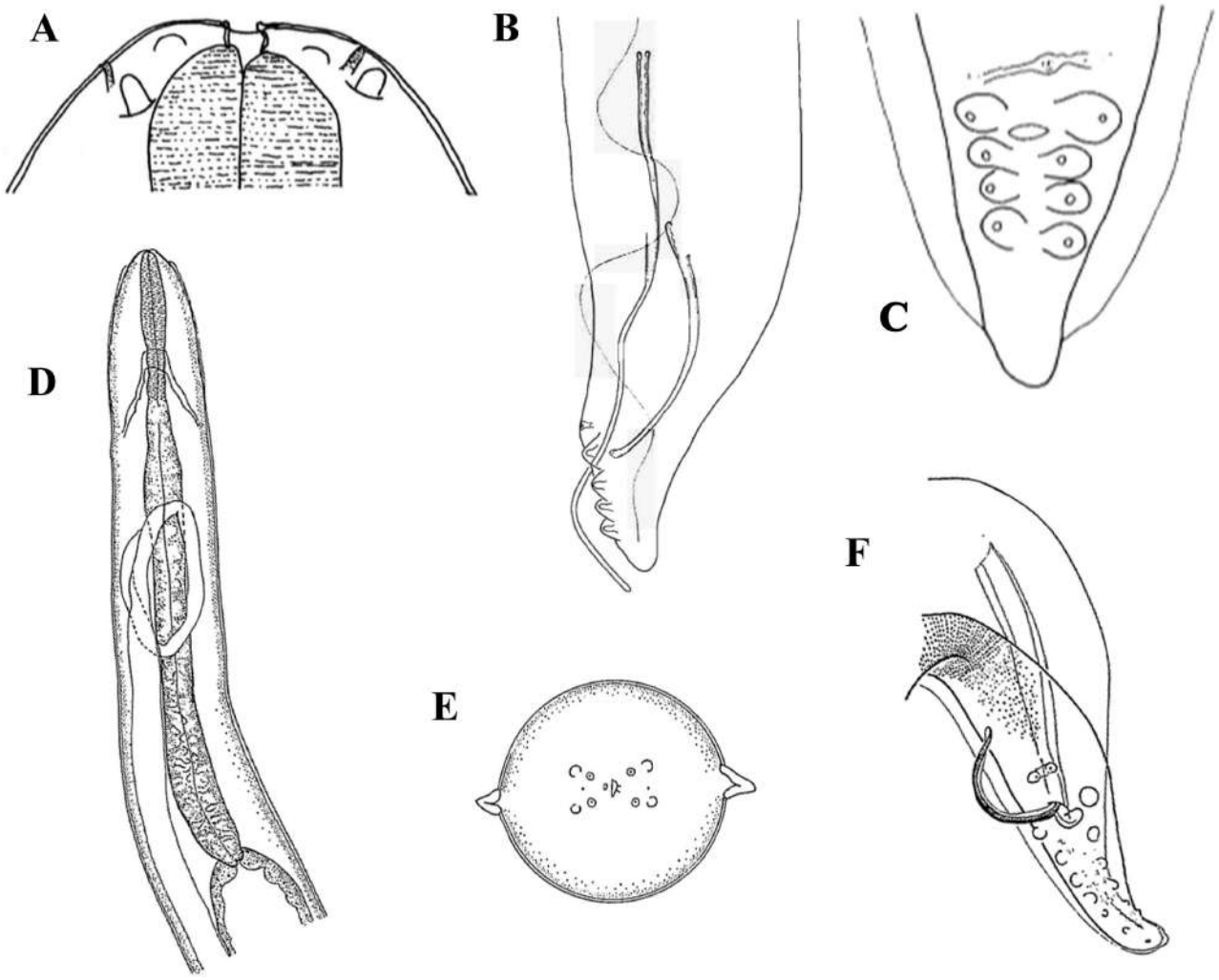

Figure 13. Waltoneliinae species. A) *Ochoterenella esslingeri* anterior extremity showing the buccal capsule and head papillae; B, C) Male posterior end of *Madochotera pichoni*. B. Lateral view showing the spicules and lateral alae; C) Ventral view with large symmetric cloacal papillae; D-F) *Foleyellides striatus*, male; D) Anterior extremity showing the divided esophagus; E) Apical view of the head showing the head papillae and the lateral alae; F) Tail with spicules and caudal papillae. Sources: A) Adapted from Souza Lima et al., 2012; B, C) adapted from Bain and Prod'hon, 1974; D) J. Notarnicola; E) adapted from Esslinger, 1986. License: CC BY-NC-SA 4.0.

Subfamily Setariinae

This subfamily comprises 2 genera: *Setaria* with more than 40 species parasitizing artiodactyls, mainly bovines, hyracoids, and equines; and *Papillosetaria* with only 3 species parasitic in artiodactyls. They are normally found in the abdominal cavity, but also rarely can be found in the eyes, lungs, and skin. Adults are medium- to large-sized, characterized by a complex cephalic structure composed of median or lateral cuticular elevations (spines) and well-developed cephalic papillae. The vulva is near the muscular esophagus, the male tail is rounded without caudal alae, and the spicules are markedly dissimilar from one another (Figure 14). Sheathed microfilariae are 200–231 μ-long; they circulate in the blood until mosquitoes feed on them (*Aedes* spp., *Culex* spp., and *Anopheles* spp.). Microfilariae invade the hemocoel and later the fat body where development takes place. After 12 days, the J_2 is developed and moves again to the hemocoel where it stays for 5 to 12 days more until it reaches the J_3 stage. The infective juveniles are 1.65–2.32 mm-long with numerous tubercules on the tip of the tail. These juveniles then invade a new host and migrate to the final location, the abdominal cavity. The prepatent period varies between 7 and 8 months, and the longevity of adult worms is 1.5 years (Osipov, 1966).

Two species are distributed worldwide: *Setaria equina*, a parasite of the abdominal cavity of horses, and *S. labiatopapillosa* from cattle. *Setaria digitata* parasitizes cattle in Asia. Adults are non-pathogenic, thus, filariasis goes unnoticed unless detected by the presence of microfilariae in blood smears.

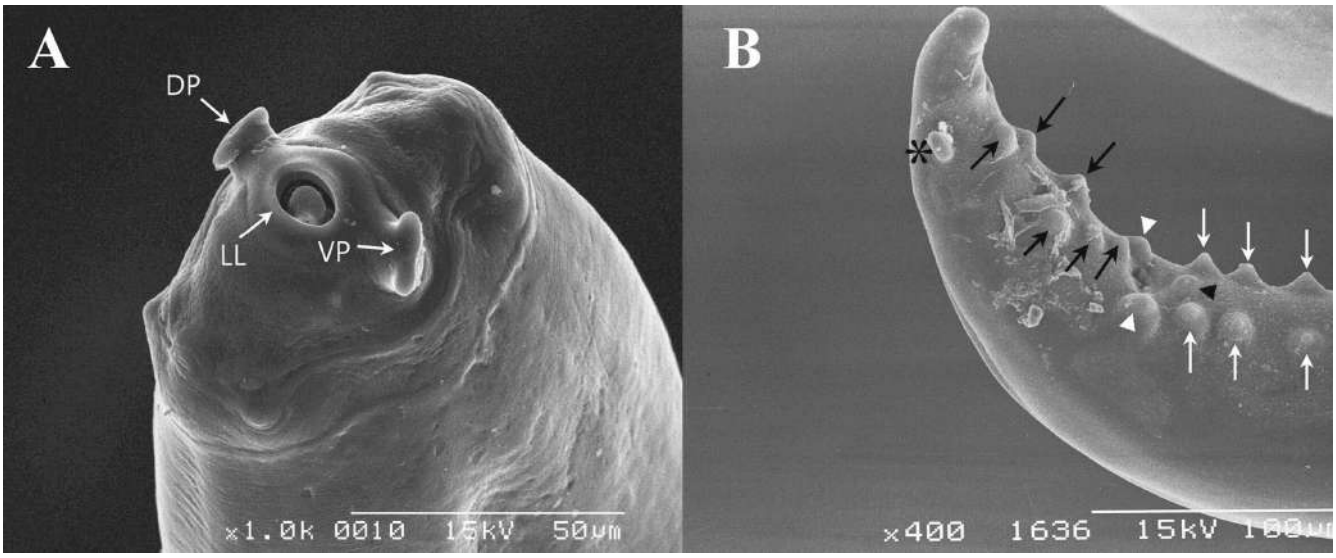

Figure 14. Scanning electron micrograph of *Setaria digitata* found in horse from Korea. A) Anterior end of a male adult showing the anterior structure; DP: Dorsal projection, LL: Lateral lips, VP: Ventral projection; B) Male tail showing the papillae and a pair of lateral appendages near the tip tail (asterisk). Three pairs of precloacal papillae (white arrows), a pair of ad-cloacal papillae (white arrowheads) and 3 pairs of postcloacal papillae (black arrows), plus a central papilla just in front of the cloaca (black arrowhead). Source: Shin et al., 2017. License: CC BY 4.0.

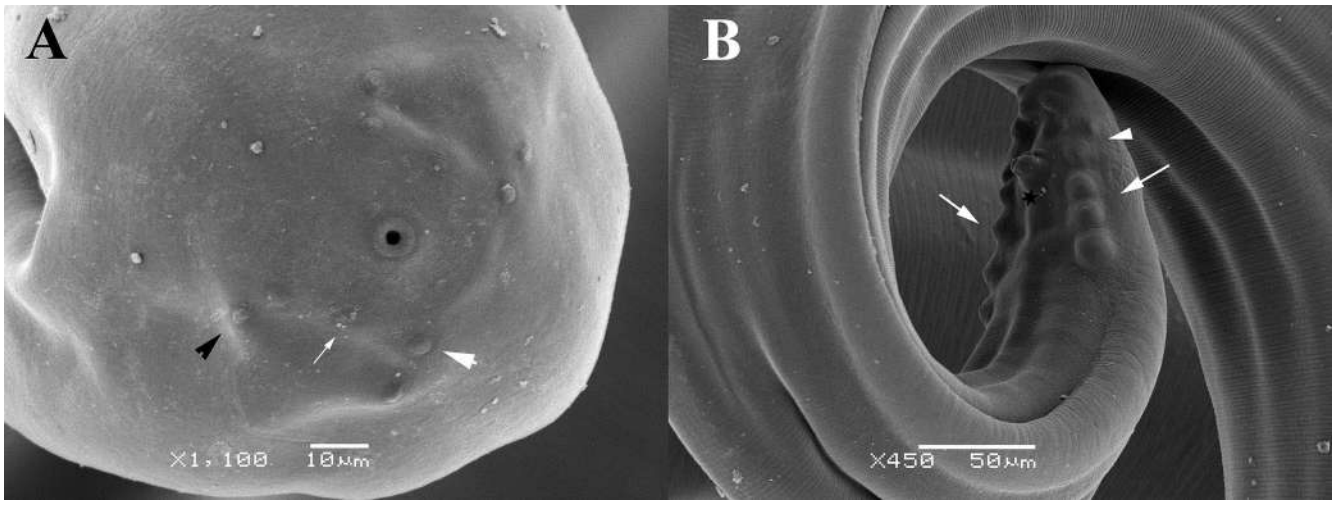

Figure 15. Scanning electron micrograph of *Pelecitus* sp. found in *Arremon flavirostris* (Emberizidae) from Argentina. A) Apical view showing 4 labial papillae (white arrowhead), 4 cephalic papillae (black arrowhead), and amphids (white arrow); B) Posterior extremity of male with protruded cloaca (black asterisk), large precloacal papillae (white arrows), and a group of 2 pairs of postcloacal papillae (white arrowhead). Source: J. Notarnicola. License: CC BY-NC-SA 4.0.

The major pathogenic effect occurs when immature stages migrate erratically in the pleural cavity, central nervous system, urinary bladder, and other organs. *Setaria cervi*, a common parasite of the body cavities from *Alces alces*, *Capreolus* spp., and *Cervus* spp. in Europe and Asia, is frequently found invading the central nervous system with concurrent infections with *Elaphostrongylus cervi* (Metastrongylidae) causing neurological disease (Blažek et al., 1968).

Subfamily Dirofilariinae

Nematodes of this subfamily include males with a short tail and a well-developed caudal alae, which distinguish them from other members of the Onchocercidae. They also have large and pedunculate caudal papillae and spicules that are markedly dissimilar from one another (Figures 15 and 16). Representatives of this subfamily include 1 genus parasitizing reptiles, 1 genus in birds, and 8 in mammals (Table 2)

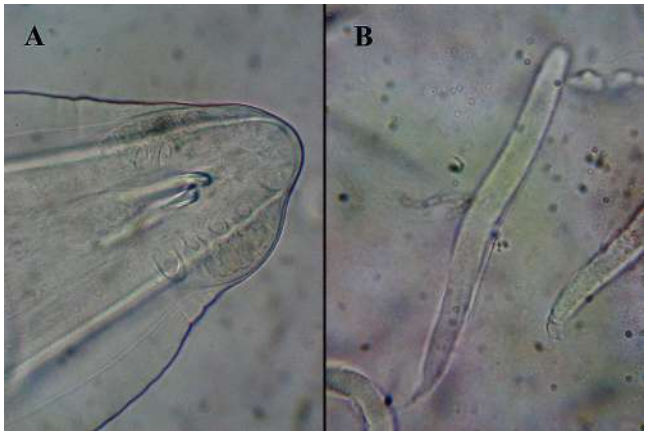

Figure 16. A) Photograph of *Pelecitus* sp. found in *Arremon flavirostris* (Emberizidae) from Argentina, posterior extremity of a male showing symmetrical caudal alae, spicules dissimilar and caudal papillae; B) Photograph of uterine microfilaria of *Pelecitus fulicaeatrae* found in *Podiceps occipitalis* (Podicipediformes) from Argentina. Source: J. Notarnicola. License: CC BY-NC-SA 4.0.

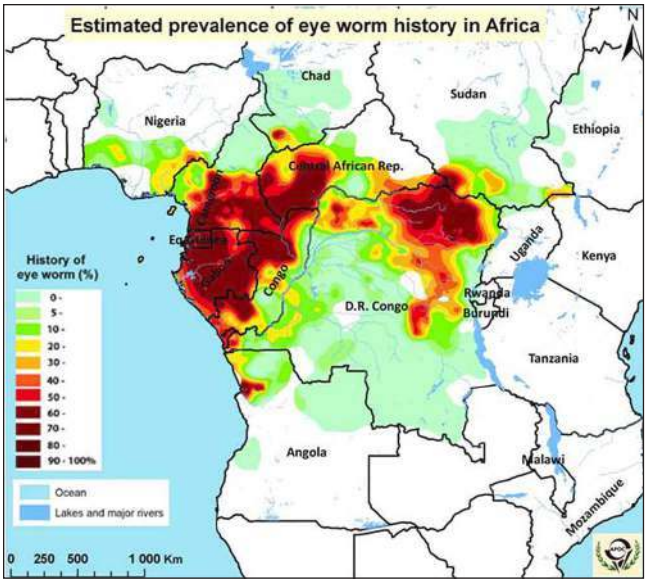

Figure 17. Distribution of *Loa loa* based on prevalence data collected in more than 4,700 villages in 11 African countries. Source: Adapted with data from WHO, 2010, https://www.who.int/apoc/raploa/Africa_EN_map.jpg?ua=1. License: CC BY-NC-SA 4.0.

(Anderson, 2000). Most of the genera are parasites of the subcutaneous tissues or muscles, with the exception of *Edesonfilaria* spp., which is located in the body cavity of arboreal dermopterans, chiropterans, and primates from the Indo-Malaysian region, and the cosmopolitan *Dirofilaria immitis* which parasitizes the right ventricle of the heart and pulmonary artery of carnivorous mammals.

This subfamily includes 2 species that are of epidemiological importance for humans: *Loa loa* and the zoonotic *Dirofilaria immitis*. Adult worms of *L. loa* live in subcutaneous tissues of humans producing edematous swellings on the body known as Calibar swellings or loiasis. Occasionally they migrate through the eyes in the conjunctiva and cornea. The species is endemic of the rainforest of West Africa and equatorial Sudan. Usually, it is diagnosed by the presence of microfilariae in blood smears or adults in the subconjunctiva. It is estimated that between 3 and 13 million people are infected at any one time with filariasis (Klion and Nutman, 2011). Infection is hidden in a large proportion of patients, which are asymptomatic. According to the World Health Organization (WHO, 2022), loiasis (also called African eye worm) is potentially endemic in 11 African countries, recording more than 40% of prevalence in Gabon, Equatorial

Table 2. List of Dirofilariinae genera with their localization and their hosts.

Genus	Localization	Hosts
Bostrichodera	Muscles	Parasites of edentates
Dirofilariaeformia	Pulmonary artery	Parasites of rodents Sciuridae
Edesonfilaria	Body cavity	Parasites of arboreal dermopterans, chiropterans, and primates
Macacanema	Muscles	Parasites of primates
Skjabinodera	Inguinal fascia and renal fat	Parasites of ungulates
Loa	Subcutaneous tissues	Parasites of primates
Foleyela	Subcutaneous and intermuscular connective tissues; body cavity	Parasites of reptiles chameleonids
Pelecitus	Tendons and muscles near leg joints and feet	Parasites of birds and mammals
*Dirofilaria**	Subcutaneous tissue and heart	Parasites of mammals
Loaina	Subcutaneous tissue and muscles	Parasites of lagomorphs

* *Tawila tawila* Khalil 1932 was transferred to *Dirofilaria tawila* by Webber, 1955.

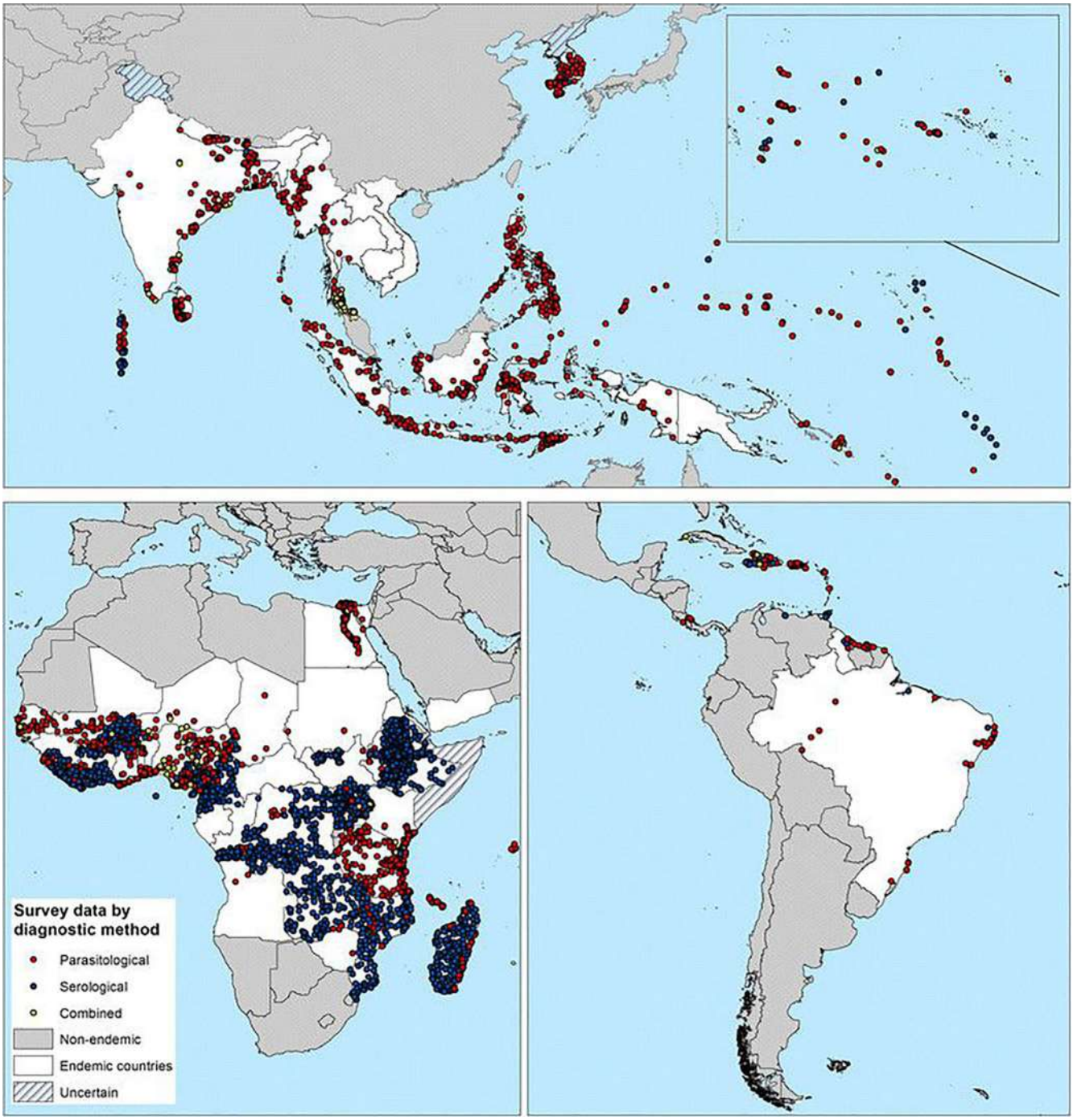

Figure 18. Global atlas of lymphatic filariasis (LF). Global distribution of LF and data points by diagnostic method. Data were identified for 66 of the 72 countries currently endemic and for a further 17 countries where LF is no longer endemic. Red = parasitological methods; blue = serological methods; and yellow = combination of methods. Source: Cano et al., 2014. License: Graphics and text, CC BY 4.0; data, CC0.

Guinea, southern Cameroon, eastern Central African Republic, the Republic the Congo, northwestern Democratic Republic of the Congo, and southwestern Sudan (Figure 17).

The heartworm *Dirofilaria immitis* is a common parasite of dogs and other mammals of several orders, including humans (Artiodactyla, Carnivora, Edentata, Lagomorpha, Perissodactyla, Primates, and Rodentia). Most animals exhibit no signs of disease when infected, however, some of them experience respiratory distress, cough, and other symptoms. Human dirofilariosis has been reported worldwide. Cases presenting with subcutaneous infestations in the Old World are attributed to *D. repens*, a subcutaneous worm from dogs,

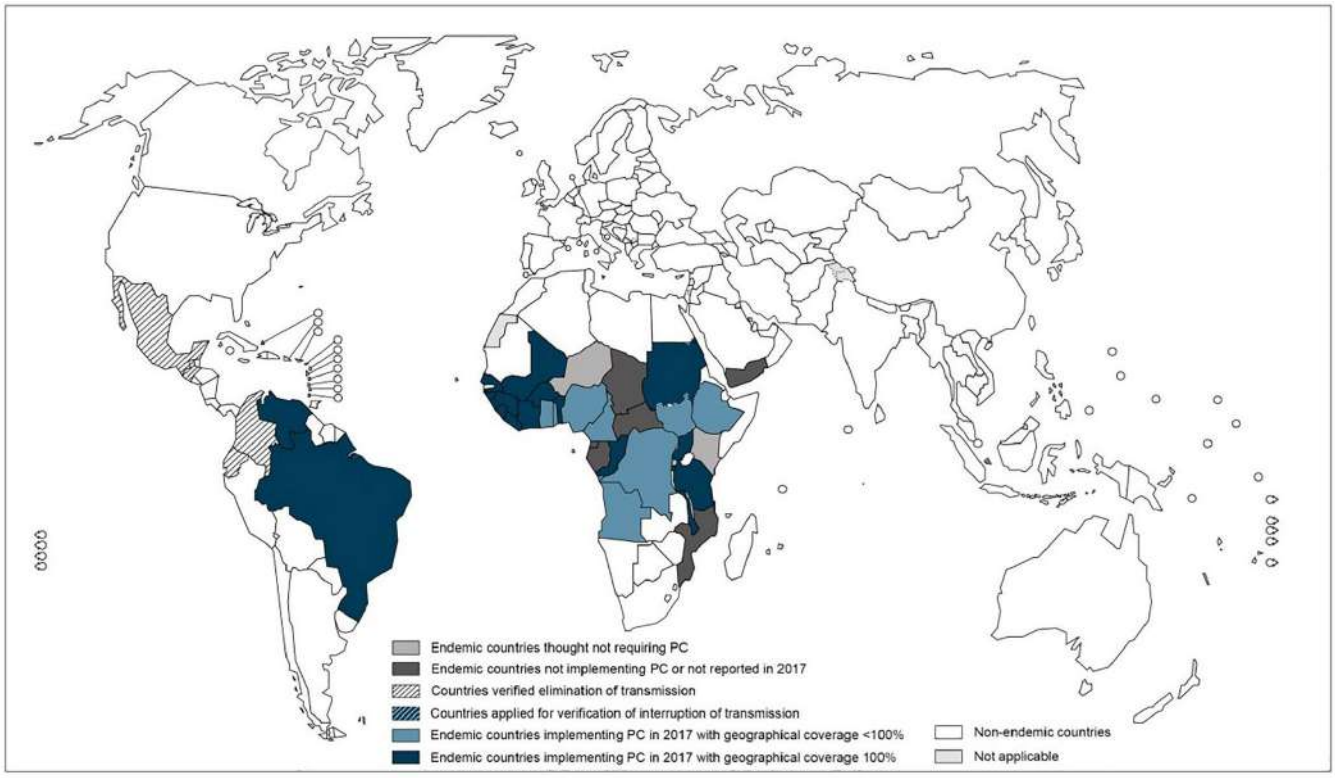

Figure 19. Map of the distribution and status of preventive chemotherapy for onchocerciasis worldwide. Source: Adapted from WHO, https://www.who.int/news-room/fact-sheets/detail/onchocerciasis. Permissions: Cf WHO terms of acceptable uses (non-commercial, educational).

whereas pulmonary dirofilariosis in the New World is associated with *D. immitis* (Dantas-Torres and Otranto, 2013).

The life cycle of filarioids within Dirofilariinae generally includes mosquitoes as intermediate hosts. In *Dirofilaria* spp. juvenile stages develop in Malpighian tubules and, depending on the country and habitat, different genera act as vectors, specifically, *Culex*, *Aedes*, *Anopheles*, *Ochlerotatus*, *Stegomyia*, *Jarnellius*, and *Aedimorphus*. In *Foleyella*, J_2s and J_3s develop in the fat body of mosquitoes, while *Loaina* prefers the hemocoel. In the case of *Loa loa* species, it develops in the horsefly, while *Pelecitus* develops in Mallophaga lice.

As an example of a life cycle, adults of *Pelecitus fulicaeatrae* are found in the tendons near the ankle of coots and grebes. Both male and female *P. fulicaeatrae* worms are short and coiled, and males possess an asymmetrical caudal ala and large pedunculate cloacal papillae. In other species of *Pelecitus*, the caudal alae are symmetrical (Figure 16). Microfilariae are about 92–122 µm-long and occur in the skin and the feathered portions of the lower leg, usually located near the feather follicles (Figure 17). The louse *Pseudomenopon pilosum* (order Amblyocera, suborder Mallophaga) inhabits the base of feathers ingesting tissues of the bird with microfilariae. Second-and third-stage juveniles (J_2 and J_3) of *P. fulicaeatrae* were recovered from the fat body in naturally infected lice. Parasitized lice can be transferred from adult coots to coot chicks, infecting the young birds. Immature adult worms can be found after 20 days post-infection in the ankles, while microfilariae appear in the skin after 7 to 8 months (Bartlett and Anderson, 1989).

Subfamily Onchocercinae: Cause of Several Neglected Diseases in Humans

This subfamily includes more than 30 genera parasitizing mammals. Some of them induce what are termed **neglected diseases** of humans, which is defined by the United States Centers for Disease Control and Prevention (CDC, 2021) as diseases caused by parasites, viruses, or bacteria that cause substantial illness for approximately 15% of the world's inhabitants that results in trapping them in a cycle of poverty.

The worms in this subfamily have a long non-alate tail and dissimilar and unequal spicules (see Figures 3 and 4 above). Adult worms are usually located in the body cavity of the host; however, some species inhabit subcutaneous tissues or the lymphatic vessels and nodes.

Seven species are responsible for infection in humans: *Wuchereria bancrofti, Brugia malayi*, *B. timori*, *Onchocerca volvulus*, *Mansonella (Mansonella) ozzardi*, *M. (Esslingeria) perstans*, and *M. (E.) streptocerca*. Each is described briefly below.

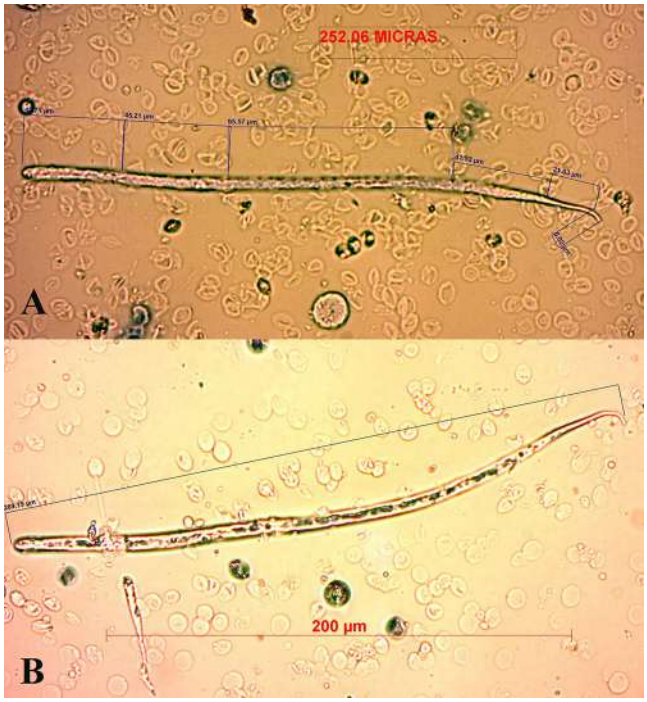

Figure 20. Microfilariae photographs. A) From *Dirofilaria immitis*; B) From *Acanthocheilonema reconditum*. Microfilariae from *D. immitis* is characterized by a cephalic end rounded, straight tip tail, and longer body length; *A. reconditum* possess a cephalic end obtuse, tail J-shaped and shorter body length. Source: S. Costa. License: CC BY-NC-SA 4.0.

Wuchereria bancrofti, Brugia malayi, and *B. timori* cause lymphatic filariasis, or elephantiasis, due to the parasites living in the lymphatic system (vessels and nodes), disrupting the system's normal function. Infection is usually acquired in childhood causing hidden damage to the lymphatic system. Later in life, people develop lymphedema and elephantiasis as well as hydrocele and scrotal elephantiasis. Patients usually suffer physical disability that contributes to poverty (Yonder and Pandey, 2023).

Ninety percent of lymphatic filariasis is caused by *Wuchereria bancrofti*; the remaining 10% by *Brugia malayi* and *B. timori*. Bancroftian filariasis is transmitted by several different species of mosquitoes: *Culex* spp., widespread in urban and semi-urban areas; *Anopheles* spp., mainly found in rural areas; and *Aedes* spp., found on endemic islands in the Pacific. *Brugia malayi* is transmitted by mosquito species of the genus *Mansonia*, whereas *B. timori* is transmitted by *Anopheles* mosquitoes (Anderson, 2000).

Lymphatic filariasis is a major problem in tropical and subtropical countries, extending throughout central Africa, the Nile delta, Turkey, India, Southeast Asia, the East Indies, the Philippine and oceanic islands, Australia, New Guinea, Brazil, Guyana, Venezuela, and some countries in Central America (Figure 18). It is estimated that 120 million people are infected worldwide; of these, almost 25 million men have genital disease and almost 15 million, mostly women, have lymphedema or elephantiasis of the leg (Anderson, 2000).

Onchocerca volvulus specimens are usually found in subcutaneous tissues producing onchocerciasis, or river blindness, which is a filariosis characterized by pruritus, dermatitis, lymphadenopathy, and ocular lesions. It is not a fatal disease; however, it can cause disfigurement of the skin and visual impairment, including permanent blindness. It is transmitted to humans through exposure to repeated bites of infected blackflies of the genus *Simulium*. The WHO (2022) reports that 20.9 million people were infected with *O. volvulus* worldwide in 2020, 14.6 million infected people had skin disease, and 1.15 million had vision loss. Onchocerciasis is distributed in 31 countries of Africa, Yemen, and some countries of Latin America (Figure 19). Implementation of different programs for control, eradication, and treatment of the disease were carried out by the WHO and governments, contributing to Colombia, Ecuador, Mexico, and Guatemala being free of onchocerciasis (WHO, 2022).

Three *Mansonella* species cause mansonellosis. *Mansonella (Mansonella) ozzardi* and *M. (Esslingeria) perstans* reside in body cavities and the surrounding tissues, while *M. (E.) streptocerca* lives in the dermis and subcutaneous tissue. Infections by *M. (E.) perstans* are often asymptomatic, however, are at times associated with angioedema, pruritus, fever, headaches, arthralgias, and neurologic manifestations. Those produced by *M. (E.) streptocerca* can cause skin manifestations, including pruritus, papular eruptions, and skin pigmentation changes. *Mansonella (M.) ozzardi* can cause arthralgias, headaches, fever, pulmonary symptoms, adenopathy, hepatomegaly, and pruritus. Adult filarioids live for several years and reside in various tissues. Biting midges of the family Ceratopogonidae transmit all 3 *Mansonella* species and blackflies of the family Simuliidae play an important role in the transmission of *M. (M.) ozzardi* in Latin America. *Mansonella (E.) perstans* is endemic in Sub-Saharan Africa as well as a northern part of the Amazon rainforest stretching from equatorial Brazil to the Caribbean coast of South America. *Mansonella (E.) streptocerca* is limited to continental Africa, occurring in the tropical rainforest areas of central and west Africa as well as in Uganda, while *M. (M.) ozzardi* has a patchy geographic distribution across Latin America. It has been recorded from southern Mexico to northwestern Argentina, but has not been reported in Chile, Uruguay, or Paraguay. The parasite also occurs on several Caribbean islands and elsewhere in Latin America (Anderson, 2000).

There is a lack of data about the prevalence of the filarioid disease mansonellosis and the morbidity and mortality

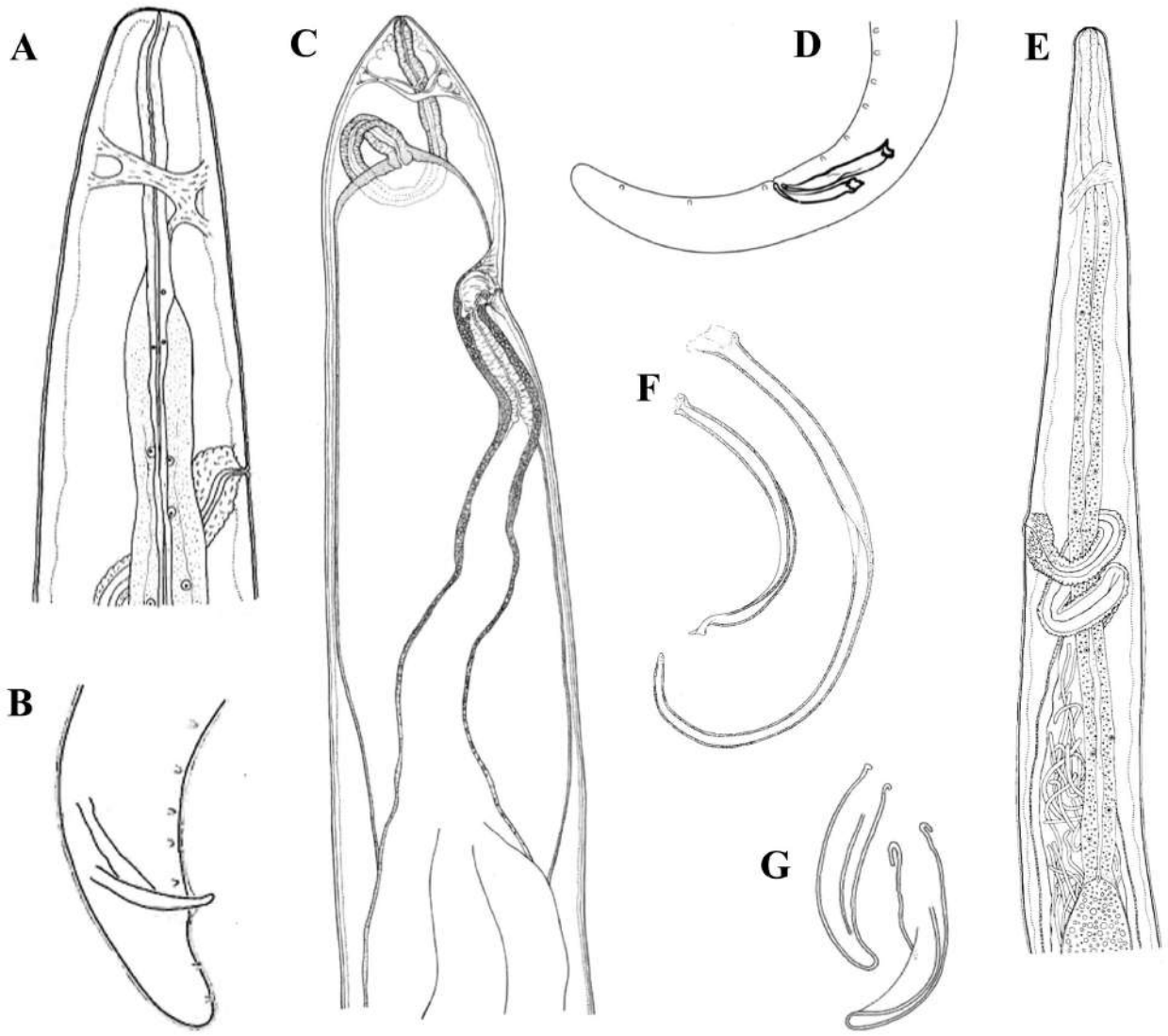

Figure 21. Examples of Splendidofilariinae species. A, B) *Dessetfilaria guianensis*; A) Female anterior extremity showing the muscular and glandular esophagus and the vulva; B) Male tail, lateral view showing the similar and equal spicules; C, D) *Meningonema peruzzii*; C) Female anterior extremity; D) Male tail, lateral view showing the spicules; E, F) *Chandlerella bushi*. E) Female anterior extremity; F) Spicules, dissimilar; G) *Splendidofilaria chandenieri* spicules, similar and equal. Sources: A, B) Adapted from Bartlett and Bain, 1987; C, D) adapted from Orihel and Esslinger; E, F) adapted from Bartlett and Anderson, 1987; G) adapted from Bartlett and Bain, 1987.

associated with it. The lack of knowledge of these effects is due in part to a high prevalence of asymptomatic cases, the lack of a clinical profile that makes diagnosis difficult, plus the similarity of the microfilariae to *Mansonella (Esslingeria) streptocerca* (in Africa) and *M. (Mansonella) ozzardi* (in Latin America) with that of *Onchocerca volvulus*. Additionally, mansonellosis parasites have been shown to interfere with some onchocerciasis immunodiagnostic assays, may interfere with the diagnostics used in other neglected tropical disease controls, and negatively affect the efficacy of vaccine programs (specifically, HIV and tuberculosis vaccines) (Ta-Tang et al., 2018).

The method for diagnosing lymphatic filariasis and *Mansonella (Esslingeria) perstans* and *M. (Mansonella) ozzardi* diseases is usually the finding of microfilariae in peripheral blood smears thick- or thin-stained with Giemsa or hematoxylin-and-eosin. Concentration techniques, such as Knott's technique or filtration through a Nucleopore® membrane, may also be efficacious in cases with a low burden of microfilariae. As lymphatic filariasis exhibits a nocturnal periodicity, an accurate diagnosis is best achieved on smears collected at night (10:00 pm–2:00 am). For *Mansonella* this is not necessary because it is a non-periodic filariasis. These

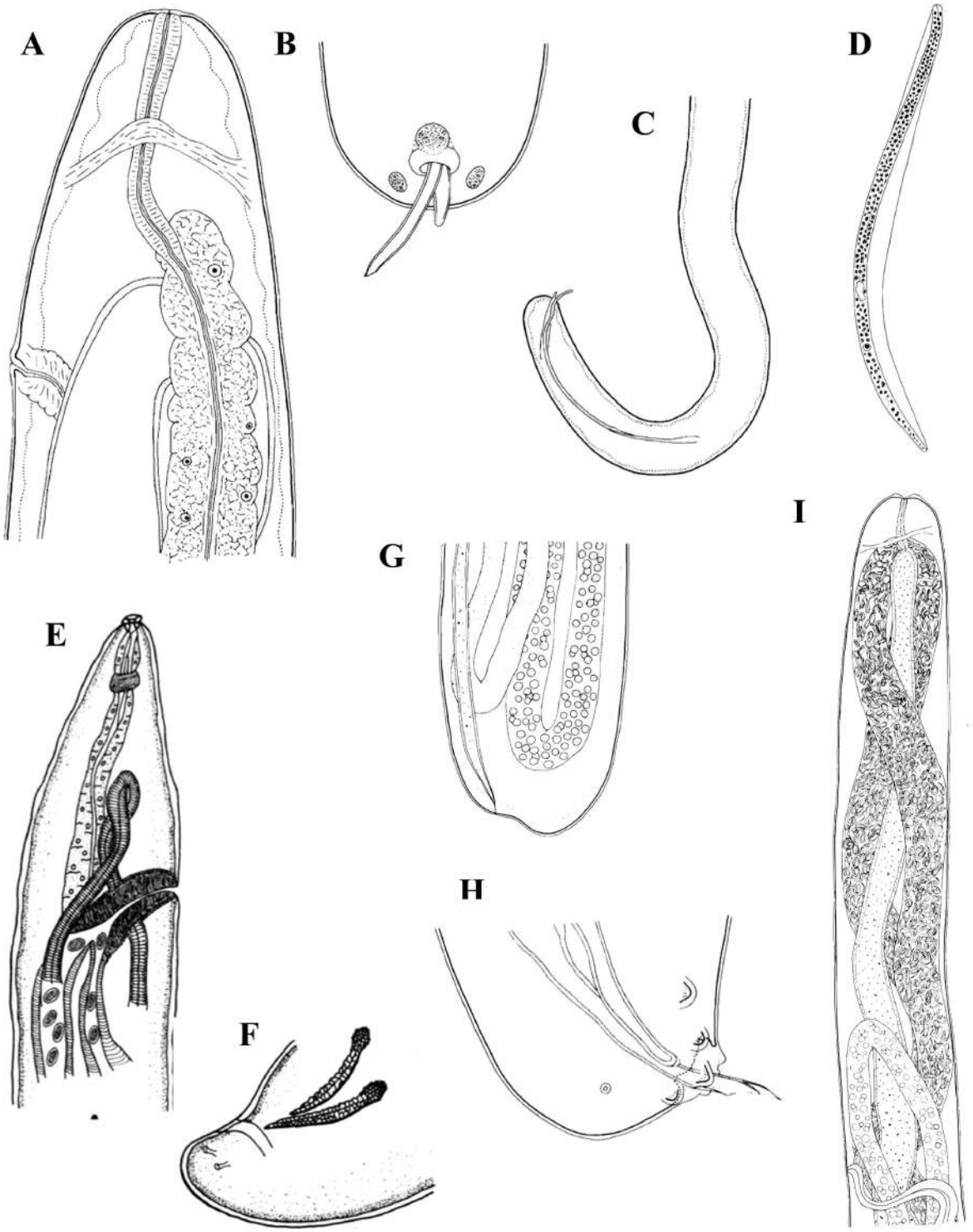

Figure 22. Examples of Lemdaniinae species. A–D) *Lemdana wernaarti*; A) Female anterior extremity showing the differentiation of the muscular and glandular esophagus and position of the vulva; B) Male tail with cloacal papillae; C) Male posterior extremity with dissimilar spicules; E, F) *Aprocta intraorbitalis*. E) Female anterior extremity; F) Male tail with similar spicules and cloacal papillae. G–I) *Eulimdana lari*. G) Female tail showing the anus at tip tail; H) Male tail with similar spicules and cloaca at tip tail; I) Female anterior extremity showing the differentiation of the muscular and glandular esophagus and uterus full of micrifilariae. Sources: A–C) Adapted from Bartlett and Anderson, 1987; E, F) adapted from Hernandez-Rodriguez et al 1986; G–I) adapted from Bartlett et al., 1985.

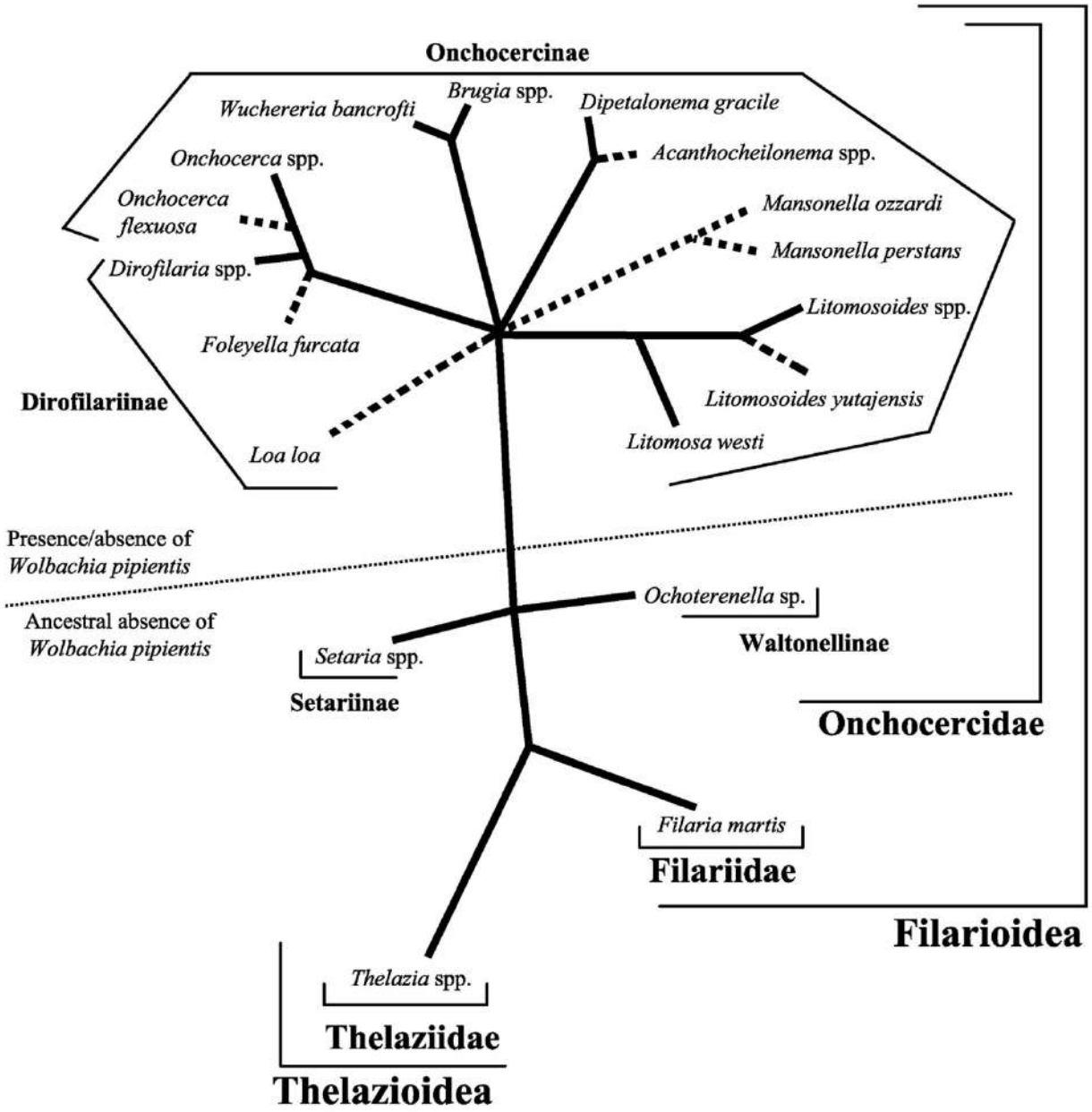

Figure 23. Hypothetical evolution of *Wolbachia pipientis* infection mapped on the phylogenetic tree of Filariae and related nematodes. *Wolbachia pipientis* could have been ancestrally absent from the lineages leading to *Thelazia* spp., *Filaria martis*, *Setaria* spp. and *Ochoterenella* spp. and it could have been acquired on the lineage leading to the Onchocercidae family, and then lost several lineages (*Litomosoides yutajensis*, *Mansonella* spp., *Acanthocheilonema* spp., *Onchocerca flexuosa*, *Foleyella furcata*, and *Loa loa*, dashed lines). The positions of *Mansonella* spp. and *O. flexuosa* are based only on their taxonomic affiliations. Source: Adapted from Casiraghi et al., 2004. License: CC BY-NC-SA 4.0.

methods are cheaper and faster than those using antigen detection, such as the immunoassay for circulating filarial antigens, which can be detected in blood samples collected at any time of day, unlike microfilariae with nocturnal periodicity. However, in many countries, antigen detection diagnosis tests are not licensed, making the diagnosis more difficult due to the similarity of the microfilariae species in areas with several filarioses. Adults may be identified in biopsied specimens of lymphatic tissue. For the pathogens causing onchocerciasis and *M. (E.) streptocerca*, diagnoses are performed by detection of microfilariae in skin snips or adults in biopsy specimens of skin nodules. Microfilariae of *Onchocerca* do not exhibit any periodicity, similarly to *Mansonella* (CDC, 2020).

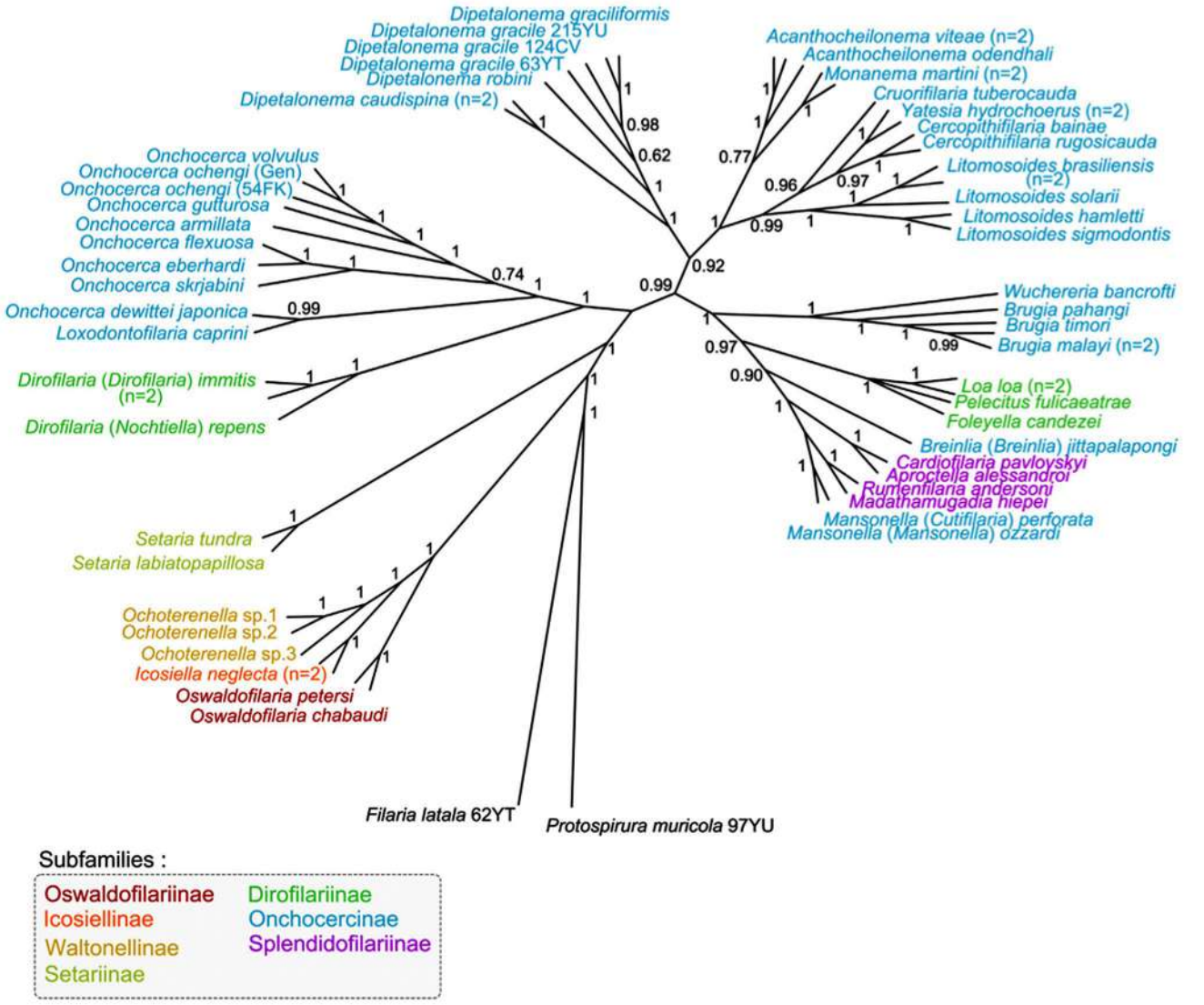

Figure 24. Phylogeny of family Onchocercidae based on partitioned concatenated datasets of 12S rDNA, coxI, rbp1, hsp70, myoHC, 18S rDNA, and 28S rDNA sequences using Bayesian inference. The total length of datasets is approximately 4,950 bp. Sixty onchocercid specimens (representing 48 species) were analyzed. *Filaria latala* and *Protospirura muricola* were used as outgroups. The topology was inferred using Bayesian inference. Nodes are associated with Bayesian posterior probabilities based on one run of 5 million generations. The onchocercid subfamilies are indicated by colors: Blue for Onchocercinae, dark green for Dirofilariinae, purple for Splendidofilariinae, pale green for Setariinae, yellow for Waltonelliinae, orange for Icosielliinae, and red for Oswaldofilariinae. Source: Adapted from Lefoulon et al., 2015. License: CC BY-NC-SA 4.0.

Among filarioids infecting dogs, *Acanthocheilonema reconditum* is a species whose microfilariae have been frequently confused with *Dirofilaria immitis* (Figure 20). This species has also a global distribution. It is important to have a correct identification of the microfilariae since *A. reconditum* is a non-pathogenic species. Adults parasitize the subcutaneous tissues and fascia of canids while microfilariae circulate in blood. The fleas *Ctenocephalides felis* and *C. canis* are the vectors for *A. reconditum*, whereas the role of ixodid ticks (that is, *Rhipicephalus*) as vectors for this filarioid species has been definitively rejected. The full development of microfilariae to the J_3 infective forms occurs in experimental infected fleas in about 15 days. Moreover, the localization and size of developing juveniles inside the infected flea suggest that this arthropod might act as an intermediate host through the ingestion of infected fleas rather than inoculation during a blood meal on dogs. This route of *A. reconditum* transmission is unique, differing from that of other filarioids, which are actively transmitted through the bites of mosquito vectors (Anderson, 2000).

The remaining filarial genera within the Onchocercinae are parasites of mammals, with the exception of *Macdonaldius* which is a parasite of reptiles. Most of the genera display a distribution restricted to a continent. For example, *Litomosoides*, a parasite of the body cavity of bats, rodents, and marsupials represented by 42 species, is distributed on the American continents from the southern United States to central Argentina, while *Dipetalonema* sensu stricto, with 6 species, and *Mansonella (Tetrapetalonema)*, with 13 species, parasitize Platyrrhinii monkeys from Central America to parts of South America. Other genera are monospecific, such as *Orihelia anticlava* which is widespread in South America and is a parasite of the body cavity from armadillos. In contrast, *Filarissima lainsoni* and *Migonella fracchiai* are only known by single unique records from a Brazilian coendou and a Paraguayan bat, respectively (Anderson, 2000).

Subfamily Splendidofilariinae

Members of this subfamily parasitize reptiles, birds, and mammals, including humans. It includes 21 genera with more than 90 species. Females contain a vulva in the anterior region of the body, the tail in both sexes is relatively long, caudal alae are absent, and the spicules are little different in size and morphology (Figure 21). Worms may be found in the body cavity of birds, sometimes in capsules in hidden locations (such as in *Madathamugadia*), while in reptiles they have been found in the mesenteric vessel of the intestine, the heart of turtles (such as in *Cardiofilaria*), or subcutaneous tissues in gecko lizards (such as in *Thamugadia*). In mammals they may be found in the body cavity (as in *Micipsella*) or the central nervous system (as in *Meningonema*) (Anderson, 2000).

The life cycle of these filarioids involves different vectors, such as mosquitoes in *Aproctella* and *Cardiofilaria*, or ornithophilic ceratophogonids in *Chandlerella* and *Splendidofilaria*. *Splendidofilaria fallisensis*, a parasite of the subcutaneous tissues of wild and domestic ducks of North America, utilize ornithophilic simuliids of the genus *Simulium* as vectors. Blackflies crawl under the feathers and engorge on infected blood. Microfilariae penetrate the stomach of the simuliid and develop in the haemocoel. After 7 to 14 days, depending on the temperature, J_3 appears near the mouthpart of the blackfly. Microfilariae appear in the blood of the ducks 30–36 days after they are inoculated with J_3, whereas in *S. californiensis*, a parasite of the heart from California quail, the prepatent period is about 6 months.

The genus *Meningonema* is commonly found parasitizing the central nervous system of African Cercopithecinae, however Boussinesq and colleagues (1995) reported the first human case in 1995. The authors recovered a fourth-stage juvenile (J_4) female from the cerebrospinal fluid of a patient from Cameroon harboring *Loa loa*, but who did not exhibit any neurological symptoms. In another study, microfilariae recovered from patients with cerebral filariasis in Zimbabwe identified as *Mansonella perstans* may have been infected instead with *Meningonema* and not the former species. In fact, microfilariae of *Meningonema* have not been confirmed in humans, although they have been found in the peripheral blood of monkeys; therefore, careful examination of blood samples might reveal that *Meningonema* infection is actually a frequent zoonosis in humans (Boussinesq et al., 1995).

Subfamily Lemdaniinae

Filarioids in this subfamily tend to parasitize birds and, less commonly, reptiles and mammals. Adults are characterized by a subterminal anus in both females and males, an absent buccal capsule, and spicules that are similar in size and form, such as in *Eulimdana*, or may be markedly different, as in *Lemdana* or *Makifilaria* (Figure 22). These filarioids develop in Mallophaga lice, such as *Eufilaria bartlettae*, a parasite of the blackbird (*Turdus merula*) and in mosquitoes of the genus *Anopheles*, such as *Saurocitus agamae*, a parasite of the lizard *Agama agama*.

***Wolbachia* Bacteria in Filarioids**

Intracellular bacteria belonging to the genus *Wolbachia* were discovered in filarial nematodes and arthropods in the 1970s with the advent of electron microscopy (Kozek and Figueroa Marroquin, 1977). In arthropods, *Wolbachia pipientis* generally induces alterations in host reproduction, acting as a tool to manipulate pest insects; while in filarioid nematodes, the evidence shows that these bacteria are required for development and reproduction (Stouthamer et al., 1999). *Wolbachia pipientis* bacteria are typically contained in a host-derived vacuole, inhabiting the hypodermic cells and the reproductive tissues of the female filarioid worm. Bacteria have also been recovered in juvenile filarioid stages, but not in male worms. This suggests a vertical mode of transmission through the cytoplasm of the nematode egg, which parallels observations in *Wolbachia* of arthropods. The bacteria may be present in the tissues alone, in small groups of bacteria, or in large groups that fill their cellular environment.

Data generated through electron microscopy and immunohistochemical examinations have helped to elucidate the presence or absence of *Wolbachia pipientis* in filarial species. In the 1990s, PCR amplification and sequencing of the *Wolbachia* genome showed that *Wuchereria bancrofti*, *Litomosoides sigmodontis*, *Mansonella ozzardi*, and all the species examined in the genera *Dirofilaria*, *Onchocerca*, and *Brugia* harbor *Wolbachia pipientis*.

Phylogenetic analysis by comparison of 16S rDNA sequences from *Wolbachia pipientis* have showed that filarial *Wolbachia* are closely related and form a separate group from the *Wolbachia* of arthropods. *Wolbachia* of filariae segregate into 2 clusters (named C and D), which diverge from the A and B clusters that are recognized for arthropods. Within the C and D filarial *Wolbachia* lineages, the bacterial phylogeny is congruent with the nematode phylogeny. However, in the rodent filaria *Acanthocheilonema viteae*, PCR consistently showed no evidence of *Wolbachia*; this was also the case for *A. reconditum*, microfilariae of *Mansonella perstans* and *Litomosoides yutajensis* (Onchocerciinae); *Loa loa*, and *Foleyella furcata* (Dirofilariinae); *Ochoterenella* spp. (Waltoneliinae); *Setaria equina*, *S. labiatopapillosa*, *S. tundra* (Setariinae), and *Filaria martis* (Filariinae) (Casiraghi et al., 2004).

Mapping the presence or absence of *Wolbachia pipientis* in different species within the subfamilies of the Onchocercidae and Filariidae will support the trees generated by molecular data of filarioids and will help to elucidate the phylogeny of Filariata.

Phylogeny of Filarioid Nematodes

A phylogeny of filarioid nematodes based on morphological characters has been proposed by Anderson and Bain (1976) and Chabaud and Bain (1994). However, due to the convergence of morphological characters among lineages, the phylogenies proposed are not sustainable at all and the proposed evolutionary scenario is weak. The major question regards the classification of the Onchocercidae, and their origin and evolution. Analyses based on molecular characters still are ongoing. A huge amount of sequence data is available for pathogenic filarioids (for example, *Wuchereria bancrofti* and *Brugia malayi*) as well as model filarial parasites (such as *Litomosoides sigmodontis* and *Acanthocheilonema vitae*) than for the remaining filarioids, for which data are scarce. The first phylogenetic analyses were conducted for the Onchocercinae and Dirofilariinae. However, biological material is scarce, impeding broad taxonomic sampling, and the markers used (12S rDNA and coxI genes) are not suitable for resolving the internal nodes which would help elucidate the evolution within the Onchocercidae. Lefoulon and colleagues (2015) proposed a robust phylogenetic hypothesis of the relationships within the Onchocercidae based on 7 loci: 2 mitochondrial and 5 nuclear genes of 48 species belonging to 7 subfamilies. These authors concluded that the tree topology is not congruent with the classic systematic delineations and the present phylogeny neither supports the monophyly of the Dirofilariinae, Onchocercinae, nor Splendidofilariinae (Figure 24) (Lefoulon et al., 2015).

Future studies including other sequence data, the presence or absence of *Wollbachia pipientis*, and more species within the order Filariata are necessary for the elucidation of the phylogeny of this group.

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Nemata

Strongyloidea and Trichostrongyloidea (Superfamilies): Bursate Nematodes

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Phylum Nemata

Superfamily Strongyloidea

Superfamily Trichostrongyloidea

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Chapter 56

Strongyloidea and Trichostrongyloidea (Superfamilies): Bursate Nematodes

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Introduction

The bursate nematodes include those within the superfamilies Strongyloidea Baird, 1853 and Trichostrongyloidea Cram, 1927. Even though there are new data showing various phylogenetic relationships among and between species in these groups, they will be discussed here under the umbrella of bursate nematodes for the sake of simplicity. Bursate nematodes are distinguished by the presence of a copulatory bursa on the posterior end of the male (**bursa** = purse or pouch; Latin).

In general—although with exceptions—bursate nematodes have relatively stout bodies with a muscular **esophagus** that is not divided into various parts but is narrower at the anterior end and more bulbous and expanded toward the posterior end where the esophagus attaches to the **intestine**. There is always an encircling **nerve ring** around the esophagus and—as in almost all nematodes—the nerve ring slants more posteriad on the ventral aspect of the nematode and slants more anteriad on the dorsal aspect. The **excretory pore** exits the **cuticle** in the general vicinity of the nerve ring and is always situated ventrally (Figure 1). A morphological feature—which is in fact a synapmorphy for the bursate nematodes—is the possession of a **copulatory bursa** in males that is composed of muscular **rays** with **cuticular membranes** connecting them (Figure 2). The copulatory bursa consists of laterally-projecting **cuticular extensions** that surround the **tail** of males in species assigned to both superfamilies, and this structure serves as a grasping/sensory organ equipped with **sensory muscular papillae** (Figure 3). The bursa grasps the female during copulation and enables the male to extend the **spicule** or spicules (Figure 4) into the **reproductive tract** of the female thus facilitating the transfer of ameboid sperms to the female reproductive system. The bursa surrounds the spicules and the **cloaca** (which is the joint opening that drains the intestinal and reproductive systems; Gardner et al., 1994b).

Most species of bursate nematodes that are parasites in the intestines of vertebrates have direct life history patterns and only the definitive host is needed for the parasite to reach sexual maturity; however, some species that occur in organs or tissues such as lungs, muscles, or the central nervous system of their vertebrate hosts, have indirect life history patterns. Examples are species of *Angiostrongylus*, which are normally parasites of rodents, but can infect people living in tropical and subtropical regions with devastating neurological consequences. These species use land-dwelling molluscs (snails and slugs) as intermediate hosts (Alicata, 1991).

Following are discussions of the 2 superfamilies and a few of the highly numerous other families. For additional information on other groups of these animals, see Travassos (1937), Anderson (2000), and the CIH keys by Anderson and colleagues (2009).

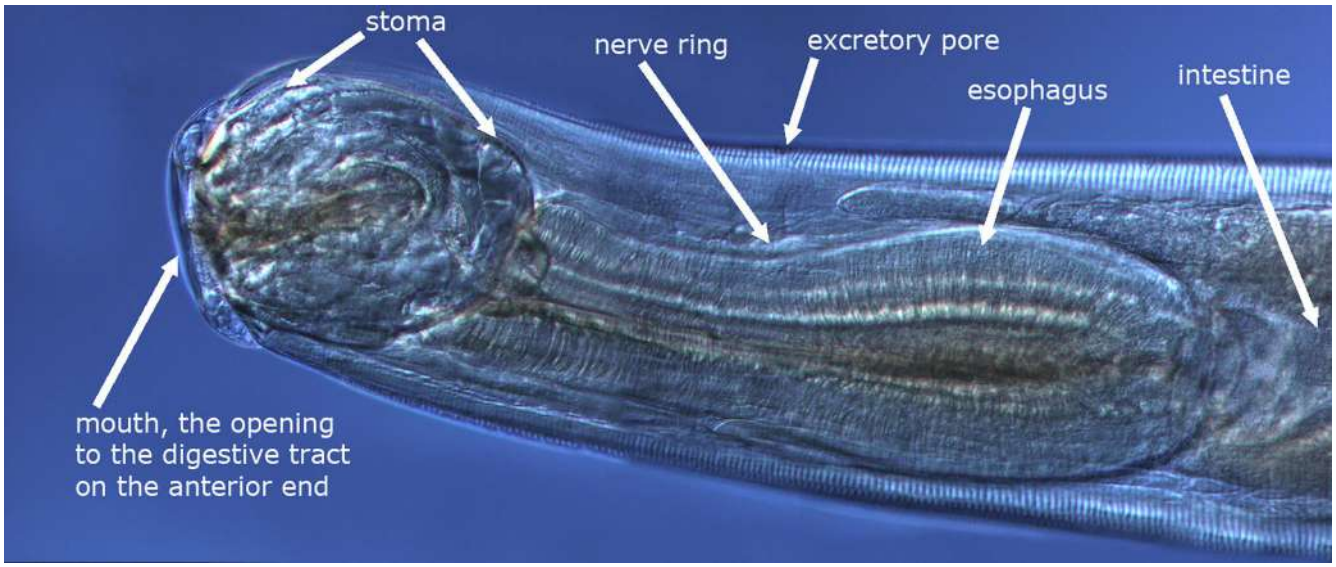

Figure 1. Anterior end of a specimen of *Ransomus rodentorum* with structures labeled. Normarsky micrograph. Source: S. L. Gardner, HWML. License: CC BY 4.0.

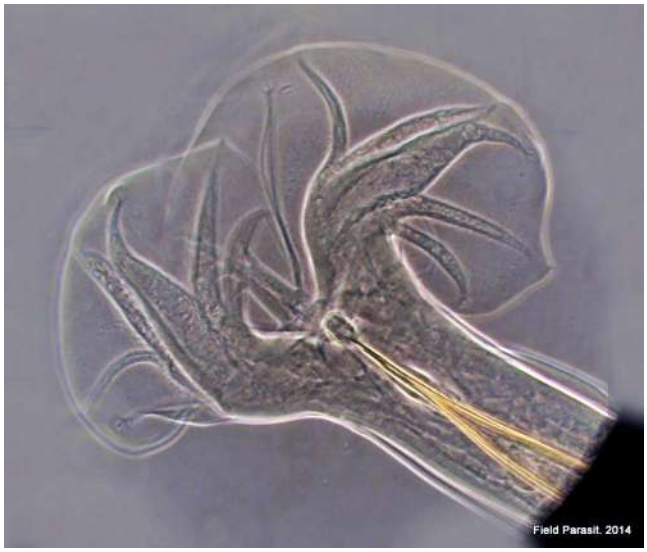

Figure 2. Copulatory bursa of *Vexillata armandae*, a parasite of the small intestine of the coarse-haired pocket mouse *Chaetodipus hispidus*. Collected and imaged at Cedar Point Biological Station, near Ogallala, Nebraska, United States. Source: S. L. Gardner, HWML, 2014. License: CC BY 4.0.

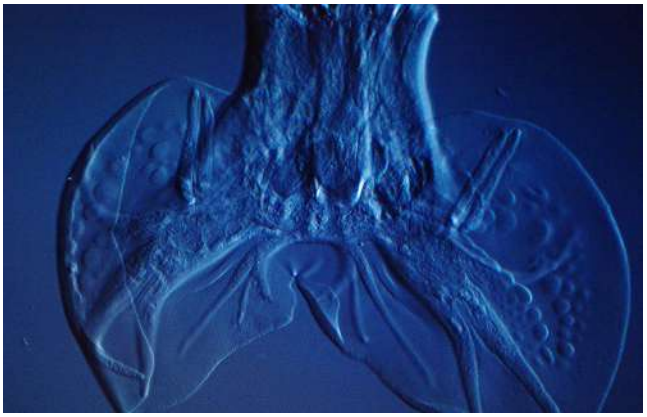

Figure 3. Copulatory bursa of a male of a species of trichostrongyloid nematode from *Ochotona princeps* (order Strongylida, superfamily Trichostrongyloidea) collected in Colorado, United States. Source: D. Tufts, HWML, 2013. License: CC BY 4.0.

Superfamily Strongyloidea Baird, 1853

The strongyloids (also known as strongyles; superfamily Strongyloidea Baird, 1853) comprise a diverse group of parasitic nematodes with a cosmopolitan distribution in vertebrates. Nematodes classified in the superfamily Strongyloidea are defined by several characteristics that are well-established **synapomorphies** (meaning, shared derived characters) for the group, as listed above.

Following are discussions of 2 families within the Strongyloidea, families **Ancylostomatidae** and **Strongylidae**, and several species within those. From the standpoint of human health, the more important of these 2 families is the Ancylostomatidae, commonly known as hookworms.

Hookworms: Family Ancylostomatidae

The ancylostomatid nematodes are commonly known as **hookworms** because of the initial name given them by Goeze (1782), who noted membranous expansions with 2 rib-like structures on the tail of the males, which were collected from the intestine of a European badger (*Meles meles*). Frölich (1789) found similar worms in foxes, also with membranous

Figure 4. Posterior end of male *Ransomus rodentorum* showing bursa and twin/paired spicules. Source: S. L. Gardner, HWML. License: CC BY 4.0.

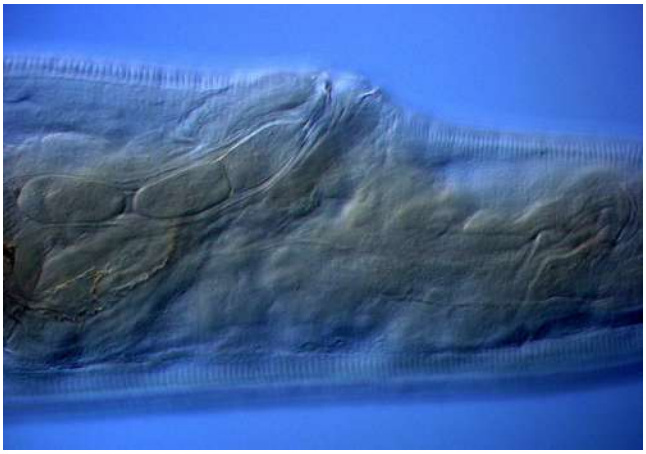

Figure 5. Posterior end showing ovijector of the same individual of *Ransomus* as shown in Figure 1 above. Eggs can be seen being expelled from the body via the muscles of the ovijector. Normarsky micrograph. Source: S. L. Gardner, HWML. License: CC BY 4.0.

expansions in the tails. Frölich called these nematodes Hakenwurm (= hookworm; German).

In their mammalian hosts (including humans), adult hookworms usually live in the duodenum (the anterior part of the small intestine), where they attach to the mucosa, pulling mucosa into their **stomata** and abrading the villi with **cuticularized cutting teeth** or **plates** (Figure 6, left). The cuticularized plates of the stoma rupture the capillaries of the mucosa and the nematode pumps blood rapidly into its intestine with the muscular esophagus. Little of the blood that is extracted from the host is digested or used by the nematode and most of the blood that is pumped out of the capillary beds of the host pass through the intestine of the nematode, out the anus, and directly back into the lumen of the host's gut. As can be seen clearly in the video (available at hookworm.vob (Japan National Institute of Health, n. d.), worms feed heavily on blood, most of which is wasted as it passes directly out of the worms out into the lumen of the intestine, where most of the blood is digested and reabsorbed.

As mentioned above, species in the family Ancylostomatidae are important human pathogens. They generally afflict people in warm-temperate, subtropical, and tropical areas of the world living without access to adequate sanitation facilities and without ready access to shoes (Loukas et al., 2016). Because the main 3 species of hookworms that reside in humans have similar life-histories, pathogenicity, control schemes, symptomatology, and epidemiology, they are treated below as a single topic, with notations where important variations occur. See Table 1 for a list of valid species of *Ancylostoma* (see Drabik and Gardner, 2019). *Necator americanus* is also of concern with respect to human health and will be discussed below, as well.

General Morphology

Ancylostomatid nematodes are robust with the anterior extremity reflexed dorsad. The esophagus is muscular and club shaped with a narrow anterior end and a more swollen posterior part. Esophageal glands are located in the pseudocoel extending posteriad. Cervical papillae or deirids are present near the nerve ring. These nematodes are dioecious with males having a conspicuous and well-developed copulatory bursa, consisting of 2 broad lateral lobes with lateral rays and a smaller dorsal lobe with a multi- or bifurcating dorsal ray (depending on the species) (Figure 6). The bursal rays found laterally are termed the externolateral rays. The dorsal ray is situated in the dorsal part of the bursal lobe. The rays

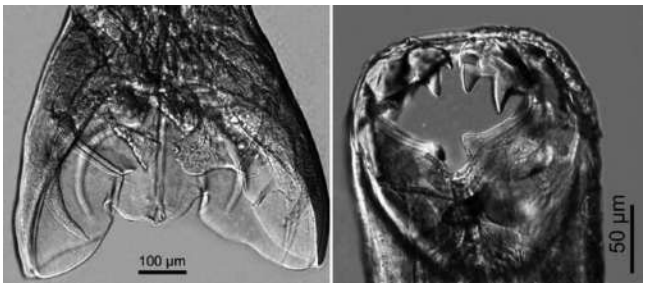

Figure 6. Left image: Posterior end of a male specimen of *Ancylostoma* from a tuco-tuco (a rodent of the genus *Ctenomys*) in Bolivia. The rays and vellum make up the copulatory bursa. Right image: Anterior end showing the cuticularized hooks of the stoma of the same species of nematode. Source: S. L. Gardner, HWML. License: CC BY 4.0.

Table 1. Species of *Ancylostoma* from mammals including known original hosts, zoogeographic region of occurrence, and nearest approximate geographic collection locality of type specimens. Zoogeographic regions follow Wallace, 1876. Source: Adapted from Drabik and Gardner, 2019. License: CC BY 4.0.

Species of *Ancylostoma*	**Host**	**Biogeographic region**	**Type locality**
A. aliuropodae Xie et al., 2017	*Ailuropoda melanoleuca* (David, 1869)	Palearctic	Fengtongzai Nature Reserve, China
			Brazil
A. bidens Molin, 1861	*Nasua nasu* (Linnaeus, 1766), *Procyon cancrivorous* (Linnaeus, 1766)	Neotropical	Brazil
A. braziliense de Faria, 1910	Canidae, Felidae	Cosmopolitan	Brazil
A. buckleyi Le Roux and Biocca, 1957	*Felis concolor* (Linnaeus, 1771), *Cerdocyon thous* (Linnaeus, 1766)	Neotropical	Leticia Amazonas, Colombia
A. caninum (Ercolani, 1859)	Canidae	Cosmopolitan	Turin, Italy
A. ceylanicum Looss, 1911	Canidae, Felidae, *Homo sapiens*	Ethiopian, Oriental, Palearctic	Sri Lanka
A. conepati (Solanet, 1911)	*Conepatus chinga* (Molina, 1782)	Neotropical	Buenos Aires, Argentina
A. ctenomyos	*Ctenomys steinbachi* Thomas, 1907, *C. boliviensis* Waterhouse, 1848	Neotropical	Bolivia
A. duodenale (Dubini, 1843)	*Homo sapiens* Linnaeus, 1758	Cosmopolitan	Milan, Italy
A. galogoi van der Berghe, 1936	*Otolemur crassicaudatus* (Geoffroy Saint-Hilaire, 1812)	Ethiopian	East Central Africa
A. genettae Macchioni, 1995	*Genetta genetta* (Linnaeus, 1758)	Ethiopian, southeast Palearctic	Scebeli River, Somalia
A. gilsoni Gedoelst, 1917	*Sciurus prevosti* (Desmarest, 1822)	Oriental	Malaysia
A. hescheleri Mönnig, 1938	*Orycteropus afer* (Pallas, 1766)	Ethiopian	South Africa
A. iperodontatum Le Roux and Biocca, 1957	*Acinonyx jubatus* (Schreber, 1775)	Ethiopian	Zambia
A. japonica Fukuda and Katsurada, 1925	*Homo sapiens* Linnaeus, 1758	Palearctic	Japan
A. longespiculatum Mönnig, 1938	*Felis silvestris* Schreber, 1777	Palearctic	South Africa
A. malayanum Alessandrini, 1905	*Ursus* sp.	Palearctic, Oriental	Southeast Asia
A. martinaglai Mönnig, 1931	*Canis mesomelas* Schreber, 1775	Ethiopian	South Africa
A. mephitis Micheletti, 1929	*Ictonyx striatus* (Perry, 1810)	Ethiopian	?
A. minimum (von Linstow, 1906)	*Prionailurus rubiginosus* (Geoffroy Saint-Hilaire, 1831)	Oriental	Sri Lanka
A. mucronatum (Molin, 1861)	*Dasypus novemcinctus* Linnaeus, 1758	Neotropical	Brazil
A. mycetis (Molin, 1861)	*Alouatta* sp.	Neotropical	Brazil
A. paraduodenale Biocca, 1951a	*Leptailurus serval* (Schreber, 1776)	Ethiopian	Zambia—Rome Zoo
A. pluridentatum (Alessandrini, 1905)	*Felis* spp.	Neotropical	Brazil
A. protelesis Macchioni, 1995	*Proteles cristatus* (Sparrman, 1783)	Ethiopian	Scebeli River, Somalia
A. somaliense Macchioni, 1995	*Canis mesomelas* Schreber, 1775	Ethiopian	Scebeli River, Somalia
A. taxidae Kalkan and Hansen 1966	*Taxidea taxus* (Schreber, 1777)	Nearctic	Manhattan, Kansas, United States
A. tubaeforme (Zeder, 1800)	*Felis silvestris* Schreber, 1777	Palearctic	—

are species specific (Drabik and Gardner, 2019). The number and general patterns of rays in the copulatory bursa are also a characteristic found in other male rhabditid nematodes, although they are much reduced in free living and insect parasitic forms (Gardner et al., 1994b). As mentioned earlier, all species of nematodes in the superfamilies Strongyloidea and Trichostrongyloidea have very well-developed copulatory bursae (Gardner et al., 1994a). The paired spicules in these nematodes are setaceous in form with a well-developed vellum (Maggenti, 1981).

Females have a simple, conical tail. The vulva is ventrally located and is usually in the posterior one-third of the body and the uterus is didelphic. About 5% of the daily output of eggs is found in the uteri at any one time; the total production is several thousand per day for as long as 14 or more years for a single female.

Life History

As far as is known, species of *Ancylostoma* and *Necator* mature and mate in the small intestine of their host. Eggs are produced by the thousands and embryos within the thin-shelled eggs develop into 2-, 4-, or several-cell stages by the time they are passed with feces. Species infecting humans cannot be identified by egg structure or size. Eggs that pass out into the environment require warmth, shade, and moisture for continued development. Coprophagous insects may mix the feces with soil and air, perhaps hastening embryogenesis, which is completed within 24 to 48 hours in ideal moist conditions. Newly hatched J_1s have a rhabditiform esophagus with a characteristic constriction at the level of the nerve ring and a basal bulb with a valve. Differentiation of hookworm juveniles from those of *Strongyloides* spp. is difficult for a beginning parasitologist.

First stage juveniles living in the feces deposited by their host feed on bacteria therein and molt their cuticle in 2 to 3 days. Second-stage juveniles (J_2), which also have a rhabditiform esophagus, continue to feed and grow and, after about 5 days, molt to the third stage (J_3) filariform-type of juvenile, which is then infective to a mammal. At this point, the second-stage cuticle may be retained as a loose-fitting sheath until penetration of a new host, or the cuticle may be lost just before the juvenile penetrates. Filariform J_3s have a strongyliform esophagus; that is, with a reduced basal bulb that is not separated from the corpus by an isthmus. It has been shown that the J_3s do not feed and they evidently survive on the stored bacterial soup stored in the intestine. Hookworm J_3s are similar to filariform J_3s of *Strongyloides* spp. but can be distinguished by the tail tip, which is pointed in hookworms and notched in *Strongyloides* spp.

Living in the upper few millimeters of soil, J_3s remain in the water film surrounding soil particles and they never survive freezing or drying out. There is a short, vertical migration in the soil, depending on the weather or time of day. When the ground surface begins to desiccate, they migrate a short distance into the soil, staying ahead of the drying soil. Under ideal conditions, they can live for several weeks using this up and down method to stay alive. When the ground surface is wet, after rain or morning moisture condensation in the form of dew, the juveniles wriggle to the surface, remaining in a resting posture until activated (Haas et al., 2005). They are stimulated into sinusoidal motion called "the dance macabre" by a variety of environmental cues, such as touch, vibration, water currents, heat, light, or carbon dioxide. Warmth and moisture stimulate them to stand upright on their tail, waving to-and-fro in a searching behavior termed **questing** or **nictation**. Warmth and fatty acids in skin induce penetration behavior (Haas et al., 2005).

Infection occurs when J_3s contact a host's skin and burrow into it, and they resume feeding at about this time (Hawdon et al., 1993). They usually shed the second-stage cuticle as they penetrate, but the presence of a cuticle does not preclude resumption of feeding (Kumar and Pritchard, 1994). Juveniles can penetrate any epidermis, although parts most often in contact with the soil, such as hands, feet, and buttocks, are most often attacked. *Necator americanus* (and probably other skin-penetrating nematodes) secrete a variety of enzymes that hydrolyze skin macromolecules (Brown et al., 1999; Crompton, 1989; Yu et al., 1995).

After gaining entry to a blood or lymph vessel, juveniles are carried to the liver via the hepatic portal vein, and then to the heart and the lungs via the pulmonary artery. In the lungs, the juveniles break from the venous capillary beds into the air spaces of the alveoli where they molt to the fourth stage (J_4), leaving behind the cuticle like an abandoned collapsed space suit. At this point, the fourth stage juvenile (J_4) now has an enlarged stoma. The fourth stage juveniles (J_4) are carried by ciliary action of the ciliated columnar epithelial cellular lining of the bronchi and bronchioles up the respiratory tree to the glottis where they coughed up by the host, and—if they are lucky—they may be swallowed and finally arrive in the small intestine. There they attach to the mucosa with their enlarged stoma, begin to grow, and then molt to the adult stage. After further growth, they become sexually mature and the male grasps the female with his copulatory bursa transferring ameboid sperm into the genital tract of the female (Williamson et al., 2003).

At least 5 weeks are required from the time of infection via penetration through the host's epidermis to the beginning of

egg production in the intestine. However, it has been shown that juveniles of *Ancylostoma duodenale* can undergo developmental arrest for up to 38 weeks, their maturation perhaps coinciding with the seasonal return of environmental conditions favorable to transmission (Behnke, 1987). *Ancylostoma caninum*, a widespread hookworm of dogs and other carnivores, manifests developmental arrest or stasis during its tissue migration and then is reactivated in female dogs when they begin lactation, resulting in transmammary transmission to pups (Arasu, 2001). Reactivation of the juveniles is modulated by estrogen and prolactin (Hotez et al., 2004).

Many species of hookworms across several genera occur in humans and domestic and wild mammals globally (Drabik and Gardner, 2019). Hookworms represented by several species infect approximately 500 million people worldwide and are responsible for much morbidity and mortality globally (Loukas et al., 2016).

Following are more details about of some the species of Ancylostomatidae, including *Necator americanus* (Stiles, 1902), *Ancylostoma duodenale* (Dubini, 1843), *A. ceylanicum* Looss, 1911, and *A. caninum* (Ercolani, 1859), especially the implications of their effect on human health.

***Necator americanus* (Stiles, 1902)**

The Latin name for this species translated literally means American killer. This species is also called the New World hookworm and was first discovered in Brazil and then Texas, United States, but it was later found indigenous in Africa, India, Southeast Asia, China, and the southwest Pacific islands. It probably came to the New World with the trade in enslaved people in the 16th through 19th centuries, with both enslaved individuals and their captors contributing to the importation of the pathogen.

This nematode has caused much human suffering and has had a significant negative impact on the economic development of the southern United States as well as other regions of the world in which it occurs (Loukas et al., 2016). Primarily a parasite of tropical and subtropical regions, *Necator americanus* is the most common species of hookworm in humans in most of the world, accounting for about 85% of recorded infections (Hotez et al., 2010). Prior to effective hookworm control in the United States, about 95% of hookworms in the southern states were this species (Behnke, 1987; Loukas et al., 2016).

Necator americanus has a pair each of dorsal and ventral cutting plates surrounding the anterior margin of the stoma (Looss, 1911). In addition, a pair each of subdorsal and subventral teeth are near the rear of the stoma. The duct of the dorsal esophageal gland opens on a conspicuous cone that projects into the stoma. Males are 5 mm- to 9 mm-long with filariform/needlelike spicules that have minute barbs at their tips and are fused distally. Females are 9 mm- to 11 mm-long and their vulva is located in about the middle of their body with a single individual producing about 5,000 to 10,000 eggs per day (Behnke, 1987; Loukas et al., 2016).

***Ancylostoma duodenale* (Dubini, 1843)**

As noted above, *Ancylostoma duodenale* (Looss, 1911; see Figure 7) has a tropical and subtropical worldwide distribution (Loukas et al., 2016). It is known in mines as far north as England and Belgium. Since Lucretius, in the 1st century CE, it was known to cause serious anemia in miners. Mines offer an ideal habitat for egg and juvenile development because of their constancy in temperature and humidity. The problem is apt to occur whenever miners defecate on the open ground, outside of established latrines (Cumming and White, 1917).

The anterior margin of the stoma of *Ancylostoma duodenale* has 2 ventral plates, each with 2 large teeth that are fused at their bases (Looss, 1911). A pair of small teeth is found in the depths of the capsule. The duct of the dorsal esophageal gland runs in a ridge in the dorsal wall of the buccal capsule and opens at the vertex of a deep notch on the dorsal margin of the capsule.

Adult males are 8 mm- to 11-mm-long and have a bursa characteristic for the species. The needlelike spicules have simple tips and are never fused distally. Females are 10 mm- to 13 mm-long, with the vulva located about a third of the body length from the posterior end. A single female can lay from 10,000 to 30,000 eggs per day (Hotez and Pritchard, 1995).

This is the first hookworm for which the life history was fully studied and understood. To demonstrate this early work on this organism, following is a lengthy excerpt from Arthur Looss (1911) from his monograph on the morphology and life cycle of *Ancylostoma duodenale*, based on his work in Egypt in 1896 he wrote:

> In order to study in greater detail the very earliest changes in the larvae after their arrival in a host, without sacrificing large experimental animals, I had attempted to introduce the larvae into rats and guinea pigs, partly along with food or drink …. These attempts never gave rise to a settlement of the larvae in the intestine of the experimental animals … [but the larvae] evidently remained alive for a long time. The subsequent history of these larvae was not investigated further, since in the meantime my attention was drawn in another direction. While engaged on one of the experiments, … a drop of the fluid fell on my left hand between the roots of two fingers. I paid no attention to this moisture which dried up of itself within a few minutes. At the

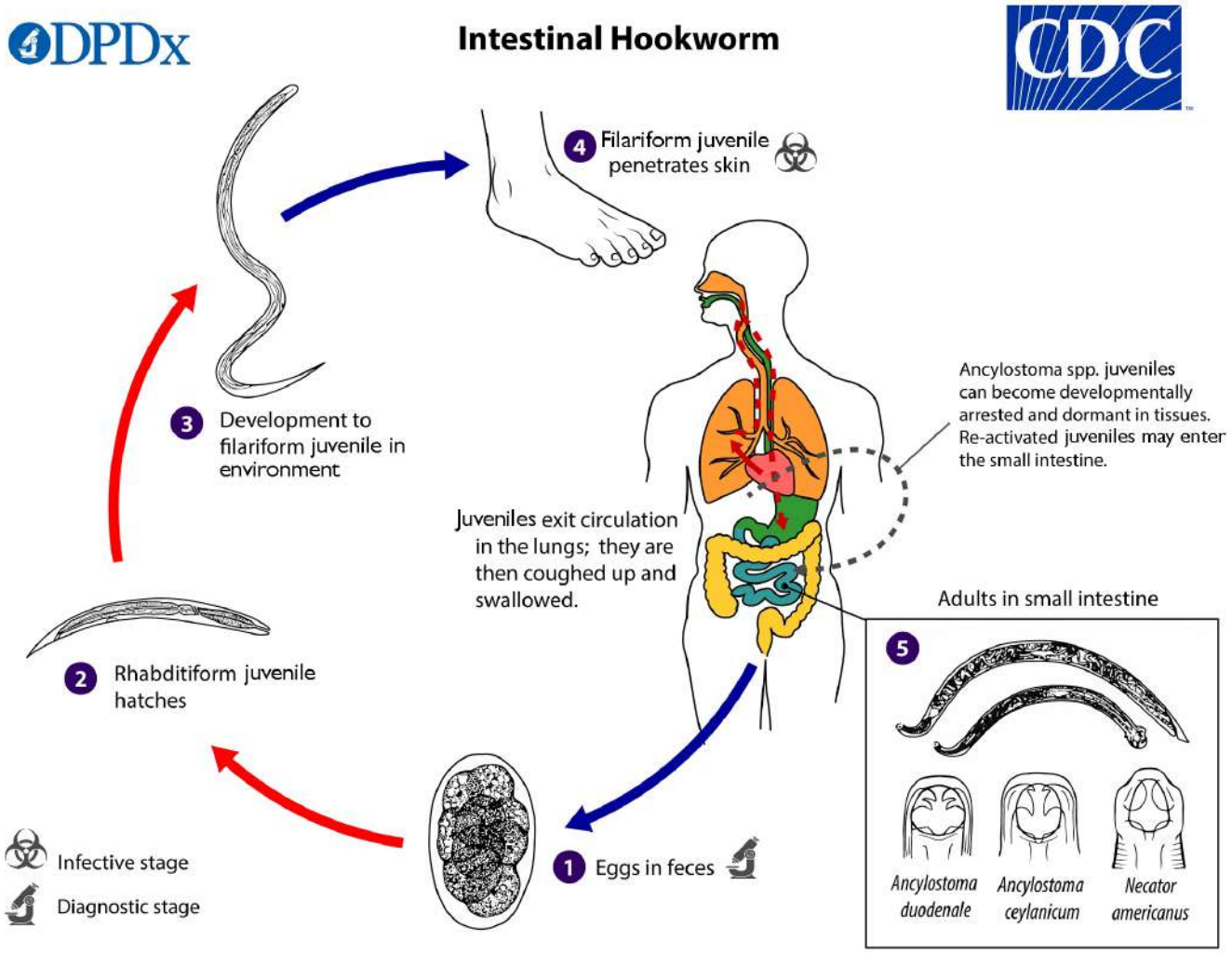

Figure 7. Hookworm (intestinal) causal agents and life cycle. Intestinal hookworm disease in humans is caused by *Ancylostoma duodenale*, *A. ceylanicum*, and *Necator americanus*. Classically, *A. duodenale* and *N. americanus* were considered the 2 primary intestinal hookworm species worldwide, but newer studies show that a parasite infecting animals, *A. ceylanicum*, is also an important emerging parasite infecting humans in some regions. Occasionally juveniles of *A. caninum*, normally a parasite of canids, may partially develop in the human intestine and cause eosinophilic enteritis, but this species does not appear to reach reproductive maturity in humans. Another group of hookworms infecting animals can penetrate the human skin causing cutaneous larva migrans (*A. braziliense*, *A. caninum*, *Uncinaria stenocephala*). Other than *A. caninum*, these parasites do not develop further after their juveniles penetrate human skin. Eggs are passed in the stool (1), and under favorable conditions (moisture, warmth, shade), juveniles hatch in 1 to 2 days and become free-living in contaminated soil. These released rhabditiform juveniles grow in the feces and/or the soil (2), and after 5 to 10 days (and 2 molts) they become filariform (third-stage) juveniles that are infective (3). These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, typically bare feet, the juveniles penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed (4). The juveniles reach the jejunum of the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, typically the distal jejunum, where they attach to the intestinal wall with resultant blood loss by the host (5). Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years. Some *A. duodenale* juveniles, following penetration of the host skin, can become dormant (hypobiosis in the intestine or muscle). These juveniles are capable of re-activating and establishing patent, intestinal infections. In addition, infection by *A. duodenale* may probably also occur by the oral and the transmammary route. *Ancylostoma ceylanicum* and *A. caninum* infections may also be acquired by oral ingestion. *Ancylostoma caninum*-associated eosinophilic enteritis is believed to result following oral ingestion of juveniles, not percutaneous infection. *Necator americanus* does not appear to be infective via the oral or transmammary route. Source: Adapted from United States Centers for Disease Control and Prevention, Division of Parasitic Disease and Malaria, 2019. Public domain.

same time, however, a burning sensation made itself felt on the spot, which grew more intense, while the skin became distinctly reddened. I am still of opinion that the most natural thing was to refer these symptoms to the *Ankylostoma* larvae, which … were present in the drop in great numbers. But the alternative existed that the active agent was either the water, in which the larvae had been kept (in this special case for a long time), and into which they might have discharged irritating products of excretion; or the irritating agency was the *Ankylostoma* larvae themselves. To test this, I let a drop of fluid without larvae fall on to another part of the hand and allowed it to dry. No reaction followed. Then a drop of fluid containing numerous larvae—as at first—was dropped in a third place on the back of the left hand, and was spread out with the handle of the scalpel so gently that the skin was only touched occasionally. Even before the fluid had quite dried up, the burning and reddening of the skin began exactly as before. It was thus clear that the irritating action proceeded from the larvae themselves. In order to see what had become of them I scraped the last remains of the fluid from the skin with the blade of the scalpel, using some pressure, and examined it under the microscope. The *Ankylostoma* larvae, previously so numerous, had disappeared, except for a few specimens; between the epithelial cells which had been scraped off innumerable empty skins were found, burst at the head end, and among them some half desiccated, still feebly motile larvae. The great majority had disappeared, and I saw no better explanation of this disappearance than the assumption that the larvae, casting their envelopes, had penetrated the skin and had thus produced the symptoms described. These symptoms, which were at first local, extended in the course of the next 24 hours over the whole hand, which also swelled considerably. The application of poultices of Goulard's water reduced the swelling in about 3 days, but it completely disappeared only after 6 days. … The fact that the mature *Ankylostoma* larvae not only possessed the power of actively penetrating into the uninjured skin of their host, but that they made energetic use of this power the moment they had an opportunity of doing so was so unusual—from the helminthological standpoint—that to regard it as a mere chance behaviour on the part of the larvae would have seemed to me simply absurd. Its true significance would not perhaps have suggested itself to me so rapidly, had I not become so strongly infected with the parasite in a manner up to that time wholly inexplicable. This infection was a fact; that it had not occurred through the mouth I also regarded as a fact; that the larvae could disappear in the uninjured skin I had just convinced myself on my own person with my own eyes; that this power of penetration into the uninjured skin was accidental and without further significance I considered as out of the question. But if the penetration of the larvae was the starting point of a second path by which they—no matter for the present in what manner—could reach the intestine of their definitive host, then indeed this phenomenon had not only a significance, as was to be expected from the outset, but my own enigmatical infection could be explained. For during my previous investigations I had certainly been careful to keep my hands away from the mouth, or to disinfect them according to the prescribed methods whenever there was the possibility of their coming into contact with the mouth (in eating, etc.); but I had thought nothing of allowing the water permeated with larvae to remain on my hands while manipulating the cultures and the material used for infection. Thus, the larvae had had an ample opportunity to affect an entry from the hands. That their penetration had never produced subjective symptoms is easy to understand; for in the first place the number of larvae entering simultaneously can, under the circumstances, never have been very large, and in the second place, even if a slight itching had been perceptible, there would have been no conceivable reason for seeking its cause in the penetration of *Ankylostoma* larvae into the skin. I may say without exaggeration that I have given earnest and prolonged consideration to all the points here enumerated before coming to a final decision; but in whatever way the facts in question were regarded they all conformed to one theory only, namely that the skin must be another starting point for the larvae from which they could reach the intestine and grow there to sexual maturity.

***Ancylostoma ceylanicum* Looss, 1911**

Ancylostoma ceylanicum was first recorded as a parasite of carnivores in Sri Lanka but is now known from people in Southeast Asia, the East Indies, and the Philippines. A morphologically similar species, *A. braziliense*, is considered to be cosmopolitan in the tropics and is found in domestic and wild carnivores. Although this species has been reported from humans in Brazil, Africa, India, Sri Lanka, Indonesia, and

the Philippines, the infections reported probably were from *A. ceylanicum. Ancylostoma braziliense* is the most common cause of cutaneous larva migrans (creeping eruption) in the southeastern United States and the tropics in the Western Hemisphere.

***Ancylostoma caninum* (Ercolani, 1859)**

Ancylostoma caninum is the most common hookworm of domestic dogs, especially in the Northern Hemisphere. It has been found in humans on at least 5 occasions, and the worm also is a common cause of cutaneous larva migrans (Figure 8). This hookworm is an important cause of eosinophilic enteritis (EE) in northeastern Australia and is now reported in the United States (Croese, 1998). EE causes abdominal pain with peripheral blood eosinophilia but with no eggs evident in the fecal examinations. Evidently the development to maturity of these nematodes in humans is inhibited, but the presence of even 1 immature worm can cause EE. *Ancylostoma caninum* juveniles have been isolated from human muscle and associated with muscle inflammation (Little et al., 1983). This species is also implicated in other pathology involving invasion of human tissues (Loukas et al., 2016).

Human Hookworm Disease

The distinction between hookworm infection and hookworm disease is important. Far more people are infected with hookworms than exhibit overt disease symptoms. The presence and severity of disease depend strongly on 3 factors: 1) Number of worms present, 2) species of hookworm, and 3) nutritional condition and immune status of the infected person. In general, fewer than 25 *Necator americanus* individuals in a person will cause no symptoms, 25 to 100 worms lead to light symptoms, 100 to 500 produce moderate symptoms and considerable damage, 500 to 1,000 result in severe symptoms and grave damage, and more than 1,000 worms cause very grave damage that may be fatal. Because *Ancylostoma duodenale* individuals suck more blood than *N. americanus* ones, fewer *A. duodenale* worms can cause greater disease; for example, 100 *A. duodenale* worms may cause severe symptoms. However, the clinical disease is intensified by nutritional condition, impairment of host's immune response, and other factors.

The human immune response to hookworm infection is complex, but it is clear that hookworms have evolved to modulate the host's defense system. Survival of hookworms appears to depend upon a balance between host immune responses that ultimately protect the parasite. When attached to the host's mucosa, mature hookworms seem to be protected from the host's immune response. In contrast to established adults, newly recruited juvenile worms appear to cause a strong eosinophilic response that expels them from the small intestine (Croese and Speare, 2006).

In addition, several potential mechanisms for evading the host's defense systems have been discovered. For example, *Ancylostoma* spp. secrete a neutrophil inhibition factor that interferes with activation of neutrophils (Pritchard, 1995). *Necator americanus* directly secretes acetyl cholinesterase, which can inhibit or decrease peristaltic movement of the intestine and possibly acts also as an anti-inflammatory factor. It also secretes glutathione-S-transferase and superoxide dismutase, substances that interfere with antibody-dependent, cell-mediated cytotoxicity (ADCC). Nine genes in *N. americanus* code for proteins similar to neutrophil inhibitory factor (Daub et al., 2000). The details involved in the possible immunomodulation by hookworms is not established definitively and is a hotly debated topic (Mortimer et al., 2006).

Epidemiology

A combination of poor sanitation and conducive environmental conditions is necessary for high endemicity of hookworm in people. The disease is restricted to warmer parts of the world (and to specialized habitats, such as mines in more severe climates) because juveniles will not develop to maturity at less than 17 °C, with 23–30 °C being optimal. Freezing temperatures kill eggs and juveniles. Oxygen is necessary for hatching of eggs and juvenile development because their metabolism is aerobic. Thus, juveniles will not develop in undiluted feces or in waterlogged soil. Therefore, soil that is loose with lots of humus and has reasonable drainage and aeration is favorable to the development and survival of juveniles. Both heavy clay and coarse sandy soils are unfavorable for the parasite, the latter because juveniles are also sensitive to desiccation. Alternate drying and moistening are particularly damaging to juveniles; hence, very sandy soils become noninfective after brief periods of frequent rainfall. However, juveniles live in the film of water surrounding soil particles, and even apparently dry soil may have enough moisture to enable survival, particularly below the surface.

Juveniles are quite sensitive to direct sunlight and survive best in shady locations, such as coffee, banana, or sugarcane plantations. Humans working on such plantations often have preferred defecation sites, not out in the open where juveniles would be killed by sun, of course, but in shady, cool, secluded spots beneficial for juvenile development. Repeated return of people to a defecation site exposes them to continual reinfection. Furthermore, use of preferred defecation sites makes it possible for hookworms to become endemic in otherwise quite arid areas. A higher average number of worms per individual will seed the soil with more eggs, so human defeca-

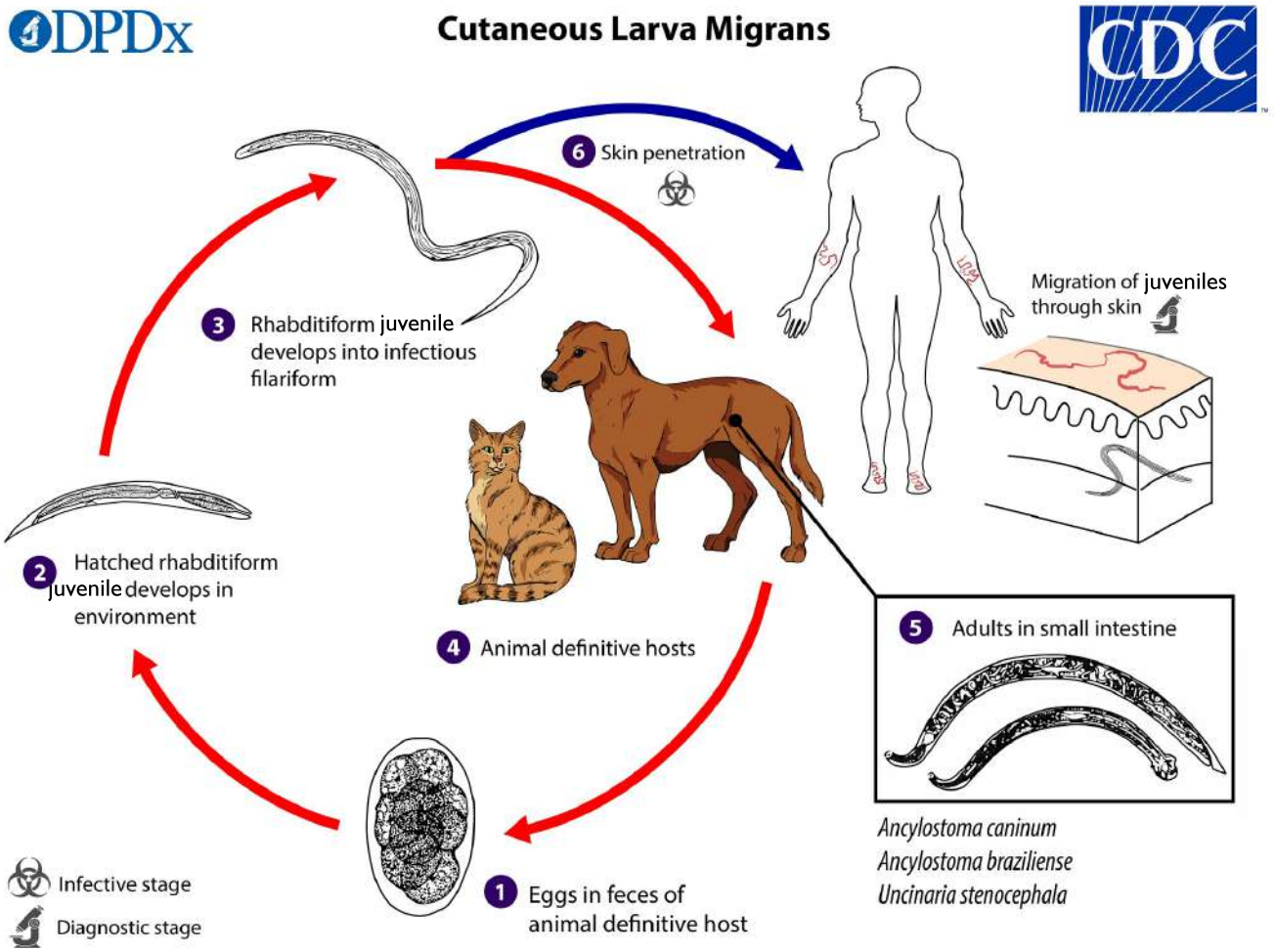

Figure 8. Zoonotic hookworm (extraintestinal) causal agents and life cycle. Some zoonotic hookworm species are capable of infecting humans, but they typically do not develop in the intestine and instead infect extraintestinal sites like the skin. Cutaneous larva migrans (CLM) has been associated with *Ancylostoma caninum*, *A. braziliense*, and *Uncinaria stenocephala*, which are all hookworms of dogs and cats. *Bunostomum phlebotomum*, a cattle hookworm, is also capable of causing short-lived CLM in humans. Cutaneous larva migrans (also known as creeping eruption) is a zoonotic infection with hookworm species that do not use humans as a definitive host, the most common being *A. braziliense* and *A. caninum*. The cycle in the definitive host is very similar to the cycle for the human species, which involves tracheal migration to the small intestine. Some juveniles become arrested in the tissues and serve as the source of infection for pups via transmammary (and possibly transplacental) routes. Mature hookworms reproduce in the small intestine, and eggs are passed in the animal definitive host's stool (1), and under favorable conditions (moisture, warmth, shade), juveniles hatch in 1 to 2 days. The released rhabditiform juveniles grow in the feces and/or the soil (2), and after 5 to 10 days (and 2 molts) they become filariform (third-stage) juveniles that are infective (3). These infective juveniles can survive 3 to 4 weeks in favorable environmental conditions. On contact with the animal host (4), the juveniles penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed. The juveniles reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall. Some juveniles become arrested in the tissues and serve as source of infection for pups via transmammary (and possibly transplacental) routes (6). Humans become infected when filariform juveniles penetrate the skin (7). With most species, the juveniles cannot mature further in the human host and migrate aimlessly within the epidermis, sometimes as much as several centimeters a day. Some juveniles may become arrested in deeper tissue after skin migration. Source: Adapted from United States Centers for Disease Control and Prevention, Division of Parasitic Disease and Malaria, 2019. Public domain.

tion on open ground keeps soil contamination high. Use of nightsoil as fertilizer for crops is an especially important factor in parts of East Asia.

Juveniles develop best in near-neutral pH, and acid or alkaline soils inhibit development, as does the acid pH of undiluted feces (pH 4.8 to 5.0). Chemical factors also have an influence. Urine mixed with feces is fatal to eggs, and several strong chemicals that may be added to feces as disinfectants or fertilizers are lethal to free living stages. Salt in the water or soil inhibits hatching and is fatal to juveniles.

Because worms penetrate the epithelial tissues of the host, a habit of going barefoot in tropical countries is an elemental contribution to transmission. The role of skin penetration presumably accounts for a general lack of high correlation of hookworm with *Ascaris lumbricoides* and *Trichuris trichiura* infections, which must be acquired by ingestion (Booth and Bundy, 1992). However, higher egg counts have been reported in instances of hookworm coinfection with *Ascaris lumbricoides*, suggesting a possible synergistic effect (Fleming et al., 2006). This finding may have important implications for control strategies.

Longevity of the worms is important in transmission to new hosts, continuity of infection in a locality, and introduction to new areas. Juveniles can survive in reasonably good environmental conditions for about 3 weeks; in protected sites like mines, they can last for a year. There is some dispute about the life span of adults, but a good estimate is 5 to 15 years. A person who moves from an endemic area loses the infection in about that time. Specifically, *Necator americanus* has been recorded to live up to 15 years and species of *Ancylostoma* have been reported to live from 12 months to 5 years (Nawalinski et al., 1978a; 1978b; Behnke, 1987).

Schad and colleagues (1984) discovered that juveniles will survive in muscles of paratenic hosts. Thus, *Ancylostoma duodenale* can be transmitted through ingestion of undercooked meat, including rabbit, lamb, beef, and pork. Pigs can also serve as transport hosts for *Necator americanus* (Steenhard et al., 2000). Similarly, dogs can be infected with the canid hookworm, *A. caninum*, by ingestion of juveniles in mice, cockroaches, and possibly other paratenic hosts that might be consumed through predation.

Pathogenicity

In addition to causing iron deficiency anemia involving the direct loss of blood from the mucosa of the intestine by the feeding action of adult nematodes, human hookworm disease manifests 3 main phases of pathogenicity: 1) The cutaneous phase or invasion period, 2) pulmonary phase, and 3) intestinal phase. When a juvenile enters an unsuitable host, after the cutaneous phase or invasion period, it may result in cutaneous larva migrans, after which the pathogenic action of the worm is halted. Cutaneous larva migrans is included in the discussion in the section on migration phase or invasion period, below.

Cutaneous phase or invasion period

The cutaneous phase or invasion period begins when juveniles penetrate the host's epithelial tissue. They do little damage to superficial layers, since they seem to slip through tiny cracks between skin scales, or penetrate sweat pores or hair follicles. Juveniles are stimulated to penetrate by host fatty acids and they must remain in a water film for successful penetration (Haas et al., 2005).

Cutaneous larva migrans may occur at this point. See the discussion of this phase, below. At this stage, infection with pyogenic bacteria may result from the nematodes penetrating into the skin and bringing bacteria with them from their previous fecally-laced habitat, causing an urticarial reaction and dermatitis, a condition known as ground itch.

Proteases released by J_3s cause an increase in cellular permeability and disruption of vascular endothelial cell junctions (Williamson et al., 2003). Once in the dermis, however, their attack on blood vessels initiates a tissue reaction that may isolate and kill the worms.

Pulmonary phase

After the cutaneous phase, or invasion period, the worms enter the hepatic portal system, migrate to the liver, then go into the heart, and then to the lungs. More specifically, the pulmonary phase occurs when juveniles break out of the lung capillary bed into alveoli and progress up the bronchi to the throat. Juveniles migrating through the liver, heart, and lungs may cause inflammation in the lungs termed pulmonary pneumonitis (Hotez et al., 2004). Small hemorrhages occur in the alveoli, and in some cases juveniles induce eosinophilic pneumonia, or Loeffler's syndrome. The pulmonary phase is usually asymptomatic, although there may be some dry coughing and sore throat.

Intestinal phase

The intestinal phase is the most important period of pathogenicity. On reaching the small intestine, young worms attach to the mucosa with their strong buccal capsule and teeth, and they begin to feed on blood. Initiation of this phase is accompanied by painful eosinophilic enteritis (see also the discussion about this phase of infection by Looss; 1911). In heavy infections, worms are found from the pyloric stomach to the ascending colon, but usually they are restricted to the anterior third of the small intestine. Worms move from

place to place, and blood loss is exacerbated by bleeding at sites of former attachment (Gilman, 2000). Hookworms produce proteins that inhibit host blood clotting factors (Gan et al., 2009), and these molecules may contribute to bleeding at former feeding sites. Ironically, such anticoagulants may have beneficial medical applications (Friedly, 1996). Worms pass substantially more blood through their digestive tracts than would appear necessary for their nutrition alone, but the reason for this is unknown. Blood loss per worm is about 0.03 ml per day for *Necator americanus* and around 0.26 ml per day for *Ancylostoma duodenale.*

Migration phase

After the cutaneous phase, or invasion period, cutaneous larva migrans (also termed creeping eruption) may occur. It is usually caused by invasive juvenile hookworms of species normally maturing in animals other than humans (however, ground itch and larva migrans may also occur with the normal human hookworms). In cases of cutaneous larva migrans, juveniles manage to penetrate the skin of humans, and although they may migrate into and through the stratum germanitivum, they are incapable of successfully completing migration to the intestine. Before they are overcome by immune effectors, they produce distressing, but rarely serious, complications of the skin.

After entering the top layers of epithelium, juveniles are usually incapable of penetrating the basal layer (stratum germinativum), so they begin an aimless wandering. As they tunnel through skin, they leave a red, itchy wound that usually becomes infected by pyogenic bacteria. Juveniles may live for weeks or months. It is known that some can enter muscle fibers and become dormant (Little et al., 1983). Juveniles can attack skin anywhere on the body, but people's feet and hands are more in contact with the ground and so are most often affected. Thiabendazole is used as a treatment for cutaneous larva migrans.

Species of hookworms from cats, dogs, and other domestic animals are likely to come into contact with people. *Ancylostoma braziliense*, a common hookworm of dogs and cats, appears to be the most common agent throughout its geographic range (Schad, 1994). Travelers from temperate regions who acquire this infection by visiting tropical beaches may encounter difficulty obtaining a correct diagnosis and medication upon returning home (Tremblay et al., 2000).

Severe Infections

Patients with severe infections may lose up to 200 ml of blood per day, but around 40% of the afflicted person's iron may be reabsorbed before it leaves the intestine (Layrisse et al., 1961). Nevertheless, a moderate hookworm infection will gradually produce iron-deficiency anemia as body reserves of iron are used up. Severity of anemia depends on worm load and dietary iron intake of a patient. Anemia during pregnancy can cause serious complications, putting both mother and child at risk. In hookworm endemic regions, iron deficiency resulting from hookworm infection during pregnancy is common (Baidoo et al., 2010). Slight, intermittent abdominal pain, loss of normal appetite, and desire to eat soil (geophagy) are common symptoms of moderate hookworm disease. Certain areas in the southern United States became locally famous for the quality of their clay soil, and people traveled for miles to eat it. In the early 1920s, an enterprising person began a mail-order business, shipping clay to hookworm sufferers throughout the country!

In very severe infections, patients suffer severe protein deficiency, with dry skin and hair, edema, and potbelly in children and with delayed puberty, mental disability, heart failure, and even death. Intestinal malabsorption is not a marked feature of infection with hookworms, but hookworm disease is usually manifested in the presence of malnutrition and is often complicated by infection with other worms and/or malaria.

The drain of protein and iron is catastrophic to a person subsisting on a minimal diet. In addition, the staple foods of some countries, such as cassava, rice, and corn, are poor sources of iron. Chronic malnutrition, particularly in the young, often results in stunted growth and intellectual disability, but treatment for the worms can significantly increase fitness, appetite, and growth (Latham et al., 1990; Stephenson et al., 1989). Impairment in ability to produce IgG results in lowered antibody response to hookworms as well as to other infectious agents.

Diagnosis

Demonstration of hookworm eggs or worms themselves in feces is, as usual for gut parasites, is the only definitive diagnosis of the disease. Demonstration of eggs in direct smears may be difficult, however, even in clinical cases, and one of the several concentration techniques should be used. If estimation of worm burden is necessary, techniques are available that give reliable data on egg counts (Cross, 2000). It is not possible to distinguish *Ancylostoma duodenale* eggs from those of *Oesophagostomum bifurcum* or *Ternidens deminutus*, and this is important in the areas of Africa where *O. bifurcum* and *T. deminutus* are widely prevalent in humans. PCR methods have been described for these identifications, and a multiplex real-time PCR test based on DNA from a 200-µl fecal sample can diagnose species in mixed infections and provide quantitative results that correlate with egg counts (Verweij et al., 2001). However, implementing ad-

vanced molecular diagnostics for routine testing is sometimes not practical (Schindler et al., 2005; Verweij et al., 2001; De Gruijter et al., 2005).

To rid a person of hookworm infection it is neither necessary nor possible to distinguish *Necator americanus* eggs from those of *Ancylostoma* spp., but care should be taken to differentiate *Strongyloides stercoralis* infections. This is not a problem unless some hours pass between time of defecation and time of examination of feces. Then hookworm eggs may have hatched, and juveniles of *Ancylostoma* spp. must be distinguished from those of *S. stercoralis*.

However, it is necessary to be able to distinguish *Necator americanus* and *Ancylostoma* spp. in studies on the efficacy of various drugs or chemotherapeutic regimens because the 2 species are not equally sensitive to particular drugs: *N. americanus* has low sensitivity to ivermectin, in contrast with *Ancylostoma* spp. (Richards et al., 1995). Differentiation can be accomplished by recovery of adults after anthelmintic treatment, culturing juveniles from feces, or molecular identification based on single eggs.

Treatment

Mebendazole or albendazole are commonly used for treatment, as they kill all nematodes. Single-dose therapy is inexpensive and convenient, but reports of drug failure and decreased efficacy for mebendazole suggest that albendazole later emerged the drug of choice (Albonico et al., 2003; Hotez et al., 2010). There is also evidence that populations of *Necator americanus* are becoming resistant to mebendazole in Africa (De Clercq et al., 1997) and *Ancylostoma caninum* shows evidence of resistance to the anthelminthic pyrantel pamoate (Kopp et al., 2007). It has also been found that routine treatment of pregnant women in areas of high hookworm prevalence significantly decreases incidence of infants with very low birthweight (Larocque et al., 2006).

Treatment for hookworm disease should always include dietary supplementation. In many cases, provision of an adequate diet alleviates symptoms of the disease without worm removal.

Control

Control of hookworm disease depends on lowering worm burdens in a population to an extent that remaining worms, if any, can be sustained within nutritional limitations of people without causing symptoms. Mass treatment campaigns do not eradicate the worms but certainly lower the so-called seeding capacity of their hosts. Education and persuasion of a population in sanitary disposal of feces are also vital. Economic dependence on nightsoil in family gardens remains one of the most persistent of all problems in medical parasitology.

Recognizing these factors, the American zoologist Charles W. Stiles persuaded John D. Rockefeller to donate $1 million in 1909 to establish the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease (Ackert, 1952). (The activities of the commission eventually led to the formation of the Rockefeller Foundation and then Rockefeller University.) Beginning state by state and then extending throughout the southeastern United States, the Commission would first survey an area. Residents of the area were examined for infection and then treated with anthelmintics. Thousands of latrines were provided with instructions on how to use and maintain them. As a result of efforts of this and other similar hygiene commissions, hookworm prevalence is now much lower in some areas of the world. Nevertheless, worldwide prevalence of hookworms is still high; between one-fifteenth and one-tenth of the Earth's human population remains infected (Chan, 1997; Hotez et al., 2010; CDC, 2023).

New molecular methods and technologies hold much promise for advances in understanding hookworm biology and implementing control measures. For example, the transcriptome of *Necator americanus* adults has been analyzed (Cantacessi et al., 2010) revealing 18 potential drug targets that lack homologues in the human genome. By inference, this means that drugs can be applied to a human population to rid the worms from humans while relatively no effect is seen on the host itself. Rapid and specific molecular diagnostic methods that clearly differentiate among different species of hookworms are needed in order to begin to achieve effective control (Clements and Alene, 2022). Deep sequencing of the genome of *N. americanus* has recently been carried out in order to identify potential drug resistant markers. Other newer methods of molecular biology are now being implemented in the ongoing battle against hookworm disease (George et al., 2022).

Family Strongylidae Baird, 1853

Family Strongylidae currently contains 1,126 species in 4 subfamilies (see Hodda, 2022).

Members of Strongylidae Baird, 1853 occur in a variety of mammals, especially herbivores such as horses, in which they are a serious veterinary problem. They are commonly recognized as large strongyles (several species of *Strongylus*, of which *S. vulgaris* is the most important) and small strongyles (mostly the numerous species of *Cyathostomum*) (Herd, 1990). Adults of both are found in the large intestine of equines. Eggs pass out in feces, hatch as J_1s, and develop in soil into infective J_3s; the latter retain the cuticle of the J_2 as a close-fitting sheath. These crawl onto vegetation and are eaten by grazing hosts. All undergo a migration and period of development in various tissues, the details of which vary with species.

Developing juveniles of *Strongylus vulgaris* migrate into the arteries of the host, especially the anterior mesenteric artery, where they cause thrombosis and arteritis. After 3 to 4 months in the arteries, young adults migrate to the intestine where they eventually enter the lumen and reach maturity.

In the past, the arterial stages of *Strongylus vulgaris* were shown to be present in 90% to 100% in horses in the United States, and it was the most feared equine parasite (Herd, 1990). *Strongylus vulgaris* remains sensitive to benzimidazole and ivermectin anthelmintics, but cyathostomes are relatively resistant to these drugs. As a result, *S. vulgaris* has almost been eradicated, and small strongyles such as *Cyathostomum* spp. are instead a much bigger problem with horse owners. To aid in diagnosis, a quantitative real-time PCR test has been developed for *S. vulgaris* (Nielsen et al., 2008).

Oesophagostomum spp. are parasites of primates, rodents, ruminants, and pigs. They are called nodular worms because developing juveniles form nodules in the walls of both the small and large intestines of the host. Adults live in the large intestine. Infections are normally acquired by ingestion of third-stage juveniles (J_3s). Infections in humans are generally considered to be accidentally caused by the zoonotic species of this genus. However, *O. bifurcum* has a high prevalence in humans and nonhuman primates in one small area in Africa (northern Togo and Ghana). Additionally, individuals with infection by species of hookworms have a higher likelihood of also being infected with *O. bifurcum* (Ziem et al., 2006). Infection of humans by these species of nematode typically shows up as a painful abdominal mass that sometimes requires surgical intervention. Eggs of *O. bifurcum* are indistinguishable morphologically from hookworm, but J_3s obtained after fecal culture show clear differences. Although morphologically indistinguishable, *O. bifurcum* from humans and 3 nonhuman primate hosts show relatively high levels of genetic divergence. This observation is consistent with low levels of gene flow between these host-associated populations (Gasser et al., 2006).

Syngamus trachea is the gapeworm of poultry and is called this because adults live in the trachea of their galliform hosts causing the host to gasp and gape with the mouth wide open. The fowl coughs up eggs, swallows them, and then passes them in feces. Juveniles molt twice in the egg to become infective J_3s. Eggs may or may not hatch in soil, and a variety of terrestrial molluscs, earthworms, and arthropods can serve as paratenic hosts.

Syngamus trachea individuals can survive several years in earthworms, and numerous wild bird species serve as reservoirs. Definitive hosts become infected when they swallow embryonated eggs or juveniles. Infective juveniles penetrate the gut wall, are carried by blood to the lungs where they break out into alveoli, and then proceed up to the trachea. At this stage, males remain attached to a female via their copulatory bursa. Young birds are most severely affected and may die with a heavy infection.

Superfamily Trichostrongyloidea Cram, 1927

The superfamily Trichostrongyloidea Cram, 1927 constitutes one of the most diverse and complex taxa within the bursate nematodes (Durette-Desset, 1985; 2009; Hoberg and Lichtenfels, 1994). The group includes more than 1,000 described species approximately 175 genera. These worms have a worldwide distribution and direct life cycle. They occur in the gut and sometimes in the stomach of almost all classes of terrestrial vertebrates (Durette-Desset, 1992).

Some of the species of medical and veterinary importance include *Haemonchus contortus*, *Ostertagia* spp., and *Trichostrongylus* spp., which are discussed briefly below.

Figure 9. Posterior end of a specimen of a trichostrongyloid nematode *Obeliscoides cuniculi*, the stomach nematode of rabbits and hares in North America. This specimen was collected from the stomach of an individual of *Sylvilagus* sp. north of Ogallala, Nebraska, United States. The contracted bursa of this male is visible with small bosses covering the cuticle. Two similar spicules are visible lying parallel which are easily seen in this Normarsky micrograph (NP2380). Source: G. Drabik and S. L. Gardner, HWML, 2018. License: CC BY 4.0.

General Morphology

Trichostrongyloids are usually small, very slender worms, with a small, non-developed **stoma**. Lips around the **mouth** are very reduced or absent, and cuticularized teeth or spines in the stoma are rarely present. The **cuticle** of the **head** may be inflated and some of them are filled with fluid containing hemoglobin that is not host-derived and may be pink when the nematodes are collected alive (Figure 9). Males have a well-developed **copulatory bursa**, and **spicules** vary from simple setaceous to extremely complex falcate or modified hamate in form (see Maggenti, 1981), depending on species and group. Females are usually considerably larger than males. The **vulva** is located anywhere from before the midbody to near the **anus**, depending on the species and group (Figure 10). Worms lay thin-shelled eggs that are in the morula stage (Durrette-Desset et al., 1999).

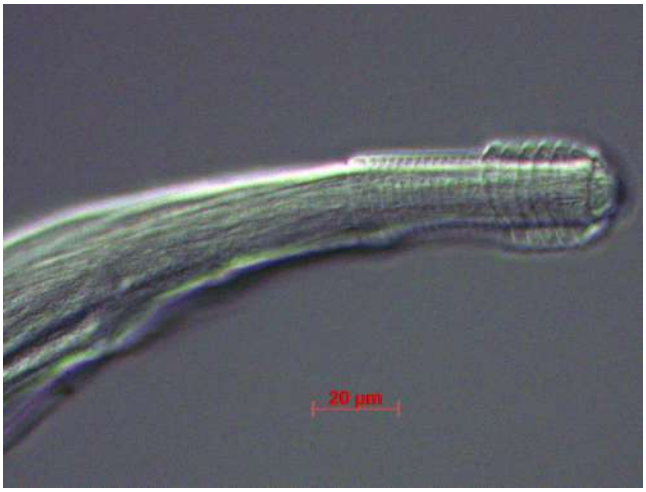

Figure 10. Anterior end of the specimen depicted in Figure 9, a trichostrongyloid nematode *Obeliscoides cuniculi*, the stomach nematode of rabbits and hares in North America. Source: G. Drabik and S. L. Gardner, HWML, 2018. License: CC BY 4.0.

Life Cycles

Life cycles are similar in all species of trichostrongyloid nematodes. For those that have been studied, no intermediate host is required; eggs hatch in soil or water and develop directly into infective J_3s. Some infections may occur through skin, but as a rule juveniles must be swallowed with contaminated food or water. Many trichostrongyloids undergo exsheathment, where J_3s escape the J_2 cuticle during initial infection. The host stimuli that induce production of exsheathing fluid by the J_3 has been extensively investigated. Enormous numbers of juveniles may accumulate on heavily grazed pastures, causing serious or even fatal infections in ruminants and other grazers. A given host usually is infected with several species since their life cycles are similar, and severe pathogenesis results from the cumulative effects of all the worms. Cost to the sheep industry in Australia, for example, is high (McLeod, 1995).

Following is a brief discussion of the families **Trichostrongylidae, Dictyocaulidae**, **Angiostrongylidae**, and **Protostrongylidae**, as well as a few noteworthy species.

Family Trichostrongylidae Leiper, 1912

Many genera and an enormous number of species comprise the family Trichostrongylidae (Durrette-Desset et al., 1999; Hoberg and Lichtenfels, 1994). They are primarily parasites of the stomach or small intestine of all classes of vertebrates, causing great economic losses in domestic animals, especially ruminants, and in a few cases causing disease in humans.

Haemonchus contortus

Haemonchus contortus lives in the so-called fourth stomach (or abomasum) of sheep, cattle, goats, and many wild ruminants. The species has been reported in humans in Brazil and Australia. It is one of the most important nematodes of domestic animals, causing severe anemia in heavy infections (Flach, 2008).

The small stoma contains a single well-developed tooth that pierces a host's mucosa (Emery et al, 2016). The blood this species sucks from this wound gives the transparent worms a reddish color. The large females have white ovaries wrapped around the red intestine, lending it a characteristic red and white appearance and leading to its common names: Twisted stomach worm and barber-pole worm. Prominent cervical papillae are found near the anterior end. The male's bursa is powerfully developed with an asymmetrical dorsal ray. Spicules are 450 µm- to 500 µm-long, each with a terminal barb. The vulva has a conspicuous anterior flap in many individuals but not in all. Frequency of occurrence of the vulvar flap seems to vary according to strain.

Infection occurs when livestock eat forage containing J_3s, which are sheathed in the loosely fitting second-stage cuticle. Exsheathment takes place in the rumen or reticulum of the host animal. Arriving in the abomasum or upper duodenum, worms molt within 48 hours, becoming J_4s with a small buccal capsule having formed. They feed on blood, which forms a clot around the anterior end of the worms. The worms molt for a final time in 3 days and begin egg production about 15 days later. Fourth-stage juveniles can undergo developmental arrest, typically in fall, with maturation to adults occurring in spring. Arrest is considered a mechanism promoting survival and transmission in temperate climates, leading to the spring rise in eggs passed in feces of sheep (Emery et al., 2016).

Anemia, emaciation, edema, and intestinal disturbances caused by these parasites result principally from loss of blood and injection of hemolytic proteins into the host's system. A host often dies with heavy infections, but those that survive usually develop immunity due to specific inflammatory responses in the intestinal mucosa.

Ostertagia Species

Ostertagia spp. are similar to *Haemonchus contortus* in host and location, but they differ in color, being a dirty brown—hence, their common name, brown stomach worm. The buccal capsule is rudimentary and lacks a tooth. Cervical papillae are present. The male bursa is symmetrical. The vulva has a large anterior flap, and the tip of the female's tail bears several cuticular rings.

Their life cycle is similar to that of *Haemonchus contortus* except that J_3s invade gastric glands and elicit nodules. J_3s molt before returning to the lumen, where they feed, molt, and begin producing eggs about 17 days after infection. *Ostertagia* spp. suck blood but not as much as *H. contortus*. Species of *Ostertagia* often undergo developmental arrest as J_4.

Some common species of *Ostertagia* are *O. circumcincta* in sheep, *O. ostertagi* in cattle and sheep, and *O. trifurcata* in sheep and goats. Economic losses in the cattle industry due to *O. ostertagi* and other nematodes probably exceed $600 million per year in the United States alone (Smith and Granfell, 1985).

Trichostrongylus Species

Trichostrongylus spp. are some of the smallest members of the superfamily, seldom exceeding 7 mm in length. Many species parasitize the small intestine of ruminants, rodents, pigs, horses, birds, and humans. They are colorless, lack cervical papillae, and have a rudimentary, unarmed stoma. The male's bursa is symmetrical, with a poorly developed dorsal lobe. Spicules are brown and distinctive in size and shape in each species. The vulva lacks an anterior flap (Anderson, 2000).

Their life cycle is similar to that of *Haemonchus* spp. except that J_3s burrow into mucosa of the anterior small intestine, where they molt. After returning to the lumen, they bury their heads in mucosa and feed, grow, and molt for the last time. Egg production begins about 17 days after infection.

Common species of *Trichostrongylus* are *T. colubriformis* in sheep, goats, cattle, and deer; *T. tenuis* in galliform birds such as grouse, pheasant, chickens, and turkeys; *T. capricola, T. falcatus,* and *T. rugatus* in ruminants; *T. retortaeformis* and *T. calcaratus* in rabbits; and *T. axei* in a wide variety of mammals. Hudson and colleagues (1998) showed that the periodic crashes in populations of British red grouse (*Lagopus lagopus scoticus*) were due to negative impact on fecundity caused by build-up of *T. tenuis* (Cattadori et al., 2005; Hudson et al., 1998).

Approximately 10 species of *Trichostrongylus* have been reported in humans, with records from nearly every country of the world. There are 9 species in Iran alone (Pearson and Schwartzman, 1991). Reported prevalence has varied from very low to as high as 69% in southwest Iran (Sabha et al., 1967) and 70% in a village in Egypt (Lawless et al., 1956).

Pathological conditions are identical in humans and other infected animals. Traumatic damage to intestinal epithelium may be produced by burrowing juveniles and feeding adults. Systemic poisoning by metabolic wastes of the parasites and hemorrhage, emaciation, and mild anemia may develop in severe infections.

Diagnosis can be made by finding characteristic eggs in feces or by culturing juveniles in powdered charcoal. Juveniles are very similar to those of hookworms and *Strongyloides* spp., and careful differential diagnosis is required. Molecular diagnostics are available for the common trichostrongylid species from ruminants (Sweeny et al., 2011).

Treatment and Drug Resistance

Treatment with thiabendazole or with pyrantel pamoate has proven effective. Cooking vegetables adequately will prevent many infections in humans. However, drug resistance in nematodes of livestock has been reported for every class of anthelminthic, and multidrug resistance (MDR) was reported in worms of sheep and goats in the 1980s (Kaplan, 2004; Shoop, 1993). MDR in trichostrongylids infecting small ruminants threatens production throughout the world, but particularly in South America, South Africa, Malaysia, and the United States. Resistance by trichostrongyles to benzimidazole drugs (for example, albendazole, mebendazole, and thiabendazole) is increasing and quite ominous (Conder and Campbell, 1995; Geerts et al., 1997).

Family Dictyocaulidae

Species in this genus are medium-sized nematodes that as adults parasitize the bronchi and trachea and are associated with bronchitis in their hosts. *Dictyocaulus filaria* is an important parasite of sheep and goats, but also infects wild antelope and deer. Adults live in bronchi and bronchioles, where females produce embryonated eggs. Eggs hatch while being carried toward the trachea by ciliary action. First-stage juveniles appear in feces and develop to J_3s in contaminated soil without feeding. Cuticles of both first and second stages are retained by the third stage until the worm is eaten by a definitive host; then cuticles of all these stages are shed together. J_3s penetrate the mucosa of the small intestine and

enter mesenteric lymph nodes. There they undergo 2 molts to become small adults (about 500 µm-long), enter the circulation by way of the thoracic duct, and parasitize the trachea and bronchi. They commonly cause death of their host (Anderson, 2000).

Fully-grown adults are slender and long, with males reaching 80 mm and females 100 mm. The bursa is small and symmetrical; spicules are short and boot-shaped in lateral view. The uterus is near the middle of the body. Other species in horses and cattle are similar to *Dictyocaulus filaria* in morphology and biology.

Family Angiostrongylidae

One of the main pathogenic organisms in the family Angiostrongylidae is *Angiostrongylus cantonensis*, also known as the rat lungworm, detailed below. Other worms in the family are also covered briefly.

Rat Lungworm: *Angiostrongylus cantonensis*

Angiostrongylus cantonensis was first discovered in pulmonary arteries and the heart of domestic rats in China in 1935. Later the worm was found in many species of rats and bandicoots, and it may mature in other mammals throughout Southeast Asia, the East Indies, Madagascar, and Oceania, with infection rates as high as 88%. As a parasite of rats, it attracted little attention, but 10 years after its initial discovery it was found in the spinal fluid of a 15-year-old boy in Taiwan. It has been discovered since in humans in Hawaii, Tahiti, the Marshall Islands, New Caledonia, Thailand, Vanuatu, the Loyalty Islands, and other places in the Eastern Hemisphere. It is now known to exist in Louisiana (United States), the West Indies, and the Bahamas (Raccurt et al., 2003).

This is another illustration of the value of basic research in parasitology to medicine, because when the medical importance of this parasite was realized, the reservoir of infection in rats already was known. Surveys of parasites endemic to wild fauna of the world remain the first step in understanding epidemiology of zoonotic diseases.

Morphology

Angiostrongylus cantonensis is a delicate, slender worm with a simple mouth and no lips or stoma. Males are 15.5 mm- to 25 mm-long, whereas females attain lengths of 19 mm to 34 mm. The bursa is small and lacks a dorsal lobe. Spicules are long, slender, and about equal in length and form. An inconspicuous gubernaculum is present. In females the intertwining of intestine and uterine tubules gives the worm a conspicuous barber-pole appearance. The vulva is about 0.2 mm in front of the anus. Eggs are thin-shelled and unembryonated when laid. Eggs are not produced in human infections.

Life Cycle

Eggs are laid in the pulmonary arteries, carried to capillaries, and break into air spaces, where they hatch. Juveniles migrate up the trachea, are swallowed, and are expelled with feces.

Many types of molluscs serve as intermediate hosts, including slugs and aquatic and terrestrial snails. Terrestrial planarians, freshwater shrimp, land crabs, and coconut crabs serve as paratenic hosts. Frogs have been found naturally infected with infective juveniles (Ash, 1968). Experimentally, Cheng (1965) infected American oysters and clams, and Wallace and Rosen (1966) succeeded in infecting crabs. All juveniles thus produced were infective to rats.

When eaten by a definitive host, J_3s undergo an obligatory migration to the brain, which they leave 4 weeks later as subadults. In rats, the time from infection to egg appearance in feces is about 6 weeks.

Epidemiology

Humans or other mammals become infected when they ingest J_3s. There may be several avenues of human infection, depending on the food habits of particular groups of people (Alicata, 1991; Cross, 1987). In Tahiti it is a common practice to catch and eat freshwater shrimp raw or to make sauce out of their raw juices. It is also possible to eat slugs or snails accidentally with raw vegetables or fruit. In Thailand and Taiwan, raw snails are often considered a delicacy. Infective juveniles escape from slugs and can be left behind in their mucus trail on vegetables over which they crawl (Heyneman and Lim, 1967; Ming et al., 2017). Such juveniles have been found on lettuce sold in a public market in Malaysia. Fish can serve as paratenic hosts in some circumstances. Thus, although the epidemiology of angiostrongyliasis is not completely known, ample opportunities for infection exist.

Pathology

For many years a disease of unknown cause was recognized in tropical Pacific islands and was named eosinophilic meningoencephalitis. Patients with this condition have high eosinophil counts in peripheral blood and spinal fluid in about 75% of cases and increased lymphocytes in cerebrospinal fluid. Neural disorders commonly accompany these symptoms, particularly cranial nerve involvement. It is now known that *Angiostrongylus cantonensis* is at least one cause of this condition.

The presence of worms in blood vessels of the brain and meninges, as well as that of free-wandering worms in brain tissue, or subdural and subarachnoid spaces, results in serious damage. Some effects of such infection are severe headache, fever in some cases, muscle paralysis and speech im-

pairment, stiff neck, coma, and death. The clinical symptoms mimic migraine, brain tumor, and psychoneurosis. In nonsusceptible hosts such as mice and guinea pigs, interleukin-5 activates eosinophils that kill the worms (Sugaya et al., 1997).

Diagnosis and Treatment

When the symptoms described appear in a patient in areas of the world where *Angiostrongylus cantonensis* exists, angiostrongyliasis should be suspected. It should be kept in mind that many of these symptoms can be produced by hydatids, cysticerci, flukes, *Strongyloides* spp., *Trichinella* spp., various juvenile ascarids, and possibly other lungworms. Alicata (1963) and Ash (1968) differentiated the juveniles of several species of metastrongylids that could be confused with *A. cantonensis*.

Albendazole shows promise in treating infection, but no anthelminthic appears reliably therapeutic. Dead worms in blood vessels and the central nervous system may be more dangerous than live ones. A spinal tap to relieve headache may be recommended (Ansdell et al., 2018.

Other Species in the Family Angiostrongylidae

Angiostrongylus costaricensis parasitizes mesenteric arteries of many species of rodents in Central America and South America, southern North America, and Cuba (Morera, 1985). Cases in humans have been diagnosed from countries in North America, Central America, South America, and several Caribbean islands. Worms mature in mesenteric arteries and their branches. In humans, most damage is to the wall of the intestine, especially cecum and appendix, which become thickened and necrotic, with massive eosinophilic infiltration. Abdominal pain and high fever are the most evident symptoms. These intestinal disorders are caused by pathogenic changes that affect blood vessels, or pseudo-neoplastic tissue thickening. No symptoms of meningoencephalitis are noted, unlike the symptoms that are typical in infections due to *A. cantonensis*.

Angiostrongylus vasorum is a serious, emerging disease of dogs (Morgan et al., 2005). It has been reported from many countries in Europe, North America, South America, and Africa. Adults localize in the right ventricle and pulmonary arteries of dogs and other canids and causes labored breathing, exercise intolerance, weight loss, abdominal and lumbar pain, heart failure, and sudden death. Snails and slugs can serve as experimental intermediate hosts, and frogs as transport hosts. However, the role of different infection sources for wild and domestic canids remains undetermined (Morgan et al., 2005). Genetic studies indicate that transmission occurs between wild and domestic canids (Jeffries et al., 2010).

Family Protostrongylidae

Protostrongylus rufescens parasitizes bronchioles of ruminants in many parts of the world. Its intermediate hosts are terrestrial snails, in which it develops to the third stage. The definitive host is infected when it eats the snail along with forage. Mountain sheep in America are seriously threatened by this and related species, which take a heavy toll on lambs every spring. Hibler and colleagues (1972) demonstrated transplacental transmission of *Protostrongylus* spp. in bighorn sheep.

Umingmakstrongylus pallikuukensis is a parasite in lungs of muskoxen in the Canadian Arctic. It has a snail intermediate host and its transmission dynamics are being radically altered by global warming (Kutz et al., 2004; 2005).

Other Trichostrongyloidea Species

In addition to species from ruminants already mentioned, *Cooperia curticei* (family Trichostrongylidae), *Nematodirus spathiger*, and *N. filicollis* (family Molineidae) often occur in the same host as other trichostrongyles and, together, cause much damage. *Hyostrongylus rubidus* (family Trichostrongylidae) is a serious pathogen of swine and can cause death when present in large numbers. *Heligmosomoides polygyrus* (family Heligmosomidae, *H. polygyrus = Nematospiroides dubius*) in mice and *Nippostrongylus brasiliensis* (family Heligmonellidae) in rats are easily kept in the laboratory, and they serve as important tools for research on nematode biochemistry, immunology, life cycles, and other topics (Anderson, 2000).

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NEMATOMORPHS

57

Nematomorpha

Nematomorpha (Phylum): Horsehair Worms

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Phylum Nematomorpha

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Chapter 57

Nematomorpha (Phylum): Horsehair Worms

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Figure 1. Free-living adult gordiids. A) Adult free-living male *Gordius* sp.; B) A typical Gordian knot containing numerous individuals of *G. terrestris*. Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

Introduction

Nematomorphs are commonly known as horsehair worms because of their resemblance to and the common myth of worms arising from horse tail or mane hairs that fall into water. Additionally, because of their habit of becoming entangled in masses of many individuals while mating, horsehair worms are also known as Gordian worms after the Gordian knot from Greek mythology (Figure 1) (Bolek et al., 2015).

The phylum Nematomorpha consists of species that can be allocated into 2 major classes, the freshwater and terrestrial Gordiida and the marine Nectonematida. These animals are unique in several ways and they are 1 of 3 entirely parasitic animal phyla that include the Cestoda—the tapeworms—and the Acanthocephala—the thorny-headed worms (Hanelt et al., 2005). At the current time, the nematomorphs include approximately 360 species that have been described globally and are included in 19 extant and 2 extinct genera (Poinar, 1999; Poinar and Buckley, 2006; Yadav et al., 2018). The 5 known marine species belong to the genus *Nectonema*, and all infect decapod crustaceans (phylum Crustacea: class Decapoda) (see Schmidt-Rhaesa, 2013). Among the Gordiida, both dioecious and parthenogenetic species are known and at least 1 species occurs in terrestrial habitats (Hanelt et al., 2012; Anaya et al., 2019). The freshwater and terrestrial gordiids have complex life cycles, which means that the life cycle can be completed by using multiple hosts with final free-living larvae and adults. Species of gordiids that infect an insect such as a cricket, appear to influence the cricket to go near or into water where the adult worm then emerges from the insect to continue its life in a free-living phase (Thomas et al., 2002; 2003). After emerging from their host, dioecious species form large mating assemblages, also called gordian knots, where they mate and females deposit egg strings on substrate in the water. Those species that are parthenogenetic immediately deposit egg strings after emerging from their host (Hanelt et al., 2012; Bolek et al., 2013a). Larvae develop in the water and infect various species of aquatic invertebrate animals (Bolek and Coggins, 2002; Hanelt and Janovy, 2003). Some of these infected animals (such as aquatic insect larvae) act as paratenic or transport hosts, and when the insects metamorphose, they can carry the cysts to a terrestrial environment where they may be consumed by omnivorous or predatory arthropods, including millipedes, orthopterans (crickets, grasshoppers, etc.), beetles, cockroaches, and mantids (Figure 2).

Horsehair worms are commonly found in domestic water sources such as swimming pools, toilet bowls, cow troughs, pet water bowls, and more, thus making human interactions with them quite common (Bolek, 2000; Hanelt et al., 2005). However, besides the trauma people experience when they discover nematomorphs in their toilet or pet's water bowl, they have no medical or economic importance, although their potential as biological control agents has been suggested (Schmidt-Rhaesa, 2013). There are a few reports of adult horsehair worms from humans, but all of these observations are most likely the result of people swallowing infected arthropods or arthropod hosts releasing free-living worms into drinking water (Bolek et al., 2015). Additionally, there is one odd report of larval horsehair worms in human facial tissue resulting in orbital tumors (Singh and Rao, 1966). However, this report is questionable because juvenile worms contain few if any morphological characteristics of gordiids (see Schmidt-Rhaesa, 2013).

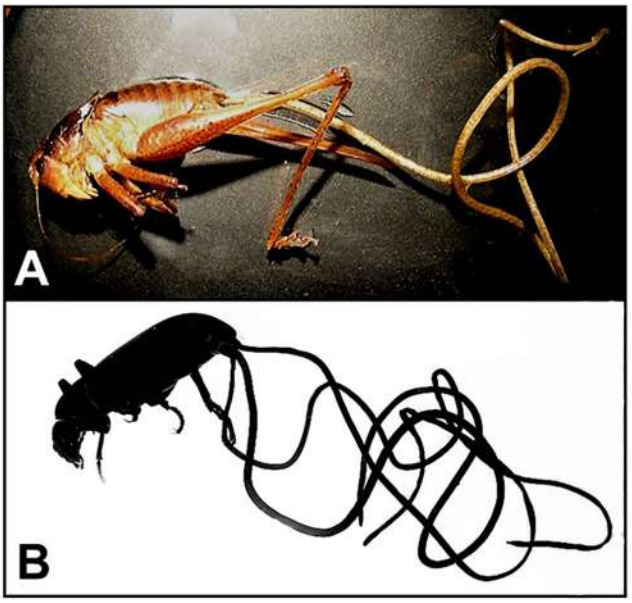

Figure 2. Examples of typical arthropod definitive hosts for gordiids. A) An undescribed species of shieldback katydid *Atlanticus* sp. with an emerging female *Chordodes morgani*; B) an unidentified tenebrionid beetle with 3 emerging individuals of a new species of *Parachordodes*. Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

Chief Morphological Characters

The Nematomorpha belong to the superphylum Ecdysozoa. As with all ecdysozoans (which means molting animals), horsehair worms molt their **cuticle** at least once during their life history. As free-living adults, nematomorphs are very long, cylindrical, and thin, and range from a few cm to over 2 m in length and less than 1 to 3 mm in diameter (Bolek et al., 2015). However, most free-living worms are 20–40 cm in length (Bolek and Coggins, 2002; Schmidt-Rhaesa, 2013).

Among the freshwater and terrestrial gordiids, adult females are usually longer and thicker than adult males (Bolek and Coggins, 2002). Adult free-living worms vary in color from white to shades of dark brown. In some species of *Chordodes*, some individuals have dark patches on a lighter background, producing a leopard-skin pattern, whereas in species of the genus *Gordius* some individuals may have white spots on a darker background (Figure 4C,D). However, color is not a good characteristic for species identification, and most species for which information is available contain various color morphs within and among populations (Schmidt-Rhaesa, 2013).

The anterior end in free-living adults is spheroid or distinctly tapering (Figure 4E,F). The **mouth** may be visible or closed (Figure 4H), and no other structures are present on the anterior end. In some species, there is a distinctly lighter colored area on the anterior end known as a **calotte**, followed by a darkly pigmented ring (Figure 4G). The posterior end is trilobed or unbranched in females and bi-lobed or unbranched in males (Figure 5A–D). In males, the **cloacal opening** is always situated on the ventral side and may contain cuticular structures, such as **circumcloacal spines**. The **cloaca** of males is usually surrounded by either post-cloacal **crescents** or **spines** and/or pre-cloacal **bristles**, and these structures are genus- and/or species-specific (Figure 5E). The cloaca in females is terminal or slightly subterminal and circumcloacal spines have not been reported for females of most species (Bolek and Coggins, 2002; Schmidt-Rhaesa et al., 2003; Bolek et al., 2010; Begay et al., 2012; Schmidt-Rhaesa, 2013).

The marine *Nectonema* species are morphologically similar to the gordiids, wormlike, long, 10–270 mm for males and 30–960 mm for females and approximately 1 mm in diameter (Schmidt-Rhaesa, 2013). The anterior and posterior ends are rounded in females, whereas the posterior end is curved ventrally and distinctly tapered in males. Unlike most gordiids, the cuticle is smooth and does not contain areoles or other surface structures, but instead the dorsal and ventral longitudinal midlines contain natatory bristles (Figure 3). In addition to the ventral longitudinal nerve cord, a dorsal nerve cord is present. The intestine is incomplete and forms a blind gut. Unlike the freshwater gordiids, the anterior end of nectonematids contains a body cavity with conspicuous large cells of unknown function known as **giant cells**. Additional work by Schmidt-Rhaesa (1996a; 1996b) and Restelli and colleagues (2002) indicates that gordiids and nectonematids differ in their muscle cell structure. Freshwater gordiids have thick and thin contractile filaments which are concentrated in bundles as thick sheets and myofibrils enclose the cell body; whereas nectonematid muscle cells are coelomtyarian, as in some nematodes.

General larval morphology. Larvae are 60–100 μm by 14–30 μm in length and width, respectively, cylindrical in shape, and superficially annulated. A septum divides the larval body into 2 regions, the pre-septum and the post-septum (Figure 10). The pre-septum contains 3 rings of cuticular **hooks** and an eversible **proboscis**, supported by 3 internal **stylets** and various sets of **muscles** (Müller et al., 2004). The outer **cuticular ring** contains 6 hooks, 1 of which is positioned ventrally and bifurcated; whereas the middle and inner rings contain 6 hooks, none of which is bifurcated (Figure 11) (Szmygiel et al., 2014). The post-septum contains 1–4 terminal spines among some gordiid genera (Szmygiel et al., 2014). Internally, the post-septum contains the **pseudointestine**, which is subdivided into unequal portions and opens to the outside of the body via a small duct (Hanelt and Janovy, 2002; Szmygiel et al., 2014). The pseudo-intestine is assumed to have a glandular function and empties during cyst formation.

Cuticular Features

In most species of Nematomorpha, the surface of the cuticle is smooth or structured into elevated thickenings called **areoles**. When present these are separated by interareolar furrows; in addition, a variety of short spines and/or bristles may be present on the surface of the cuticle. In most gordiid species, 1 or 2 types of areoles are present. These are known as simple areoles that form a regular pattern on the cuticle (Figure 5F). In species of some genera, such as those in the genus *Gordius*, areoles are lacking or are weakly developed, while in species of other genera, such as those in the genus *Chordodes*, up to 6 different types of areoles can be present and include the characteristic crowned areoles which define the species in this genus (Figure 5I–J) (Schmidt-Rhaesa et al., 2003; Bolek et al., 2013b). Finally, in some species, 2 or more areoles may be fused and form structures referred to as mega-areoles and super-areoles, and these are considered synapomorphies that group species into defined genera (Figure 5G,H).

Sexual dimorphism is common in the areole pattern among gordiids. For proper descriptions and to make firm identifications to the level of species, it is necessary to have examples of and descsribe the characters of both sexes for complete species descriptions (Bolek and Coggins, 2002; Bolek et al., 2010; 2013b).

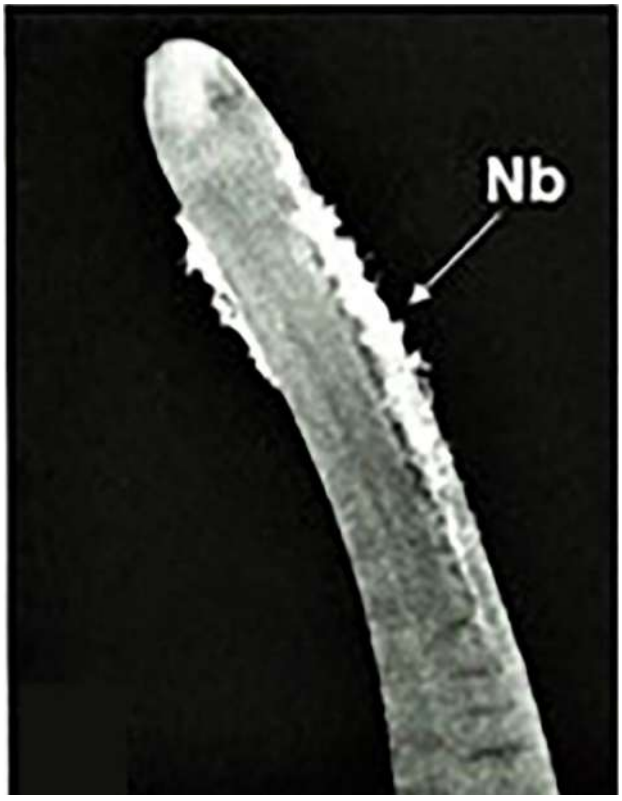

Figure 3. Light micrograph showing natatory bristles (Nb) on the anterior part of a mature fee-living gordiid.

Body Wall

The body wall of adult gordiids is composed of a thick cuticle containing an outer homogeneous region and an inner fibrous region. The fibrous region consists of 25–45 layers of thick **fibrils** that are arranged in a crisscross pattern alternating at an angle of 60–65° (May, 1919; Schmidt-Rhaesa, 1997; 2013). Studies on the chemical composition of the cuticle by Brivio and colleagues (2000) and Protasioni and colleagues (2003) indicate that the makeup of the fibrils is not collagen but some other proteinaceous components. Below the cuticle is a very thin epidermis, which secretes the cuticle layers during development within the definitive host (Schmidt-Rhaesa, 2013). The musculature in all nematomorphs consists of **longitudinal muscles** and, as in species of the phylum Nemata, circular muscles are absent (Schmidt-Rhaesa, 1996a; 1996b; Restelli et al., 2002). The body cavity in free-living adults is mostly filled with **gonads** and vacuolated **parenchyma cells** filled with lipids and glycogen, and the **digestive track** is greatly reduced (Reutter, 1972).

Nervous System

The nervous system consists of a **brain** (basically a circumesophageal **nerve commisure**), a ventral longitudinal **nerve cord**, which emerges from the ventral part of the brain, and a number of peripheral basi-epidermal **nerves**. The brain forms a ring-like structure similar to those possessed by species in the phylum Nemata and surrounds the anterior region of the **alimentary canal** (Schmidt-Rhaesa, 2013). The ventral nerve cord is connected to the epidermis by a thin lamella (Schmidt-Rhaesa, 1997). Simple **sensory organs** in the cuticle are not fully understood, but some studies indicate that integumentary receptors are present. However, it is unclear if these function as mechanoreceptors or if they have other functions (Schmidt-Rhaesa, 2013).

Digestive System

In free-living adults, a **mouth** may or may not be present and, depending on the gordiid species, the **pharynx** can be a cuticularized tube (Figure 4H), cellular in structure, or absent altogether. The **intestine** is located dorsally to the ventral nerve cord and consists of layers of cuboidal cells that manifest **microvilli** on the lumen side of the tissue. Work on *Paragordius varius* indicates that the organization of the intestine changes during development in the definitive host, decreasing in free-living adults compared to parasitic juveniles. In both sexes, the intestine and reproductive system fuse and form the **cloaca**, which is lined with **cuticle** (Schmidt-Rhaesa, 1997; 2005; 2013).

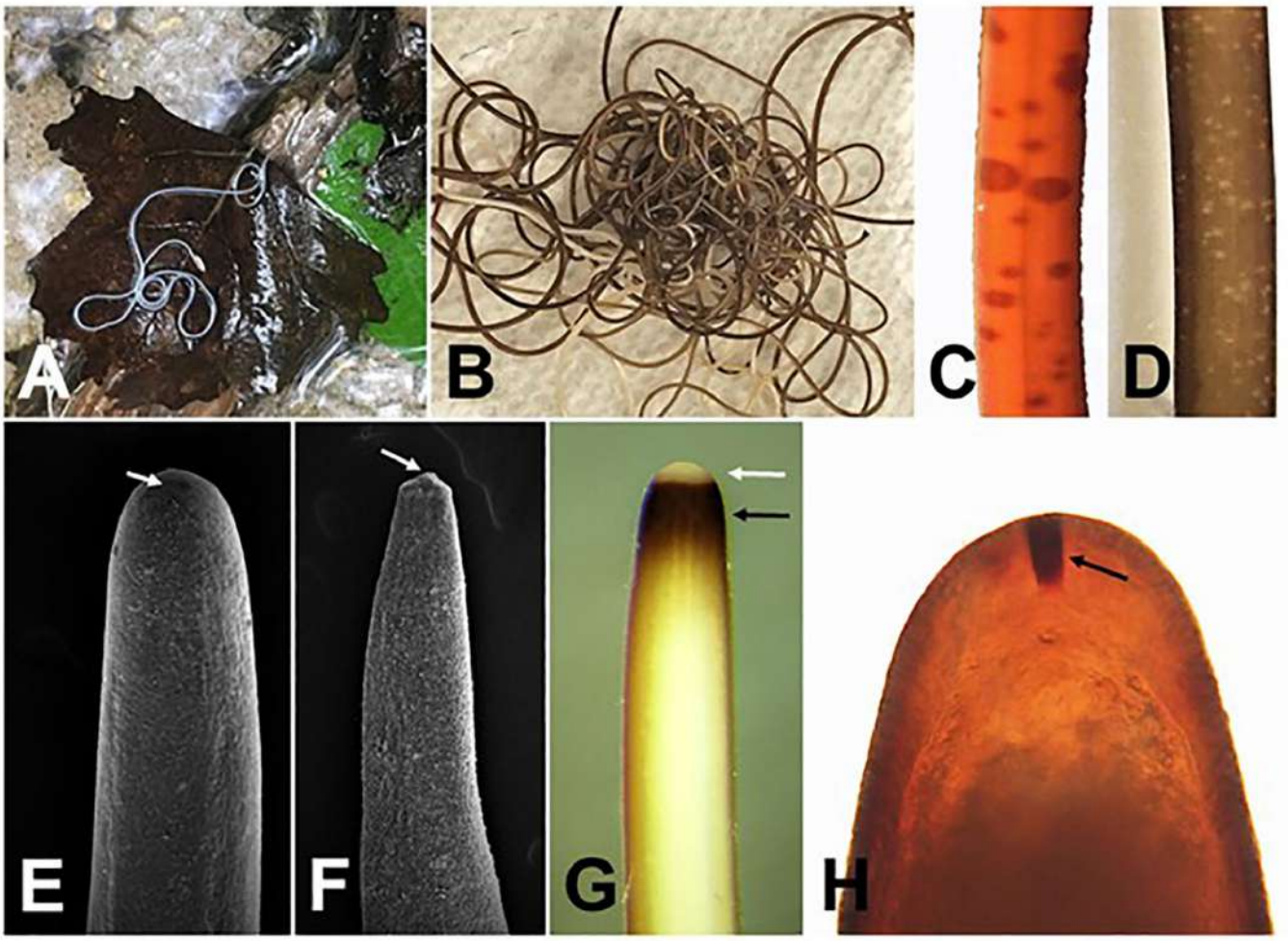

Figure 4. Color and anterior morphology of adult free-living gordiids. A) Typical white color of a female *Gordius difficilis*; B) a Gordian knot of *G. terrestris* showing variation in color of worms ranging from white to dark brown; C, D) mid-body region of a free-living adult female *Chordodes morgani* showing the leopard pattern and the mid-body region of a free-living adult male *G. terrestris* showing white spots on a darker background; E, F) scanning electron micrograph of the E) anterior region being spherical in *G. difficilis* and F) distinctly tapering in *C. morgani*. Note the degenerate mouth (white arrows); G) anterior end of a female *G. terrestris*. Note the calotte (white arrow) followed by a by a dark pigmented ring (black arrow); H) anterior end of a male *C. morgani*. Note the cuticularized pharynx (black arrow). Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

Reproductive System

The **gonads** are arranged as 2 long dorsolateral tubes, surrounded by **parenchymal cells**, and extend almost the entire length of the body. In mature males, the 2 **testes** are full of spermatozoa but may be empty after males complete mating with several females. In developing females, the 2 dorsolateral tubes contain **ovarial ducts** with numerous extensions called **ovaries** (Schmidt-Rhaesa, 1997).

Little information is available about the structure of gonads in the marine Nectonema species, including no information on mature spermatozoa (Schmidt-Rhaesa, 2013).

Reproduction

Once worms enter water, in dioecious species, male and female worms must find each other to mate. Observations on *Paragordius varius* in the laboratory indicate that male worms begin mating with females even before they completely exit their arthropod definitive host (Hanelt and Janovy, 2004b). However, field studies indicate that most arthropods that are shown to be infected by horsehair worm larvae are infected with a single worm and these individuals must somehow find the opposite sex for copulation after they emerge from their hosts (Bolek and Coggins, 2002; Looney et al.,

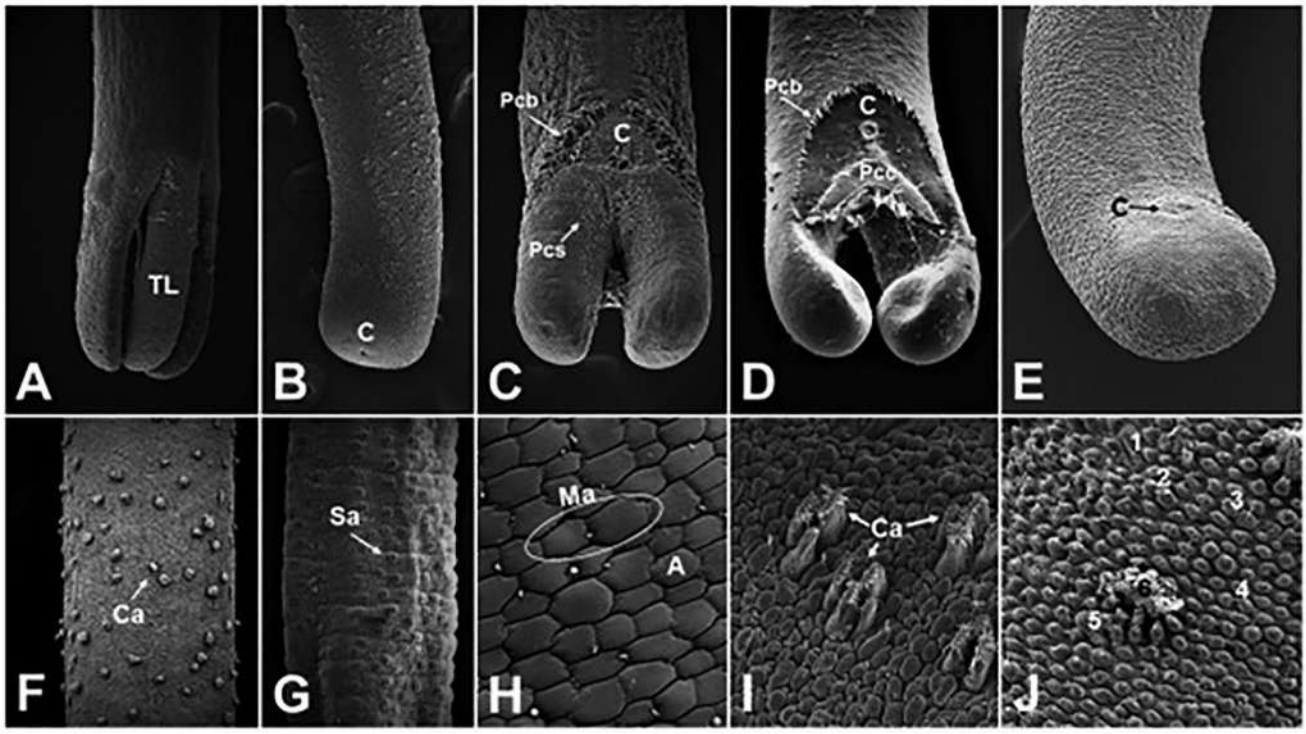

Figure 5. Scanning electron micrographs of the posterior ends and cuticle structures of adult free-living gordiids. A) Posterior end of a female *Paragordius* sp. Note the 3 tail lobes (TL); B) posterior end of a female *Chordodes* sp. showing a round posterior end and a terminal cloaca; (C); C) posterior end of a male *Parachordodes* sp. showing 2 tail lobes, cloacal spines surrounding the ventrally located cloaca (C) and rows of precloacal bristles (Pcb), and postcloacal spines (Pcs); D) posterior end of a male *G. difficilis*. Note the ventrally located cloaca (C), precloacal bristles (Pcb), and postcloacal crescent (Pcc); E) a male *Neochordodes* sp. Note the absence of distinct tail lobes and the cloacal opening (C); F) mid-body region of an adult free-living female *Chordodes* sp. Note the distinct crown areoles (Ca) among simple areoles; G) mid-body region of an adult free-living male *Parachordodes* sp. showing characteristic super-areoles (Sa); H) higher magnification of the mid-body cuticle of a male *Neochordodes* sp. Note the simple (A) and mega-areoles (Ma); I, J) higher magnification of the mid-body cuticle of 2 species of *Chordodes* showing interspecies variation in crown areoles (Ca and 6). Note the 6 types of areoles (1–6) on the cuticle of the *Chordodes* species in (J). Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

2012). Currently, it is unknown whether both sexes find each other by the use of attractants, such as pheromones, or simply by chance. However, field studies using daily collections of individual female *Gordius difficilis* indicate that most females mate within a day of emerging from their host (Bolek and Coggins, 2002).

Both field and laboratory observations indicate that male and female worms initiate typical Gordian knots within hours to days of being placed together (Bolek and Coggins, 2002; Bolek et al., 2013b). During mating, males move up and down the female's body with their coiled posterior end. In genera with bi-lobed posterior ends, such as *Gordius*, a male will spread its tail lobes and glide along the female's body (Figure 8). Once a male's cloaca is in proximity of the female cloaca, the male deposits a mass of sperm referred to as a **sperm drop** or **spermatophore** (Figure 8). Field studies on *Gordius difficilis* indicate that sperm drops can remain on the posterior region of females for at least a week (Figure 8) (Bolek and Coggins, 2002).

The spermatozoa of gordiids are unique and change shape during sperm transfer between a male and a female. They contain a **nucleus** and compartments, which have been named the **acrosomal tube**, **acrosomal sheath**, and **multivesicular complex** (Figure 8). It is unclear if and how these spermatozoa move because they lack a flagellum or pseudopods (Schmidt-Rhaesa, 2013).

After dioecious species mate, females produce up to 8 million eggs during their 2-week to 2-month adult life span (Bolek and Coggins, 2002; Hanelt, 2009). Females in the genus *Gordius* and *Acutogordius* deposit short pieces of **egg strings** approximately 1–2 cm in length on the substrate or while within Gordian knots. In contrast, females of species of *Euchordodes*, *Chordodes*, and *Neochordodes* deposit their egg strings in a zigzag pattern to objects such as sticks or

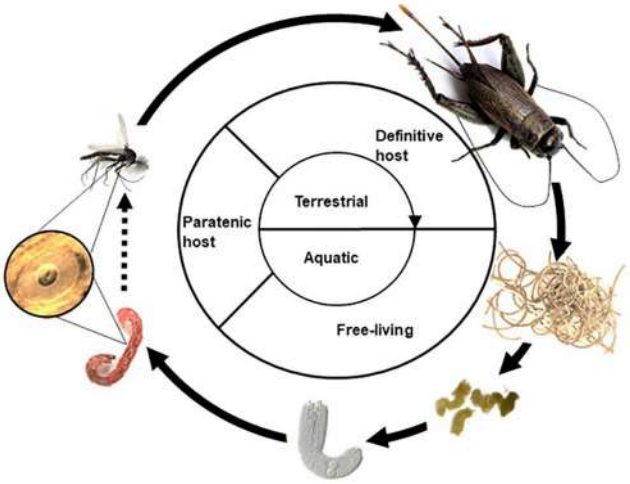

Figure 6. Diagram of a typical gordiid life cycle. The life cycle takes 4–8 weeks to complete in the laboratory depending on the species of nematomorph involved. Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

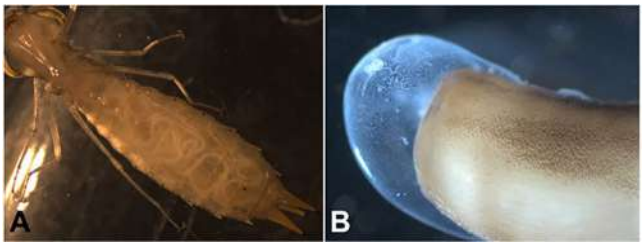

Figure 7. Development of gordiids in their arthropod definitive host. A) An infected larval comet darner *Anax longipes*, showing developing *Neochordodes* species within the hemocoel; B) a developing worm removed from the hemocoel of an arthropod host, showing the thin larval cuticle. Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

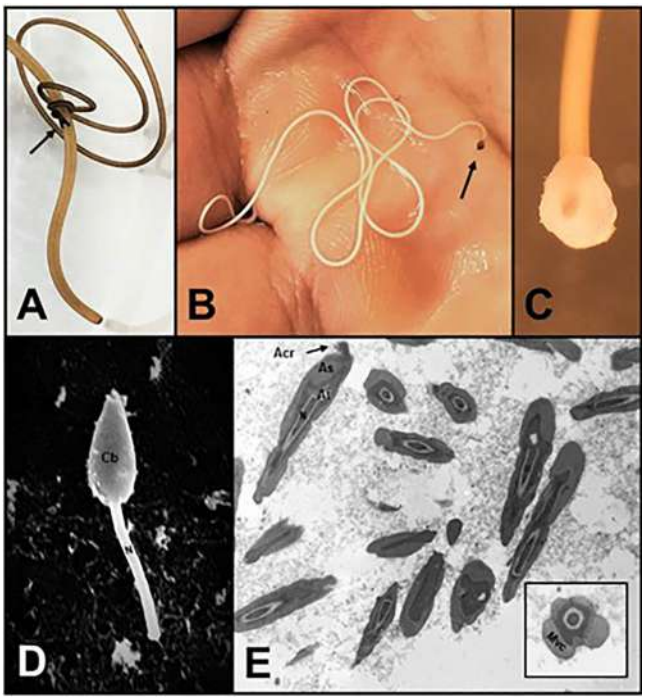

Figure 8. Mating behavior and the morphology of the sperm drop and sperm of gordiids. A) A male *Gordius terrestris* in the process of initiating mating with a female. Note the thinner size and bi-lobed posterior end (arrow) of the male; B) a field-collected female *G. difficilis* with a sperm drop (arrow) on the posterior end; C) a higher magnification of the posterior end of a female *Paragordius varius* with a deposited sperm drop; D) a single sperm on the posterior region of the cloaca of a female *G. difficilis*. Note the round end (Cb) and rod-shaped end (N) where part of the nucleus is located; E) cross- and longitudinal sections of a spermatozoon from the reproductive system of a *Gordius* sp. Note the numerous compartments and organelles, including Acr = acrosome, As = acrosomal sheath, At = acrosomal tube, Mvc = multivesicular complex, and N = nucleus. Sources: A–C) M. G. Bolek; D) Bolek and Coggins, 2002; E and insert) A. Schmidt-Rhaesa. License for all: CC BY-NC-SA 4.0.

rocks in water. Finally, females of *Paragordius* species deposit a single long egg string approximately 1–5 times the length of the worm's body in the water column and/or in algal mats (Figure 9) (Poinar, 2010; Szmygiel et al., 2004; Bolek et al., 2015; Chiu et al., 2017).

Eggs are elliptical to round in shape, with a distinct shell and a thin inner membrane surrounding the developing larva. This inner membrane is relatively thin in aquatic species but is much thicker in gordiids that reproduce in terrestrial habitats (Figure 10) (Anaya et al., 2019). After hatching, the free-living gordiid larvae are semi-sessile and not capable of moving great distances.

In order for aquatic gordiid larvae to reach their terrestrial arthropod hosts, 3 transmission strategies have been proposed. These include: 1) Direct consumption of larvae by the definitive hosts while drinking water; 2) larvae encysting on vegetation/detritus and being ingested accidently while the definitive host ingests vegetation/detritus; and 3) larvae entering and encysting within a paratenic host, which is preyed on or scavenged by the definitive hosts (Hanelt et al., 2005; Bolek et al., 2015). A number of studies show that when definitive arthropod hosts ingest suspended larvae when they drink water, the definitive host becomes infected (May, 1919; Inoue, 1962; Hanelt and Janovy, 2004b). However, comparative work by Inoue (1962) and Hanelt and Janovy (2004a) strongly suggests that prevalence and intensities of these infections in definitive arthropod hosts are much lower compared to those that occur when definitive hosts are exposed to gordiid cysts in paratenic hosts. Finally, observations on gordiid larvae of European and North American *Gordius* species

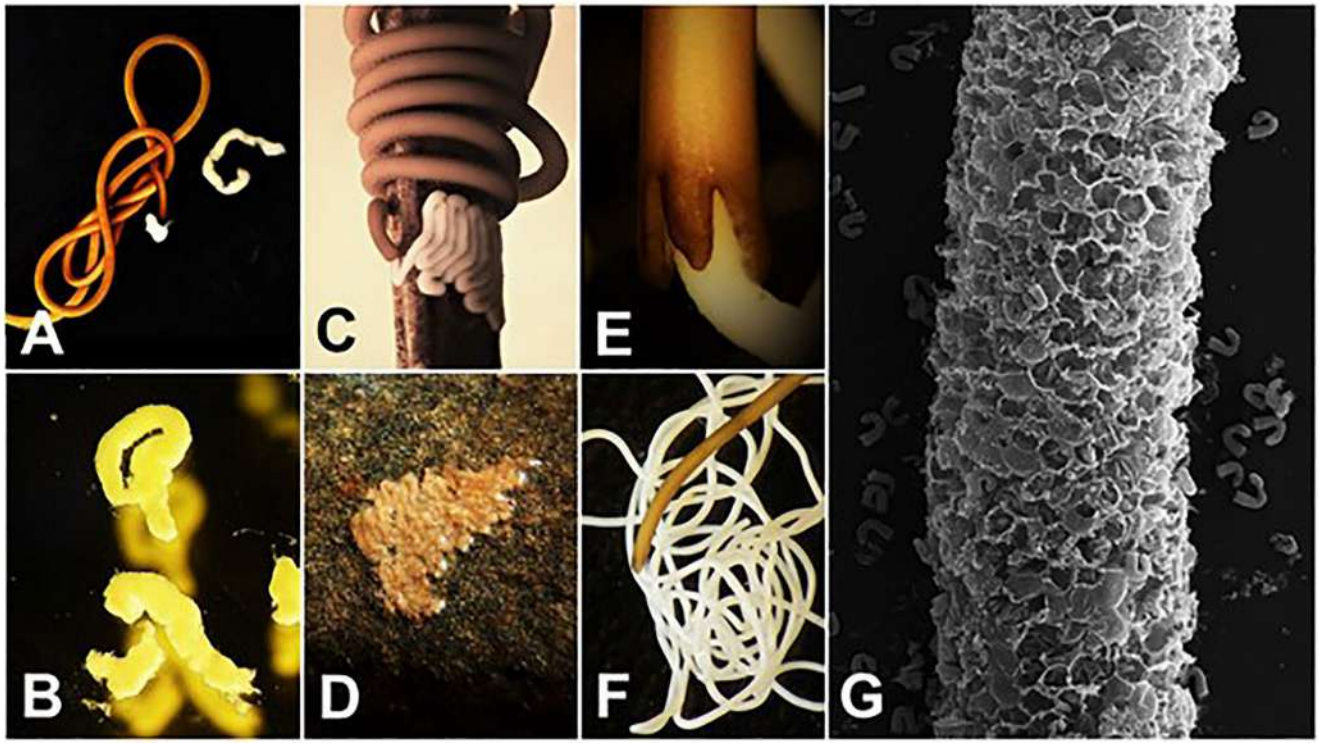

Figure 9. Examples of gordiid egg strings and eggs. A) A female *Gordius terrestris* (brown) in the process of depositing short pieces of egg strings (white); B) higher magnification of pieces of *G. terrestris* egg strings; C) a female *Chordodes kenyaensis* in the process of laying an egg string on a stick. Note the zigzag pattern of the egg string; D) egg string of *Chordodes* sp. deposited on a rock in a zigzag pattern; E) posterior end of a female *Paragordius varius* showing an egg string (white) excreted from between the 3 tail lobes; F) posterior end of a female *P. varius* (tan) in the process of depositing a single and very long egg string (white); G) a scanning electron micrograph of a partial egg string (ES) of *P. varius*, with hatched larvae scattered around the periphery of the egg string. Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

and Asian *Acutogordius* species indicate that these larvae can encyst freely on the surface of aquatic vegetation, or within the egg strings deposited in the environment, but the role of these cysts in transmission is unclear (Dorier, 1930; Bolek et al., 2015; Chiu et al., 2017).

Studies on larvae of other gordiid species indicate that larvae of these species never encyst on vegetation or detritus (May, 1919; Inoue, 1960; Hanelt and Janovy, 2002; Bolek et al., 2010; Hanelt et al., 2012; Bolek et al., 2013a; Szmygiel et al., 2014). In fact, most reports of gordiid cysts have been reported from aquatic metazoan animals including molluscs, annelids, arthropods, fish, and amphibians (Harkins et al., 2016; Chiu et al., 2016; Yamashita et al., 2017). More importantly, experimental studies by Hanelt and Janovy (2004a) demonstrate that 3 phylogenetically distinct species of gordiids indiscriminately infect and form cysts in a variety of aquatic invertebrates and fish. These authors also demonstrated that within non-biting midge paratenic hosts, gordiid cysts survived metamorphosis of these aquatic insects, and when these insects were fed to crickets, the crickets became infected and released adult worms. Taken together, the numerous reports of gordiid cysts infecting a variety of aquatic animals, their ability to survive insect metamorphosis and their ability to infect terrestrial arthropod hosts, supports the paratenic host strategy in the life cycles of gordiids.

Once paratenic hosts ingest larvae, the larvae penetrate the gut and begin forming cysts within the tissue of their paratenic host. During cyst formation, larvae empty the contents of their pseudo-intestine. Laboratory studies by Dorier (1930), Poinar and Doelman (1974), Hanelt and Janovy (2003), Hanelt et al. (2012), and Bolek et al. (2010; 2013a; 2013b) report that during cyst formation, larvae secrete a jelly-like material from the pseudo-intestine and a clear halo-like structure appears around the folded larva (Figure 11). Transmission electron microscopy studies of gordiid cysts in tadpole paratenic hosts indicate that the clear halo-like cyst wall is multilayered (Poinar, 2010). Cyst development can take a few days up to a few months (Hanelt and Janovy, 2002; De Villalobos and Ronderos, 2003). More importantly, if an animal other than a definitive host ingests a paratenic host, the cysts are digested out and the larvae re-penetrate into the

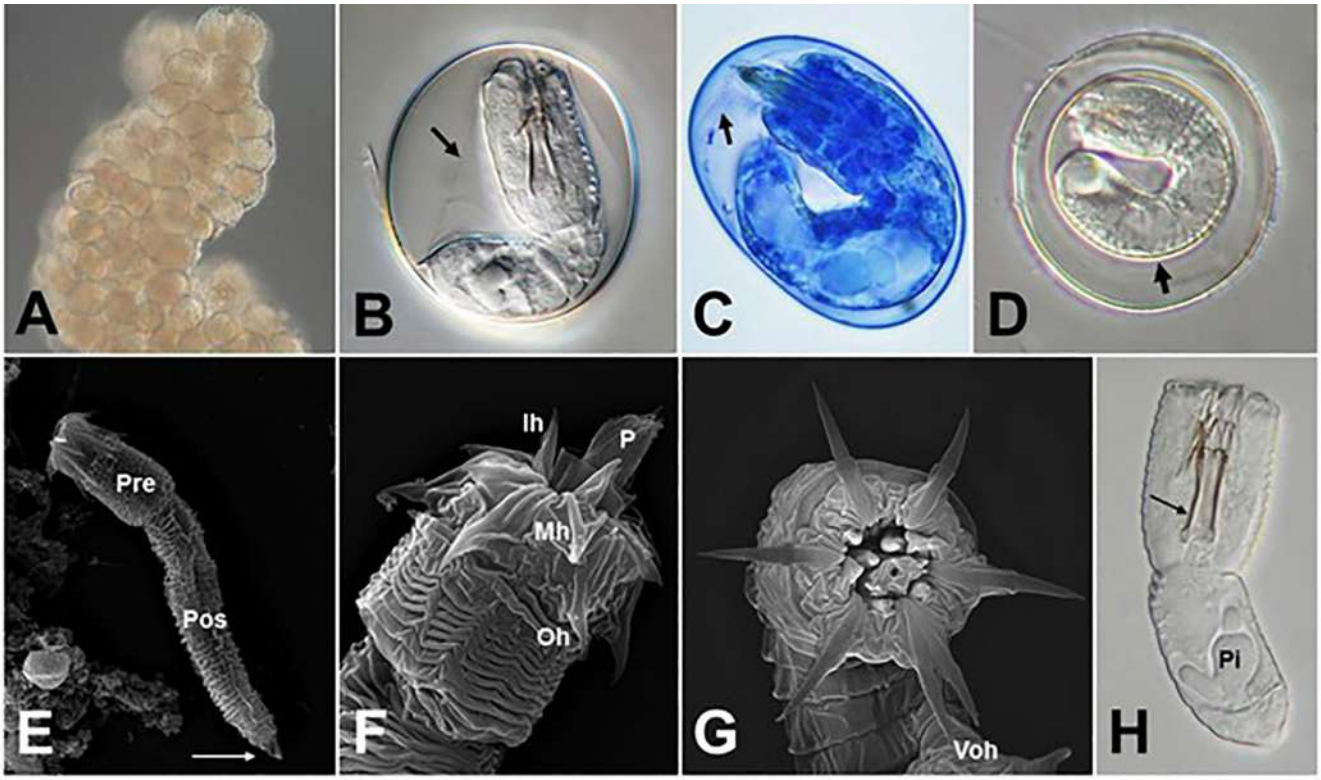

Figure 10. Eggs and larvae of gordiids. A) Higher magnification of pieces of *Gordius difficilis* egg strings showing the concentration of eggs; B, C) typical eggs of freshwater gordiids. Note the developed larva surrounded by a thin inner membrane (arrow) in each egg; D) an unusual egg of *G. terrestris*, a gordiid that lays eggs in the soil. Note the outer shell separated by distinct space from a thick inner membrane (arrow) surrounding the larva; E) scanning electron micrograph of a typical *Gordius* larva. Note the pre-septum (Pre), post-septum (Pos), and terminal spine (arrow) on the post-septum; F) scanning electron micrograph of the anterior end of a *Gordius* larva. Note the proboscis (P) and 3 rings of cuticular hooks including the inner, middle and outer hooks (Ih, Mh, Oh); G) scanning electron micrograph of the anterior end of a *Paragordius varius* larva. Note the dorsoventrally compressed proboscis in relationship to the ventral outer hooks (Voh); H) larva of *Chordodes kenyaensis.* Note the 3 internal stylets (arrow), and V-shaped pseudo-intestine (Pi). Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

second paratenic hosts and re-encyst (De Villalobos and Ronderos, 2003; Hanelt and Janovy, 2003).

It is unknown if nematomorph larvae have any impact on mortality of paratenic hosts in nature. However, several laboratory studies and field observations indicate that insect paratenic hosts mount some type of immune reaction to horsehair worm larvae and cysts (Poinar and Doelman, 1974; De Villalobos and Ronderos, 2003; Hanelt and Janovy, 2003). Host reactions usually involve humoral mediated melanization of larvae (Figure 11) and/or cysts. Melanization of gordiid larvae and cysts have been reported in a variety of aquatic larval insects including mosquitoes, chironomids, caddisflies, mayflies, stoneflies, as well as larval beetles (Poinar and Doelman, 1974; Poinar, 1991; Hanelt and Janovy, 2003; Bolek et al., 2015).

Most definitive hosts for gordiids are predaceous or omnivorous arthropods, which capture infected paratenic arthropod hosts after they metamorphose from an aquatic habitat or scavenge on dead infected paratenic hosts (Figures 2, 6, and 7). Laboratory studies indicate that the maturation of gordiids within the definitive host takes several months. For example, the development within the definitive arthropod hosts can take as long as 8 months for *Gordius tolosanus* (Svábeník, 1925), 2 to 3 months for *Chordodes japonensis*, *C. kenyaensis*, and *Paragordius obamai* (Inoue, 1962; Hanelt et al., 2012; Bolek et al., 2013a), to as short as 1 month for *P. varius* (Hanelt and Janovy, 2004b).

Field studies indicate that after worms emerge from their hosts, only the gut remains within the host's body cavity (Linstow, 1891; Thorne, 1940), whereas, other studies indicate that the production of eggs by female definitive hosts is inhibited or absent altogether (Tanner, 1939; Baker, 1985; Studier et al., 1991; Chiu et al., 2015). Only 1 report found that naturally infected female hosts might be capable of reproducing (Poulin, 1995). A more recent experimental study by Biron et al. (2005b) using naturally infected crickets showed

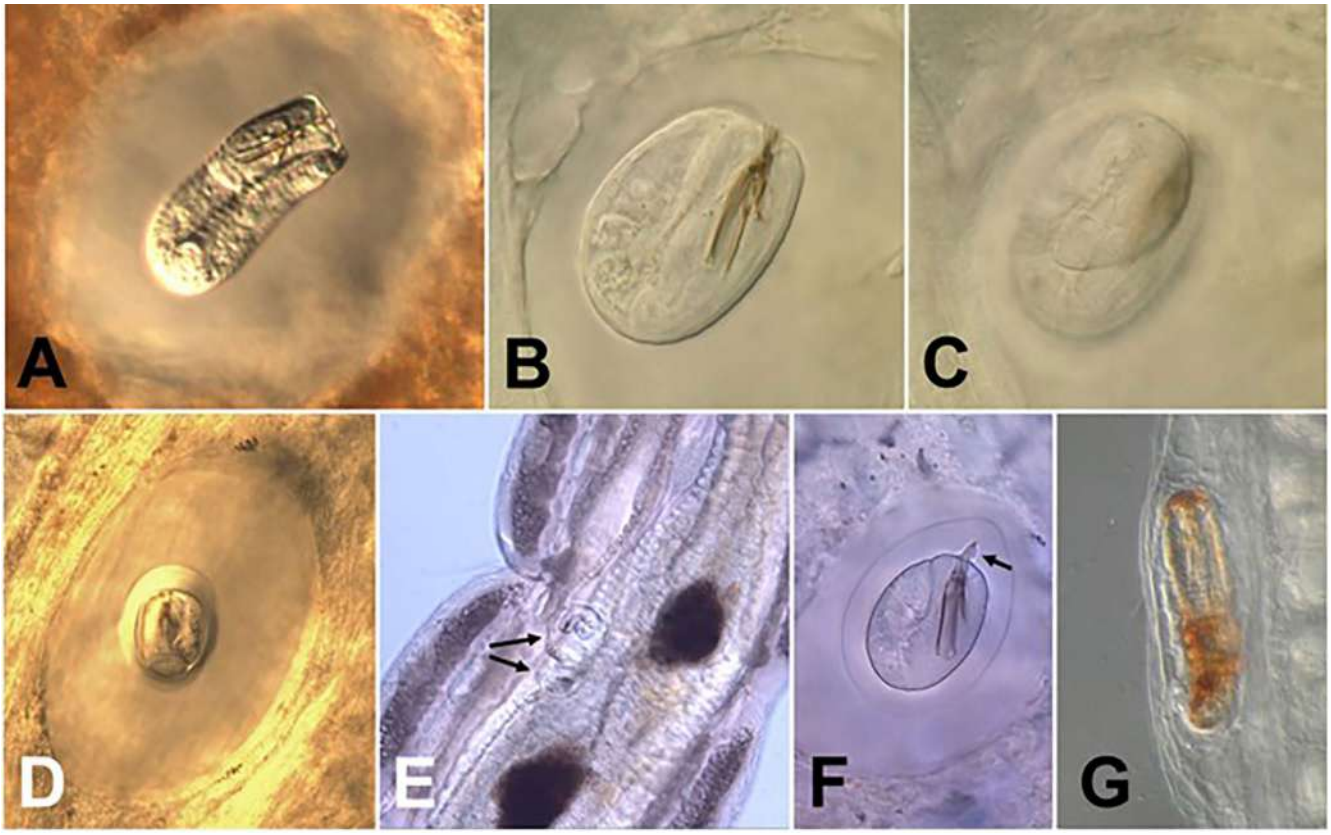

Figure 11. Cysts of gordiids. A) A larval *Gordius terrestris* in the process of folding into a cyst in its earthworm paratenic host; B, C) typical *Gordius* type cyst, not the folding pattern of the larva; D) fully developed *Gordius* type cyst. Note the clear halo-like structure surrounding the folded larva; E) 2 types of gordiid cysts (arrows) in the hemocoel of a non-biting midge larva; F) a *Paragordius varius* cysts in the tissue of an aquatic snail. Note the prominent spines on the folded larva within the cyst, which are characteristic for the genus *Paragordius*; G) a *Chordodes* like larva on the outside gut wall of an aquatic beetle larva in the process of being melanized (orange-brown pigment). Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

that female crickets were capable of producing eggs only after they released worms and were provided with food ad libitum. However, all female crickets that released worms and produced eggs had difficulties mating with male crickets and/or ovipositing. In contrast, all infected male crickets were castrated by horsehair worms and did not regain the ability to produce sperm after they released worms.

Life Cycle

One fascinating aspect of gordiid biology is their complex life cycle which includes both free-living and parasitic phases (May, 1919; Inoue, 1962; Hanelt and Janovy, 1999; 2004b; Hanelt et al., 2012; Bolek et al., 2013b; Swanteson-Franz et al., 2018) (Figure 6). As juveniles, gordiids are parasites of terrestrial arthropod hosts from which free-living adults emerge into aquatic or semi-aquatic environments, such as waterlogged fields, streams, rivers, and lakes (Hanelt et al., 2005; Anaya et al., 2019). Three species of gordiids (*Paragordius varius*, *P. obamai*, and *Chordodes kenyaensis*) have been domesticated in the laboratory including dioecious and parthenogenetic species (Hanelt and Janovy, 2004b; Hanelt et al., 2012; Bolek et al., 2013a; 2013b). Studies on these domesticated nematomorphs indicate that life cycles of gordiids involve 5 distinct life stages (Figure 6) including: 1) Egg strings, 2) free-living larvae, 3) parasitic cysts, 4) parasitic juveniles, and 5) dioecious or parthenogenetic free-living adults (Hanelt and Janovy, 2004a; 2004b; Hanelt et al., 2012). Juvenile gordiids are obligate parasites of predominantly terrestrial arthropods, whereas a number of species of aquatic animals serve as paratenic hosts for the cyst stage (Hanelt et al., 2001; Bolek and Coggins, 2002; Hanelt and Janovy, 2003; 2004a).

As noted above in general, gordiids commonly infect 4 major groups of terrestrial arthropods, including beetles, orthopterans, praying mantids, and cockroaches. Additional confirmed records exist from earwigs (Dermaptera) and aquatic larval trichopterans and larval dragonflies (Schmidt-Rhaesa, 2013). All gordiids develop in the hemocoel of their

arthropod host where they grow from a small length of 60–100 μm to a length of over 2 m for some species (Schmidt-Rhaesa, 2013) (Figure 7). During development in the arthropod definitive host, 2 cuticles are present, a thin white larval cuticle which is replaced by a robust dark adult cuticle (Figure 8) (Schmidt-Rhaesa, 2005). Before emergence from their host, adult gordiids form an open wound on the posterior end of the host's abdomen and once the infected arthropod enters water, the worms emerge head-first (Hanelt and Janovy, 2004b; Hanelt et al., 2012; Bolek et al., 2013a).

As noted, field observations indicate that infected terrestrial arthropods, such as crickets and beetles, deliberately enter water, suggesting that worms may be manipulating the behavior of their arthropod hosts (McCook, 1885; Müller, 1926; Jolivet, 1945; 1948). More recently, Thomas and colleagues (2003) discovered differences in the brains and concentrations of neurotransmitters among infected and uninfected field-collected crickets. Additional studies by Biron and colleagues (2005a; 2005b; 2006) show that several brain proteins are altered in crickets infected with gordiids. It is not known whether the gordiids' mere presence or something emitted by the gordiids affects the hosts' behavior.

Distribution and Diversity

Within the freshwater/terrestrial Gordiida, approximately 360 species of horsehair worms have been described worldwide from 18 extant and 2 extinct genera (Poinar, 1999; Poinar and Buckley, 2006; Yadav et al., 2018). In addition, 5 species of marine horsehair worms have been described from a single genus (Schmidt-Rhaesa, 2013). However, current estimates suggest that only 18% of the horsehair worm diversity has been documented across the world, with another 2,000 species awaiting discovery (Poinar, 2008). The earliest reported and credible fossil Nematomorph, was described from 100 million year-old Lower Cretaceous Burmese amber and belongs to the extinct species *Cretachordodes burmitis* (Poinar and Buckley, 2006). Additionally, 2 individuals of the fossilized species *Paleochordodes protus* (Poinar, 1999) emerging from a cockroach have been described from Dominican amber dated between 15 and 45 million years-old (Figure 12). However, obtaining knowledge on the diversity of horsehair worms has been difficult due to their unusual life cycles, where free-living adult worms exit their hosts, and the lack of reliable ways of collecting the free-living adults over large geographic areas from aquatic and terrestrial habitats (Bolek and Coggins, 2002; Bolek et al., 2013a; Bolek et al., 2015).

The freshwater and terrestrial horsehair worms have been reported from all continents except Antarctica; whereas the marine genus *Nectonema* is known from several locations worldwide including both coasts of the northern Atlantic Ocean,

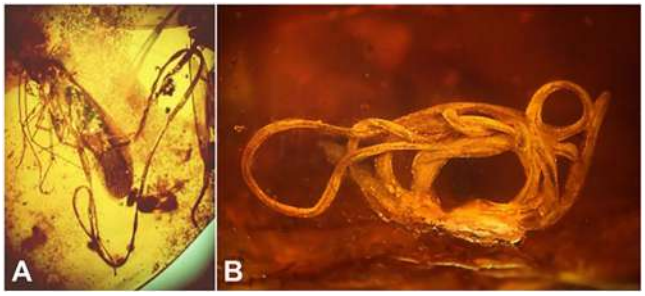

Figure 12. Fossil nematomorphs. A) Two specimens of *Paleochordodes protus* in the process of emerging from a cockroach host in Dominican amber, 15–45 Ma (= million years old); B) the oldest known hairworm fossil *Cretachordodes burmitis* recovered from Early Cretaceous amber, 100 Ma, from Myanmar. Source: G. Poinar. License: CC BY-NC-SA 4.0.

as well as the Indian Ocean, and the Pacific Ocean from the southern coast of New Zealand (Poinar and Brockerhoff, 2001; Hanelt et al., 2005; Bolek et al., 2015). The fauna of North American, European, and Argentinean nematomorphs has been relatively well studied. However, nematomorph diversity in Africa, Asia, and most of South America has received comparatively little attention (Hanelt et al., 2005; Schmidt-Rhaesa, 2013; Bolek et al., 2015; Schmidt-Rhaesa et al., 2016; Swanteson-Franz et al., 2018; Anaya et al., 2019; Zanca et al., 2020). Among the Nearctic freshwater and terrestrial horsehair worms, 24 species from 7 genera have been described (Schmidt-Rhaesa et al., 2003; Poinar and Chandler, 2004; Begay et al., 2012; Swanteson-Franz et al., 2018; Anaya et al., 2019). However, evidence from molecular barcoding techniques indicates there are numerous hidden, cryptic species. For example, the common Nearctic species *Gordius robustus* represents a large species complex composed of at least 8 distinct genetic lineages (Hanelt et al., 2015). These recent molecular studies indicate the importance of genetic data and limitations of morphological characters in determining some gordiid species within the phylum.

Taxonomy and Phylogeny

The phylum Nematomorpha consists of 2 subphyla, the marine Nectonematida and the freshwater and terrestrial Gordiida comprising 1, and 18 extant and 2 extinct genera, respectively (Schmidt-Rhaesa, 2013). Nematomorphs have few morphological characters that can be used by taxonomists for species determination. Macroscopic characters include the shape of the posterior end being bilobed or round in males, and trilobed or round in females. In addition, the presence of cuticular structures such as crescents near the cloacal opening and/or areas of dense bristles and/or spines on the posterior region of worms are useful for some genera delimitations

(Figure 4) (Schmidt-Rhaesa, 2013). All other characters, including areoles and intra-areoles spaces are found on the cuticle, many of which are so small that scanning electron microscopy (SEM) is necessary to visualize these characters. As such, SEM has become the standard protocol for nematomorph identification (Hanelt et al., 2005; Bolek et al., 2015).

Horsehair worms are placed in the superphylum Ecdysozoa and are considered the sister phylum to the phylum Nemata (Hanelt et al., 2005). However, and unlike nematodes, cephalic papillae, lateral epidermal cords, secretory-excretory systems, amphids, and spicules are lacking in horsehair worms. Other differences between horsehair worms and nematodes include genital openings located on the posterior end of female horsehair worms instead of near the middle of the body as in nematodes. Additionally, and unlike nematodes, these animals have a true larval stage that undergoes drastic morphological tissue reorganization during development in their host (Schmidt-Rhaesa, 1997; 2013).

Of 3 phylogenic hypotheses based on molecular (DNA) sequencing discussed here, the ancestor-descendant relationships of multiple genera and species within the Nematomorpha were analyzed (Bleidorn et al., 2002; Chiu et al., 2017; Tobias et al., 2017). Bleidorn and colleagues (2002) uses a combination of morphological and molecular (18S rRNA gene) data indicating a sister-group relationship between the marine genus *Nectonema* and the freshwater Gordiida. However, within the Gordiida, all species within the basal genus *Gordius* and all species within the sister genus *Paragordius* are monophyletic. The remaining derived genera are not well supported, and some appear as polyphyletic. For example, the more derived *Neochordodes occidentalis* is nested within species of *Chordodes*. More recent molecular phylogenetic analyses using mitochondrial markers (*CO1*) and/or nuclear markers (8S rRNA) indicate that the freshwater genus *Paragordius* is basal to the remaining freshwater and terrestrial gordiids. More importantly, these molecular phylogenetic hypotheses are in agreement with the traditional morphological relationships of freshwater and terrestrial gordiids including the genera *Gordius* and *Acutogordius* within the family Gordiidae and the remaining genera within the family Chordodidae (Chiu et al., 2017; Tobias et al., 2017).

Ecology and Behavior

The ecology of nematomorphs is closely tied to the biology of their arthropod definitive hosts and the aquatic or terrestrial habitats of the adult free-living worms. However, few studies have sampled for free-living adult worms throughout the year and even fewer studies have examined multiple arthropod species for nematomorph infections (Bolek and Coggins, 2002; Poinar and Weissman, 2004; Looney et al., 2012). As a general rule and depending on the gordiid species, nematomorphs vary in their definitive host specificity, and free-living adults are seasonal and have a male-biased sex ratio (Hanelt et al., 2005; Bolek et al., 2015).

Host specificity for most nematomorph species is poorly understood, and most host records are based on field observations (Schmidt-Rhaesa, 1997; 2013; Schmidt-Rhaesa et al., 2003). Field studies indicate that some, but not all, nematomorph species appear to be host-specific at the definitive arthropod host level (Poinar, 1991; Bolek and Coggins, 2002; Schmidt-Rhaesa et al., 2003; Chiu et al., 2011; Looney et al., 2012). For example, the North American *Chordodes morgani* has been reported from 4 phylogenetically distinct orthopteran and cockroach species, suggesting that some horsehair worms are generalists at the definitive host level (Schmidt-Rhaesa et al., 2003). However, other species, particularly in the *Gordius* cf. *robustus* complex, appear to be more specific at the definitive host level and are restricted to a single or a few closely related species of arthropod hosts (Hanelt et al., 2015). Molecular evidence from mitochondrial (*CO1* and *cytB*) and nuclear (partial 28S, ITS1, 5.8S, and ITS2) DNA suggests that at least 8 species occur across North America. However, this group is paraphyletic, since the European *G. aquaticus* and *G. balticus* group among the *G. robustus* lineages form 2 distinct clades, A and B (Figure 13B). When all known arthropod definitive hosts are mapped onto this phylogeny it appears that species within clade A infect various species of orthopterans; whereas species in clade B infect millipedes and ground beetles (Figure 13).

Once emerged from their arthropod hosts, free-living adult worms are seasonal (Bolek and Coggins, 2002; Schmidt-Rhaesa et al., 2005; Salas et al., 2011; Anaya, 2019). For example, Bolek and Coggins (2002) reported the occurrence of free-living adults of *Gordius difficilis* in Wisconsin, United States from June to October; whereas Anaya (2019) reported *G. terrestris* (incidentally, the only known species of gordiid consistently collected from terrestrial habitats) from Oklahoma, United States during October through March. Additionally, Salas and colleagues (2011) examined the seasonal occurrence of free-living individuals of 4 species of sympatric gordiids over a 1-year period from a stream in Argentina. In their study, free-living worms of all 4 species occurred in the stream during the fall, winter, and spring. However, *Noteochorododes cymatium*, *N. talensis*, and *Pseudochordodes dugesi* were most abundant during the winter and spring; whereas *Chordodes brasiliensis* was most abundant during the fall.

The explanation for these seasonal patterns includes the short life span of free-living adult worms (2–8 weeks) and the abundance of their arthropod definitive hosts. For example, in a 3-year study, Schmidt-Rhaesa and colleagues (2005)

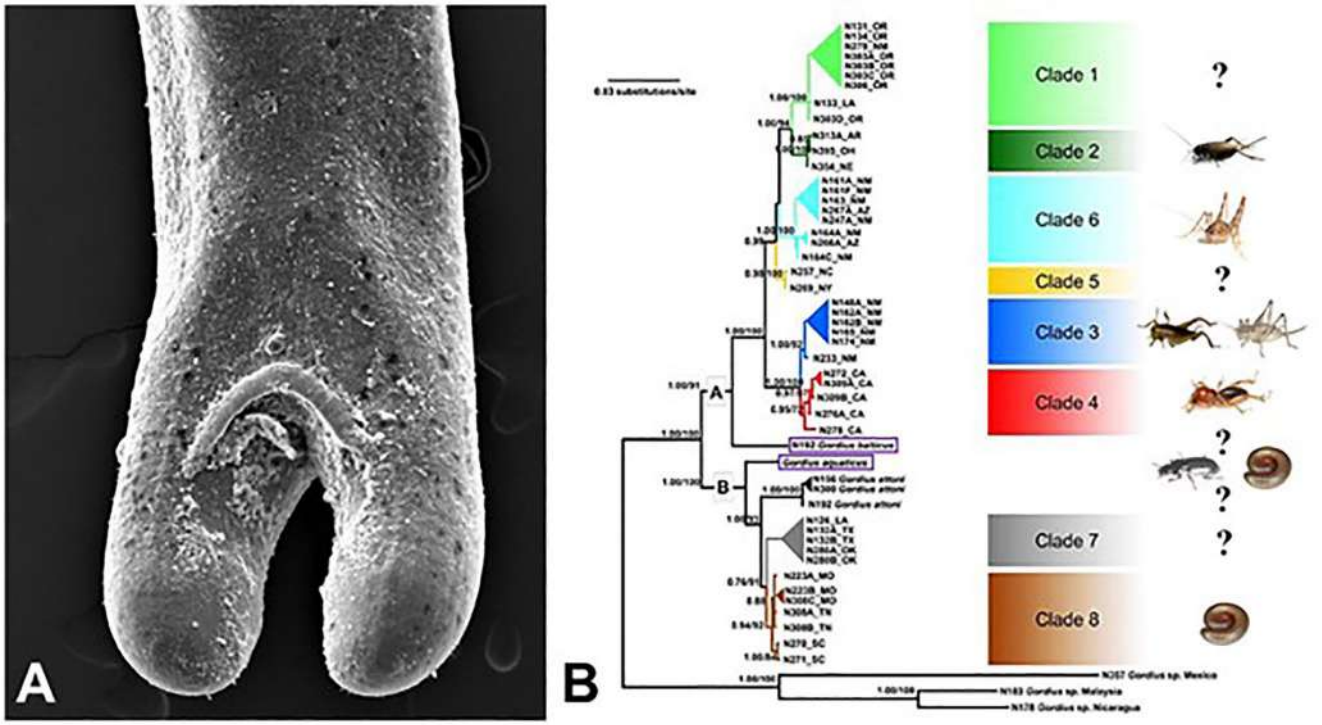

Figure 13. Hosts and phylogenetic relationships of the *Gordius* cf. *robustus* complex. A) Posterior end of a male *Gordius* cf. *robustus*, with the characteristic bilobed end, post cloacal crescent, and poorly developed areoles on the cuticle; B) phylogenetic hypothesis (partial CO1 and cytB sequences) of the *Gordius* cf. *robustus* group and diverse group of arthropod definitive hosts. Note that the *Gordius* cf. *robustus* linages are paraphyletic with European species (purple brackets) group among the *G. robustus* lineages and form 2 distinct clades A and B. Species in clade A appear to infect orthopteran arthropod hosts, whereas, species in clade B infect ground beetle and millipede arthropod hosts. Sources: A) M. G. Bolek; B) adapted from Hanelt et al., 2015. License: CC BY-NC-SA 4.0.

collected data on recently emerged adults of 2 species of nematomorphs and their definitive arthropod hosts around a swimming pool in southern France. Most adults of *Pseudochordodes tricuspidatus* emerged from their hosts during June through August, whereas most adults of *Spinochordodes tellinii* emerged from their hosts during August through September. At their study site, both gordiid species infected different species of definitive hosts and their occurrence was correlated with the abundance of these hosts.

Free-living adults do not feed and are found in various aquatic habitats including water sources in caves, puddles, ponds, lakes, and small and large streams and rivers (Reeves, 2000; Hanelt et al., 2005; Schmidt-Rhaesa, 2013; Bolek et al., 2015). Within these habitats, free-living worms can be located in the sediment, among moist fallen leaves, under rocks, in algal mats, and/or on aquatic vegetation where they form Gordian knots and mate (Hanelt et al., 2005; Bolek et al., 2015). Additionally, free-living adults of *Gordius terrestris*, a terrestrial species, appear during rain events on wet lawns and pools of water on streets and sidewalks, where the worms copulate. After the rains stop, adult free-living worms can be found entangled in the roots of grasses and in the soil where females deposit egg strings (Anaya, 2019; Anaya et al., 2019; Figure 14).

The sex ratio of free-living adult gordiids is usually but not always male biased, with a few field studies indicating equal sex ratios (De Villalobos and Camino, 1999; Valvassori et al., 1988; Salas et al., 2011). For example, Cochran and colleagues (1999) reported that of 1,391 individuals of *Gordius difficilis* collected during a 32-year period in 6 Midwestern states of the United States, 1,205 were males. In contrast, Watermolen and Haen (1994) reported 67 individuals of *G. robustus* from Wisconsin of which 66 were females. These field-skewed sex ratios are in contrast to laboratory life cycle studies on dioecious nematomorph species. Hanelt and Janovy (2004b) and Bolek and colleagues (2013b) each found no statistically significant differences in the sex ratios of *Paragordius varius* or *Chordodes kenyaensis* emerging from laboratory-reared and -infected cricket definitive hosts.

A few hypotheses have been proposed for this strongly skewed sex ratio in the field, including differences in the development times of male and female worms in their final hosts,

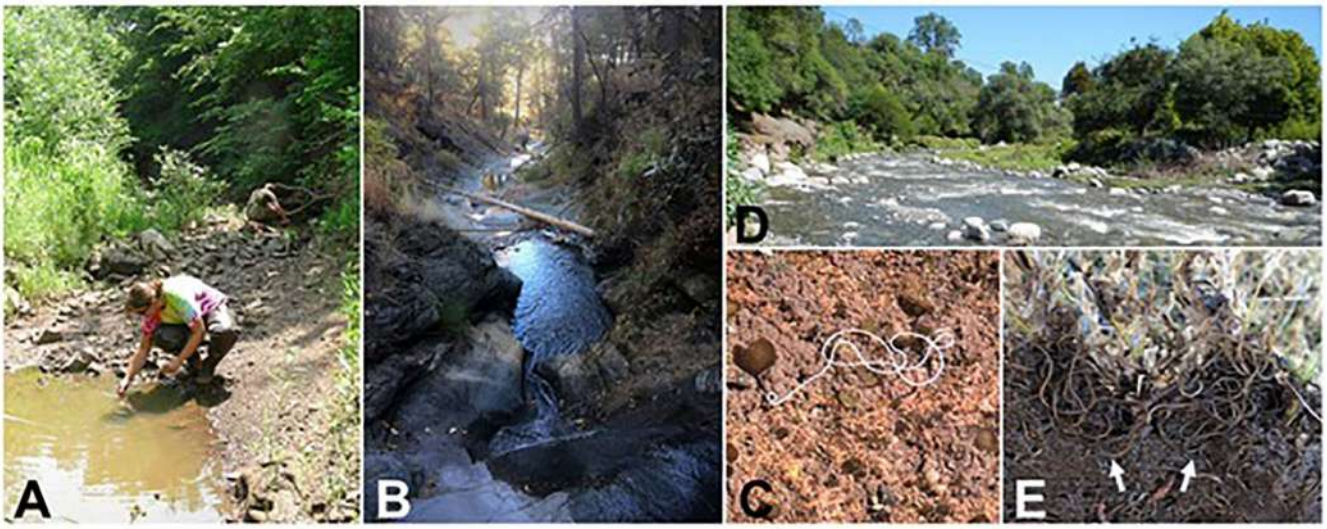

Figure 14. Typical habitat for free-living adults, larvae, and cysts of freshwater and terrestrial gordiids. A) Second-order stream in Payne County, Oklahoma, United States; B) a typical first-order stream in the Chiricahua Mountains, Arizona, United States; C) a white free-living adult male *Gordius* sp. on the bottom of a first-order stream in the Chiricahua Mountains, Arizona, United States; D) a third-order stream in the Córdoba, Argentina. Many of the nematomorphs glue their egg strings on rocks in this habitat; E) adult free-living *G. terrestris* entangled in grass roots under the soil, in a suburban environment, Stillwater, Oklahoma, United States. Source: M. G. Bolek. License: CC BY-NC-SA 4.0.

and/or behavior differences among free-living males and females (Poulin, 1996; Bolek and Coggins, 2002). More recently, Anaya (2019) documented behavioral differences among male and female *Gordius terrestris*, a species with an extremely male biased sex ratio (5.4:1.0) observed in the field. In the laboratory, when male and female worms are placed on the surface of the soil, significantly more females burrow into the soil than males. Once females burrow, they begin ovipositing. This observation is important and provides a plausible explanation for the extremely male-biased sex ratio observed for *G. terrestris* in the field. Taken together, these observations suggest that unlike males, after mating female horsehair worms may be moving to specific locations in the environment to oviposit and be more difficult to locate than males.

Little information is available on the physiological constraints of free-living stages of horsehair worms to their external environment. However, Bolek and colleagues (2013b) indicate that in laboratory cultures adult free-living *Chordodes kenyaensis*, worms die within 24 hours if they emerge from their hosts in cages without a water source, suggesting that adult free-living worms must remain moist to survive. In addition, Achiorno and colleagues (2008) examined the survival of eggs, larvae, and free-living adults of *C. nobilii* to extreme temperatures. They demonstrated that all eggs, most larvae, and all adult gordiids die at a high temperature of 40.5 °C, and all eggs and most adult worms (89%) die at a low temperature of −3 °C. In contrast, larvae frozen at −3 °C for 48 hours survived freezing and are capable of infecting mosquito larva paratenic hosts. Finally, Bolek et al. (2013a) evaluated the survival of larvae and cysts of North American and African gordiids in the genus *Paragordius* when super cooled and/or frozen at −20 °C or −80 °C for up to 7 months. Their work demonstrates that post-frozen larvae and cysts of these species have the ability to infect and develop in the next host in the life cycle. It is currently unclear why larvae and cysts of gordiid species from Africa have the ability to survive super cooling and/or freezing for such periods.

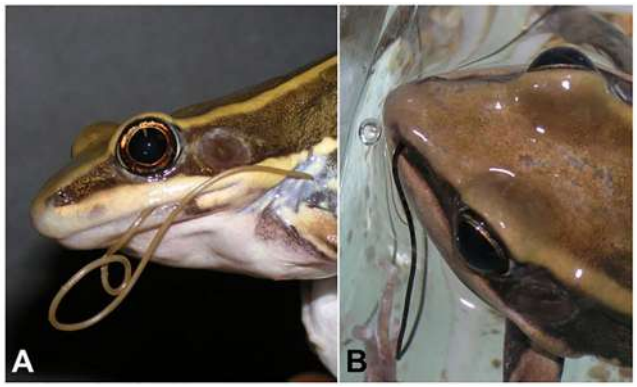

Figure 15. Predators and epibionts of gordiids. A, B) A free-living *Paragordius tricuspidatus* escaping from the mouth (A) and nose (B) of the common green frog (*Rana erythraea*) after it ingested an infected cricket. Source: F. Thomas. License: CC BY-NC-SA 4.0.

Finally, birds, fish, and frogs occasionally eat free-living adult nematomorphs and their infected hosts (Cochran et al.,

1999; Bolek and Coggins, 2002; De Villalobos et al., 2008; Fair et al., 2010). However, work by Sato and colleagues (2008; 2011) in Japan indicates that gordiids are only found in the stomachs of trout (*Salvelinus leucomaenis japonicus*) when those fish also consume their camel cricket hosts. As a result, Sato and colleagues (2011) hypothesized that horsehair worm infections and their manipulations of terrestrial arthropod hosts can increase energy inputs into aquatic ecosystems. Using estimates of seasonal prey abundance, they argue that, during worm peak emergence times, infected orthopterans account for 60% of the annual energy intake of Japanese trout (Sato et al., 2011). Other studies by Ponton and colleagues (2006a; 2006b) indicate that when trout and frogs consume orthopterans infected with these nematomorphs, 18–35% of the ingested nematomorphs can escape the predators of their hosts, through the mouth or nose of frogs and the mouth or gills of fish (Figure 15).

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ACANTHOCEPHALA

58

Acanthocephala

Acanthocephala (Phylum)

Scott Monks

Phylum Acanthocephala

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Chapter 58

Acanthocephala (Phylum)

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Introduction

Members of the phylum Acanthocephala are parasitic worms generally referred to as thorny-headed or spiny-headed worms because both larvae and adults have an invertible proboscis at the anterior end. However, this common name is incorrect because acanthocephalans do not have heads! Although some consider the term head as only a general concept, it is not particularly useful in the area of invertebrate biology, except with those groups (such as Arthropoda) that actually have heads. For example, Maggenti and colleagues (2017) define a head as "The anterior body region." which is not very useful to a biologist; however, in the same entry for head, 3 more definitions are included: Definition 2, referring to the polychaete annelids: The prostomium and the peristomium; definition 3, referring to the Arthropoda: Bearing the eyes, antennae, and mouth parts; definition 4, referring to the Nemata: Comprising the lips and sensory organs, oral opening, and supporting head skeleton. Here, each definition is slightly more specific, focusing on the presence of particular structures and sensory organs as part of a head; these are more applicable to biology.

Why get so involved with definitions before acanthocephalans have even been described? There are several reasons, but only one very important reason is mentioned here. It is a complete theme in itself, namely, the concept of **homology**, meaning that 2 characteristics (structures, features, behaviors, and so on) are derived (evolved directly) from the same origin. Or, features, such as organs or structures in 2 or more taxa that can be traced back to the same feature in the common ancestor of these taxa. The concept of homology is in play every day when structures or characters or features are called by the same name, indicating that they are the same thing, having similar features. Because what a name—such as head—means to us, we expect that it is similar to all other heads by having those important, recognizable features, such as having sensory organs clustered in that particular structure. Other obvious misnomers are the uterus, vagina, and penis of acanthocephalans, which are all names borrowed from vertebrate organs. Nevertheless, because they have been used since the first studies of acanthocephalans, scientists must use them or risk confusion.

To bring this back to animals that are known as belonging to the phylum Acanthocephala, the anterior ends of species in this group do not have a concentration of sensory organs—there are no eyes, no mouth, or any other elaborate sensory structures. Thus, to reiterate, the name spiny-headed worm is not appropriate because they have no heads! These conundrums of homology are problematic when trying to discover the relationships of this group to others, but is discussed as the phylogenetic relationships among the acanthocephalans, and the hypotheses about which groups might be close relatives, are considered.

Morphology of the Acanthocephala

Compared to the bodies of members of many phyla of invertebrates, acanthocephalans are rather simple. However, the terminology relating to simple versus complex and primitive versus advanced are relative terms that are not often used by modern biologists for comparisons. This is because of the very nature of this comparison. For example, an acanthocephalan may be considered simple compared to a more complex annelid, but that same annelid is simple compared to most species of vertebrates. Thus, defining a species of organism as simple without context relative to the comparative morphological complexity of other species is futile.

With respect to simplicity versus complexity, this applies to acanthocephalans in relation to presence and absence of sensory structures and organs. First, consider what all species of the Acanthocephala don't have: First, there is no digestive system, second, there are no sensory structures related to light detection and there are no sensilla (as in the Nemata) for pressure detection that have yet been found. They have no organs or organ systems for the exchange of oxygen and carbon dioxide and the majority of species investigated do not have protonephridia for excretion or water regulation. What they do have is discussed in the following sections.

When it is said that acanthocephalans do not have elaborate sense organs, this does not mean that they cannot detect their environment. For instance, the larvae of many species are

known to break out of their cysts in the stomach or anterior region of the small intestine of their vertebrate host, the region where bile empties into the intestine. However, this is not necessarily the site where they will establish themselves to begin to mate and produce eggs (Leadabrand and Nickol, 1993; Esch, 2000). One of the better-studied species is *Leptorhynchoides thecatus*, a parasite of the green sunfish *Lepomis cyanellus*. Detailed studies have shown that the young worms migrate through the intestine to the cecae of the fish (Richardson and Nickol, 1999; Richardson et al., 2008). They sense their surroundings, probably following chemical cues in the intestine and its contents and move anteriad and enter the cecae.

Hypotheses concerning how the Acanthocephala came to exist without these structures is discussed in a later section. Suffice it to say for now that the lack of common, or homologous, morphological structures or characters makes it difficult to estimate the phylogenetic relationship of species in this phylum with other groups of invertebrates.

Superficial External Features

Adult worms of most species of Acanthocephala are fairly small—about 1 cm in length—but some individuals of many species are much smaller and individuals of some species may be really huge and can reach lengths of 70 cm (Miller and Dunagan, 1985a). Unstained by their surroundings, they are white, although some species can be colored yellow to orange by the carotenoids ingested by the intermediate (Figure 1) or the definitive host (Nickol, 1985). In the host, the body of most species is somewhat flattened, but when the specimens are killed and fixed for study the osmotic pressure of this process fills the body cavity with liquid and it assumes a more cylindrical shape (Pritchard and Kruse, 1982).

Figure 1. *Anamesia uniformis* (Blattoidea: Blattidae: Polyzosteriinae) cockroach from Barrow Island, Western Australia. Cockroaches may serve as intermediate hosts for acanthocephalans. Scale bar = 5 mm. Source: L. Gibson and S. McCaffrey, Museums Victoria, Australia, 2006. License: CC BY-NC 4.0.

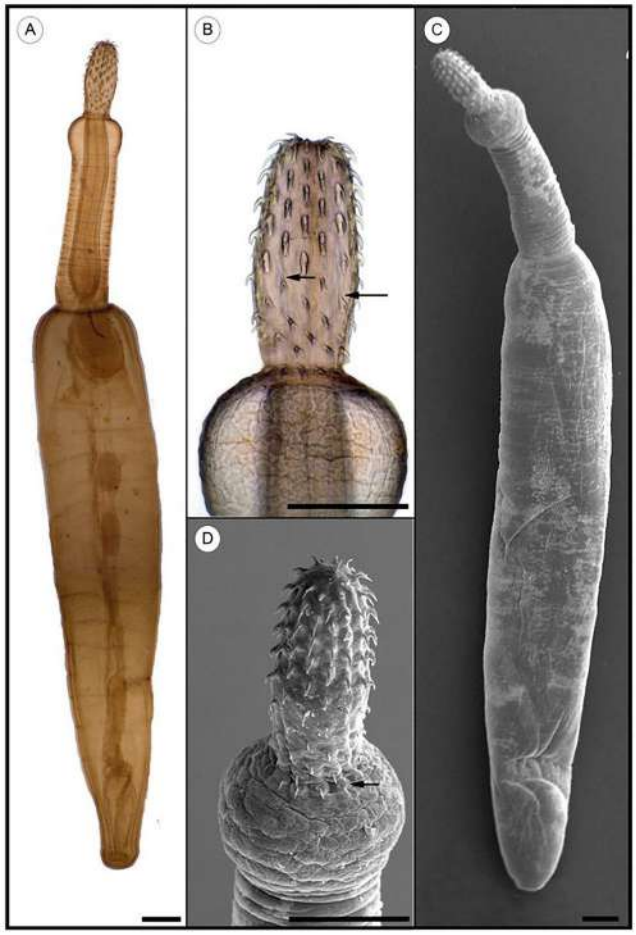

Figure 2. Larval stage (cystacanth) of the fish parasite *Pomphorhynchus tereticollis* isolated from the paratenic host *Neogobius melanostomus*. A, C) Habitus of *Pomphorhynchus tereticollis*, light- and scanning electron microscopy; B, D) detail of proboscis. Number and species specific structure details (arrows) of the proboscis hooks are clearly visible. Scale bar = 500 µm. Source: S. Emde et al., 2012. License: CC BY 4.0.

Cystacanths (the larval stage infective to the definitive host, specific to acanthocephalans, see Figure 2) are similar to adults except that the internal structures (reproductive organs and so on) are not fully developed. The cystacanths have developed into a form that is infective to the definitive host and then the development stops. Instead of being flattened, like adults of many species, the body of a cystacanth is more cylindrical in cross section. Mature cystacanths that are infective to the definitive host can be identified when the proboscis is completely inverted into the proboscis receptacle; the proboscis stays inverted until the definitive host ingests the cystacanth.

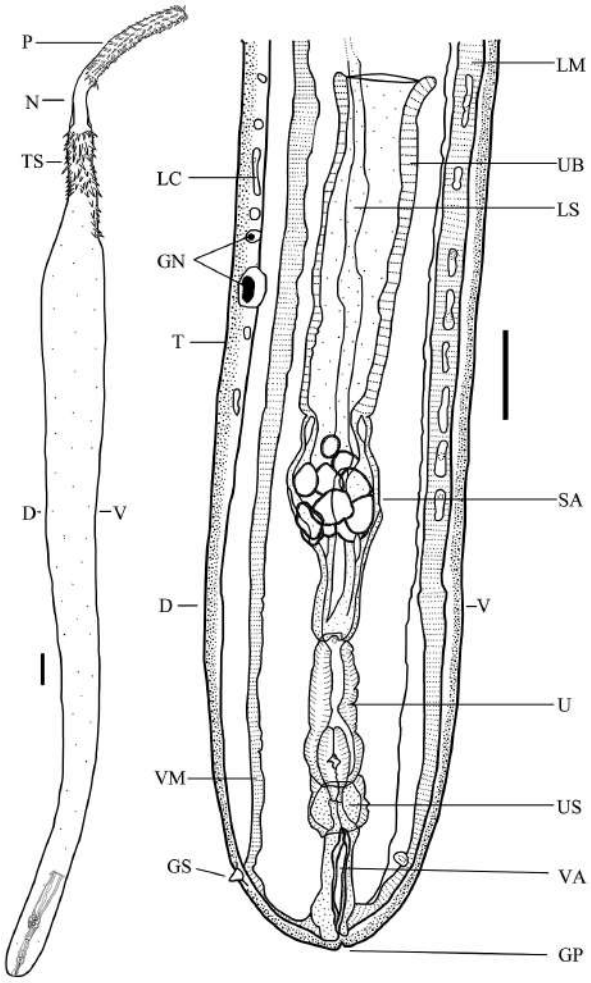

Figure 3. Drawing of the body and reproductive system of a typical female Palaeacanthocephala, *Dollfusentis* sp. D = Dorsal side of worm; GP = genital pore; GS = genital spine; LC = lacunar canal; LM = longitudinal muscles; LS = ligament sac; N = neck; P = proboscis with hooks; SA = sorting apparatus; T = tegument; TS = trunk spines; U = uterus; UB = uterine bell; US = uterine sphincter; V = ventral side of worm; VA = vagina; and VM = vestibular muscle. Source: S. Monks. License: CC BY-NC-SA 4.0.

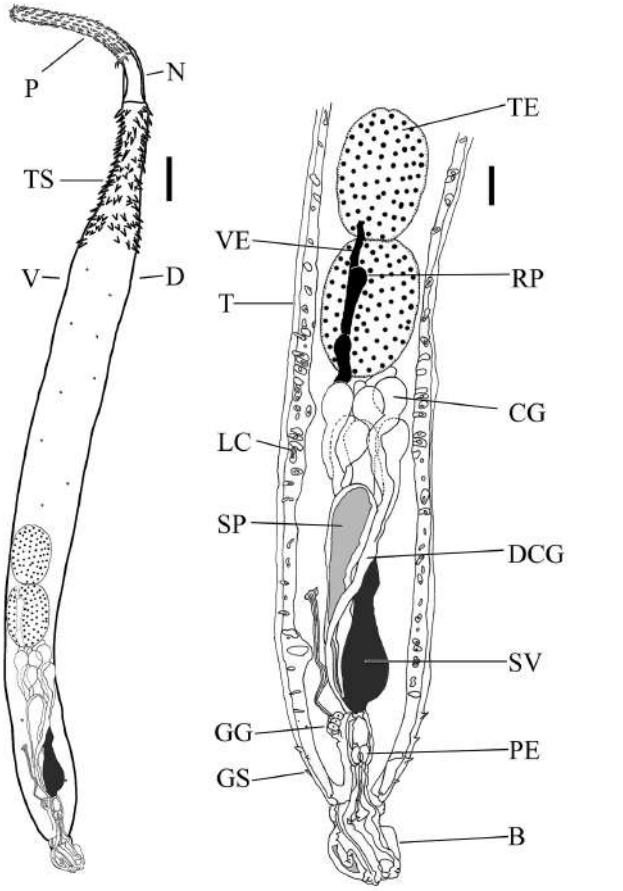

Figure 4. Drawing of the body and reproductive system of a typical male Palaeacanthocephala, *Dollfusentis* sp. B = Copulatory bursa (partially invaginated); CG = cement glands (8) (nuclei not visible); D = dorsal side of worm; DCG = ducts of cement glands; GG = genital ganglion; GS = genital spines; LC = lacunar canals; N = neck; P = proboscis; PE = penis; RP = pouch reservoir containing sperm; SP = Saeftigen's pouch (gray color represents liquid in pouch); SV = seminal vesicle (black color represents sperm); T = tegument; TE = anterior testis; TS = trunk spines; V = ventral side of worm; and VE = vas eferens (black color represents sperm). Source: S. Monks. License: CC BY-NC-SA 4.0.

The outer surface of the body (called the **trunk**) of acanthocephalans can either be smooth or can have **spines** in the tegument. Spines are similar to hooks in composition but lack a root, which, in acanthocephalans, is an important taxonomic character. The spines generally are in the more anterior part of the body, but in some species they also occur in the posterior part of the trunk in the area around the **genital pore** (Monks and Pérez-Ponce de León, 1996; Monks, et al., 1997). The distribution of the spines can be continuous or in various patterns. These patterns are often characteristic for a species and can be used for identification (see the key in Amin et al., 2011).

General Structures of the Body

All acanthocephalans are quite similar in the general structure of the body (Figures 3 and 4). The body of all acanthocephalans is composed of either 2 or 3 sections depending on whether the classic or modern designations are used.

In classic terminology, the **body** (also called the **trunk**; see Figure 5) is considered to be divided into 2 major regions, the **praesoma** and the **metasoma**. The praesoma comprises the armed (containing **hooks**) **proboscis**, **proboscis receptacle**, **cerebral ganglion** (Note: This should not be called a brain! Only vertebrates have brains), **lemnisci**, associated **muscles**, and the unarmed region be-

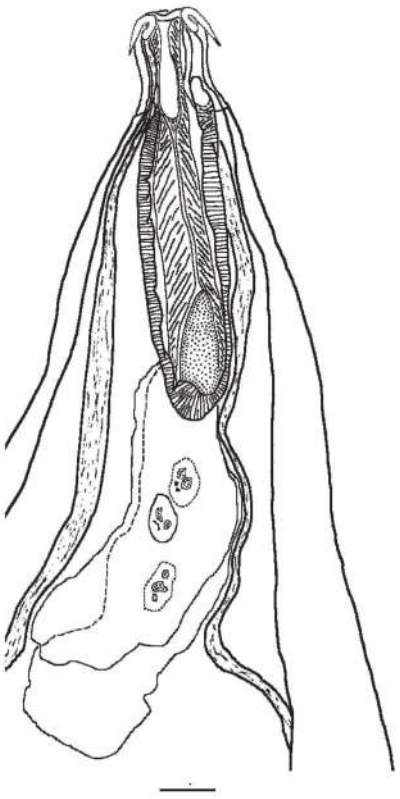

Figure 5. Anterior region of the body of a specimen of *Neoechinorhynchus brentnickoli*. Source: S. Monks. License: CC BY-NC-SA 4.0.

tween the proboscis and the rest of the body that is referred to as the **neck** (also, not a very good name for this region of the body). The metasoma is the hollow trunk of the body. The body wall, or **tegument**, of the metasoma encloses the body cavity.

The tegument was previously called a pseudocoel (Miller and Dunagan, 1985a) but is now referred to as a persistent **blastocoel**, based on more recent studies of development (Brusca and Moore, 2016). A blastocoel is the hollow cavity that forms during embryonic development comprising a ball of cells. One part of this ball of cells then **invaginates**, forming a mouth or an anus (depending on which group of animals are being discussed); this is called **gastrulation** and the larval form is called a **gastrula**. The body cavity of an acanthocephalan is the remnant of this hollow ball of cells that did not completely become lined with mesodermally-derived tissues during embryogenesis.

The tubular channels that constitute the **lacunar system** infiltrate the entire tegument of the metasoma and 2 major canals extend from the anterior to the posterior end of the trunk. Species included in some groups have **spines** that are distributed over the trunk in various patterns. These spines are similar to hooks but are smaller and do not have roots. Within the body cavity are the **proboscis receptacle**, the associated muscle bands mentioned above, and the reproductive organs.

Acanthocephalans are **gonochoristic**. Associated with the reproductive organs of males are **cement glands**, **Safftigen's pouch**, an evertible/retractable **bursa** at the posterior end, associated ducts, among other structures. Females lack these structures. Each group of structures and organs is discussed separately.

Tegument is living tissue that is a syncytium of cells without nuclei which includes dense fibers and connective tissue. The body of acanthocephalans is covered by a multilayered tegument, the overall structure of which resembles that of rotifers (Herlyn et al., 2003; Weber et al., 2013; Sielaff et al., 2016; but see Dunagan and Miller, 1991 for a traditional interpretation). Underneath the tegument are circular and longitudinal muscles, many of which are tubular rather than a dense solid mass. The outer surface of the tegument contains numerous **micropores** that connect to fine canals leading to a complex system of tubes that extend throughout the tegument in patterns specific to particular groups of acanthocephalans. As mentioned above, the system of tubules is called the lacunar system. Finally, the tegument may contain **nuclei**, called **giant nuclei** in some taxa. There can be a few very large/giant nuclei or more numerous **branched nuclei** (for examples of giant nuclei see figures in Monks et al., 2011, and branched nuclei in those of Monks et al., 1997).

Morphology of the Praesoma

Proboscis

The proboscis is one of the distinctive structures of acanthocephalans. The **armament** (**hooks** and **spines**; Figure 6) is a distinctive feature of the proboscis. It can be withdrawn into the **proboscis receptacle** (within the body cavity) by turning it inside out. The hooks make the invagination process necessary.

The hooks somewhat resemble the thorns on the stem of a rose. In most species the hooks are curved, although in some, the more posterior hooks may extend almost perpendicular to the proboscis rather than be curved posteriad (see the figures in Amin et al., 2011). Some species, such as *Koronacantha mexicana*, have rootless spines posterior to the hooks (see the figures in Monks et al., 1997). Each hook consists of a **root** (the part which anchors the hook to the proboscis) and the **blade** (the pointed part of the hook). The root is only an anchor for the hook, not to be confused with the root of a plant. That is, there are no other structures or muscles that might enable the movement of the hook. Spines on the proboscis do not have roots; those on the trunk also don't have

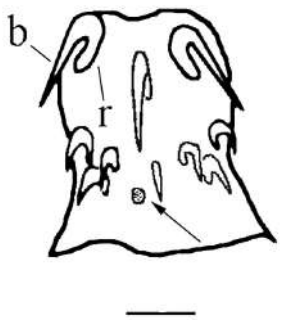

Figure 6. Proboscis and neck of a specimen of *Neoechinorhynchus brentnickoli*. b = blade of hook; r = root of hook; arrow indicates lateral sensory pore. Scale bar = 25 μm. Source: S. Monks. License: CC BY-NC-SA 4.0.

roots. This distinction, that hooks have roots and spines do not have roots, seems clear, but in real life the difference often is blurred. Finally, some prefer to call the spines on the proboscis rootless hooks (Muñoz and George-Nascimento, 2002), leaving the term spines for the armament on the trunk.

When the hooks are fastened into the intestinal wall of the definitive host of the worm, there is no way to dislodge the hooks. This is why the process of invagination of the proboscis is necessary. To visualize the retraction of the proboscis back into the body cavity of the acanthocephalan, if one imagines that a hand is inserted into a tight-fitting glove, it is obvious that the hand is not easily withdrawn from the glove. The easiest way to remove the glove is to turn the glove inside out, removing it by pulling the part nearest the wrist distally toward the fingers and finally over the fingers and off the hand, leaving the glove inside out. The folding inside out of the glove is similar to what happens to the proboscis in invagination.

In the definitive host, the worm forces its proboscis into the tissue of the wall of the host's intestine. Once inserted, the hooks prevent removal and protect the worm from being dislodged by movements of the intestine or its contents. However, acanthocephalans move and migrate within the host's intestine (Leadabrand and Nickol, 1993; Richardson et al., 2008) so they need to be able to unhook themselves. Long strands of inverter muscles extend from inside the body cavity anteriorly to the most anterior point of the proboscis. Normally they are relaxed, permitting the proboscis to remain inserted firmly into the wall of the host's intestine. When an individual acanthocephalan prepares to move, the inverter muscles contract and pull the proboscis inside of the receptacle, disconnecting each ring of hooks as the proboscis is invaginated. This smoothly removes the hooks opposite the way they went in rather than by forcibly tearing them out.

The proboscis of acanthocephalans has different shapes in the different members of the phylum. In general, the proboscis is cylindrical or spherical. In a phylogenetic analysis of members of the phylum, Monks (2001) identified different shapes of the proboscis of the species included in his analyses, including: Round, elliptical to oval, elongate to fusiform, clavate, and cone-like. These shapes were sufficient to differentiate between the taxa in those analyses. Although other shapes are known, such as, spindle-shaped, meaning, wide in the middle and tapering toward each end (see the figures in Richardson et al., 2010, for an example) that would be needed if more species had been included in the analyses by Monks (2001).

The function of the proboscis is to provide attachment to the intestinal wall of the definitive host by penetrating the intestinal wall. To date there is no hypothesis that relates the qualities of the intestinal wall to a particular shape of proboscis. One might think that the length and shape of the proboscis would be related to the structure of the host's intestinal wall—that is, thickness, muscularity, presence of thick connective tissue, and so on—but this does not seem to be true. For example, the proboscis of *Macracanthorhyncus hirudinaceous*, which is a parasite of pigs, has a relatively small and round proboscis (similar to that of *Neoechinorhynchus brentnickoli*). In contrast, species of *Pomphorhynchus*, which are parasites of fish, have a medium-sized, cylindrical proboscis and a long neck that penetrates the relatively thin intestinal wall and extends into the body cavity (see photos and discussion at http://alchetron.com/Pomphorhynchus-laevis).

Other than the hooks, the extent of which marks the posterior margin of the proboscis, few other structures are included in the proboscis (Miller and Dunagan, 1985a). Internally, at the anterior end of the proboscis is a small group of cells called the **apical organ** (Miller and Dunagan, 1983; 1984). The apical organ varies in shape depending on the group, and in some there is a small pore leading outside of the proboscis (Dunagan and Miller, 1983). Note that the apical organ discussed here is not homologous with the apical organ that is found in all species of tapeworms of the genus *Hymenolepis*. In all species of Acanthocephala that have been examined there are 2 large nuclei in the posterior area of the apical organ).

Located opposite each other on the lateral sides of the proboscis, usually near the posterior-most ring of hooks, is a pair of **sensory pores** (Figure 6), or **lateral sense organs** (Herlyn et al., 2001), that open on the surface of the proboscis. Internally, the pores are connected to the sensory support cell complex (Miller and Dunagan, 1983; 1984; 1985a). The function of these cells is not well understood and it is not known what they detect.

Neck

The neck is relatively featureless and there are no hooks or spines present. The posterior margin of the proboscis includes the posterior-most hooks or spines, and thus is not the neck. The neck is tubular, hollow, and connects the proboscis to the trunk. Structures (muscles, nerves, and in some cases the proboscis receptacle) pass through the hollow center of the neck, but they are not fastened to it. Species of *Pomphorhynchus*, which are parasites of adult fish, are one exception (see the photographs and life cycle diagrams available at https://alchetron.com/Pomphorhynchus-laevis). These species have the neck enlarged to form a bulb. The proboscis penetrates the intestinal wall of the host fish, often extending into the body cavity, and the bulb expands to prevent the proboscis from being dislodged.

Morphology of the trunk (metasoma)

The proboscis may be the most notable structure of acanthocephalans, but the trunk, which constitutes the rest of the body, contains the majority of structures. The trunk is divided from the neck by the attachment of the proboscis receptacle.

The **lacunar system**, which is the canal system of the trunk, starts anteriorly at the neck-trunk junction and extends to the posterior end of the body. Longitudinal canals run dorsally and ventrally, or laterally to link circular canals (Miller and Dunagan, 1985a; 1985b). It is thought that the liquid in the canals circulates as a result of body movements.

As mentioned above, many species have spines on the surface of the trunk, distributed in various patterns. Only recently, studies of the manner of attachment to the intestinal wall by species of *Corynosoma* have shown that the spines assist in providing a secondary attachment (Aznar et al., 2002; 2016).

The **proboscis receptacle** is attached to the anterior portion of the trunk. The receptacle, as the name implies, is a structure in which the proboscis is retracted into when it is inverted, but this seems to be only a secondary function because the proboscis could just as well be drawn into the body cavity. The receptacle is a sac, open at the anterior end and most commonly attached at the neck-trunk junction. However, in some taxa the receptacle is attached at the posterior ring of proboscis hooks (see the figures in Amin et al., 2017), or, in a few groups, in the middle of the proboscis (see the figures in Richardson et al., 2010). The wall of the receptacle is composed of 1 or 2 layers of muscle. The muscles of each layer have fibers that are circularly, longitudinally, or spirally oriented (Monks, 2001).

Long **retractor muscle bands** attach to the anterior end of the proboscis and they extend posteriad through the posterior end of the receptacle and are attached to the inner surface of the body wall. When these bands contract, they pull the proboscis into the receptacle. There are no antagonistic muscles that can pull the proboscis back out. Eversion of the proboscis is accomplished by contraction of the muscular receptacle walls, evidently forcing the proboscis out by hydrostatic pressure. Several other muscle bands pass through the receptacle, but most importantly, the **cerebral ganglion** is found in the receptacle. The cerebral ganglion hangs from nerves that run anteriad from it. These nerves exit the receptacle and attach to the inner wall of the trunk, running posteriad. The ganglion is composed of a small number of neurons (around 100 of them), although this knowledge is based on studies of just a few species (Miller and Dunagan, 1985a).

The paired **lemnisci** are connected anteriorly at the neck/trunk junction. Each is a long, spongy organ with a few **giant nuclei**. The function of the lemnisci is unknown, leading some investigators to associate them with the lost digestive system, and possibly with the salivary glands of rotifers (Miller and Dunagan, 1985a). Moore (1946) observed that the lemnisci develop as evaginations of the hypodermal layer of the trunk in *Moniliformis moniliformis*. To date, the best interpretation of the function of the lemnisci is derived from observations of the lemniscal **plasmalemma membrane** that has numerous infoldings that greatly amplify the free surface area exposed to the metasomal (body) cavity (Wright, 1970). Thus, it is thought to have an important physiological role in transporting material relative to the metasomal cavity. However, the precise nature of the materials being transported has not been investigated.

Morphology of the reproductive organs

As in all parasitic worms, the reproductive organs are well-developed structures and are obvious in stained, cleared, and mounted specimens. In acanthocephalans, this is especially true because in the majority of species, the trunk cavity is almost empty except for the presence of the reproductive system. As mentioned above, these animals are **dioecious** or **gonochoristic**, meaning that the sexes are separate, with male and female individuals.

Associated with the reproductive system are the **genital ganglia** and the **protonephridia**. The genital ganglion is a small nexus of neurons that is presumed to control the male reproductive organs (Dunagan and Miller, 1978; Dunagan and Price, 1985); however, in most descriptions of species, the genital ganglia are not mentioned. Most species of acanthocephalans do not have protonephridia, but in a few species, females possess protonephridia comprising **flame cells** (Miller and Dunagan, 1985a).

Female reproductive organs.

The main reproductive organs of females are, from anterior to posterior: **Ovary**, **uterine bell**, **sorting apparatus**, **uterus**, **vagina**, and **gonopore**. The **ligament sac** is associated with the reproductive system and is a hollow, membranous tube—in some groups there are 2 sacs—that runs from the proboscis receptacle to the uterine bell. As the worms mature, the sacs persist in species classified in the Archiacanthocephala but they rupture in species of both Palaeacanthocephala and Eoacanthocephala. In some species almost no remnants can be found. The ovaries develop within the ligament sac. The evolutionary origin of the ligament sac is uncertain, but the presence of the ovaries within the lumen of the sac precludes identifying them as the missing intestine.

When female acanthocephalans are immature, they first have 1 ovary that fragments into groups of cells called **ovarian balls**, which subsequently continue fragmentation into ova (unfertilized eggs) and finally, when fertilized in mature females, into shelled eggs, which are also called **shelled acanthors** because the embryo is called an **acanthor**. The unfragmented ovary can only be seen in female cystacanths or very immature adults. Asaolu (1980) and Asaolu and colleagues (1981) completed detailed studies of this process, including scanning electron micrographs.

While developing, fertilized ova circulate within the unbroken ligament sac or within the body of those species in which they do not persist. Eventually, eggs with shells, both immature and mature, enter the uterine bell. Note that the eggs are mature when they are infective to the intermediate host and only mature eggs are passed into the intestine and then out into the external environment; this is the function of the sorting apparatus. The chemical or physical indicators of maturity are unknown, but the apparatus has 2 openings, one leading back to the body cavity and one leading to the uterus. Based on whatever clues are used, the sorting apparatus sorts the eggs, with the immature ones being routed back to the body cavity for further development and the mature ones being sent on to the uterus.

Mature eggs in the uterus pass to the vagina and then, one by one, out to the environment (which comprises the fecal material in the intestine of the definitive host). Although not a part of the reproductive system, all females have muscles located near or around the gonopore (see Monks and Pérez-Ponce de León (1996) for drawings of the vestibular muscle of *Koronacantha mexicana*). Monks (2001) identified 10 different types of muscle of what has been called the genital vestibule. The acanthocephalans have not been studied sufficiently to identify patterns of the evolution of the different forms of vestibular muscles, but these structures may be important in protecting females of one species from being inseminated by males of a different species. Despite the many different forms, all appear to have the same function—to change the shape of the region around the genital pore in order to prevent copulation until the female is ready or not to permit the bursa of males to fit over the posterior end of females, which, in turn, prevents the penis of males from connecting to the genital pore (see figures in Monks et al., 2008).

Male reproductive organs.

The principle reproductive organs of males are (from anterior to posterior): **Testes**, **sperm ducts**, **sperm reservoir**, and **penis**. Associated structures are: **Cement glands**, **cement reservoirs** (if present), **Saeftigen's pouch** (also spelled Seftigen), **genital ganglia**, and **bursa**. A thorough study of the morphology of the reproductive system of males is provided by Asaolu (1981).

Male acanthocephalans have 2 testes, variable in location but always located some distance anterior to the remaining organs. As noted by Monks (2001), they are located generally in tandem with one another but they can be almost in line or more diagonal, distant from each other, or somewhat overlapping, but they are never opposite one another. Each testis is connected by the **vas efferens** to either the **vas deferens** or directly to the **seminal vesicle**, depending upon which group they belong to. A duct connects the seminal vesicle to the penis. The vasa efferentia may be expanded in some region to provide additional storage for sperm. Occasionally, males may only possess a single testis, a **monorchia**, although it is not common. Miller and Dunagan (1985a) provide a list of reports of monorchidism in various species.

The male members of many invertebrate phyla, and some females of those phyla, possess **cement glands**. Typically, the cement is used to bond an organism to a substrate, anchoring the organism so it is not dislodged. The cement from the glands of male acanthocephalans also is used for anchoring, but not to a substrate; instead, it is used to glue them temporarily to a female during copulation. The cement also serves to close the gonopore of females, although it is only temporary and it subsequently deteriorates, allowing females to mate again at a later time.

Several types of cement glands are known: A single syncytial gland, usually with 8 giant nuclei; a small number (usually 2–8) of glands, each with a single giant nucleus; or a small number of glands that have numerous fragments of nuclei in each (Van Cleave, 1949).

Contrasting views of the evolution of the cement glands have been suggested, but modern phylogenetic analyses indicate that separate glands with single nuclei are plesiomor-

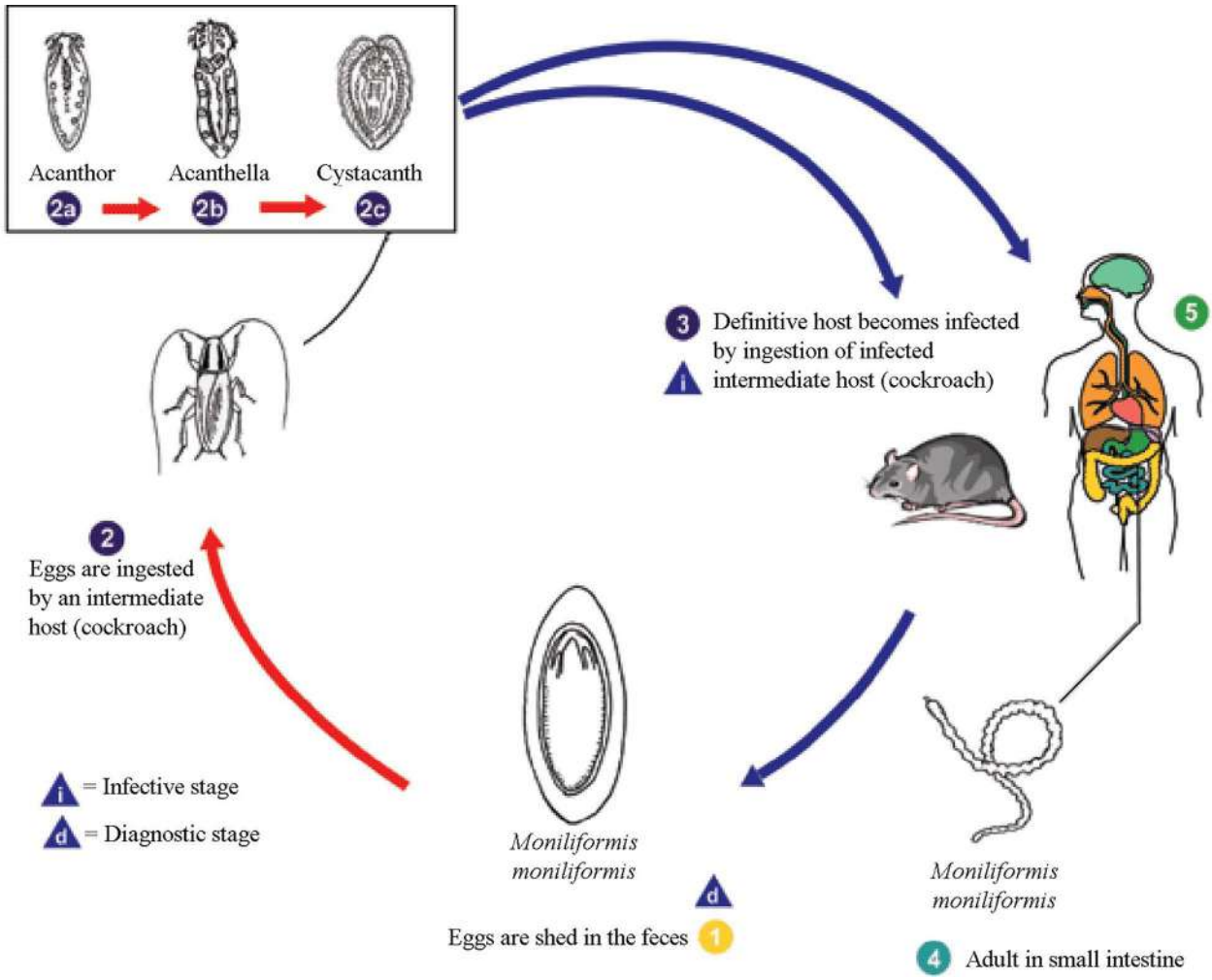

Figure 7. Life cycle of *Moniliformis moniliformis*. 1) Eggs are shed in the feces of the definitive hosts, which are usually rats for *M. moniliformis*, although carnivores and primates, including humans, may serve as accidental hosts. The eggs contain a fully-developed acanthor when shed in feces. 2) The eggs are ingested by an intermediate host, which is an insect (cockroaches, *Periplaneta americana,* for *M. moniliformis*). Within the haemocoel (persistent blastocoel) of the insect, the acanthor (2a) molts into a second larval stage, called an acanthella (2b). After 6–12 weeks, the worm reaches the infective stage, called a cystacanth. The definitive host becomes infected upon ingestion of intermediate hosts containing infective cystacanths (2c). Note that the proboscis is inverted. 3) The definitive host becomes infected by consuming an infected intermediate host. In the definitive host, larvae are liberated from their cysts and they attach to the wall of the small intestine. 4) Here they mature and mate in about 8–12 weeks. 5) In humans, the worms seldom mature, or, when they do mature, will rarely produce eggs. Source: Adapted from DPDx, United States Centers for Disease Control and Prevention, 2019. Public domain.

phic and a syncytial gland has been shown to be a synapomorphy for the species included in the Eoacanthocephala (see Monks, 2001).

Saeftigen's pouch is an expandable vesicle that is connected to the **bursa**. The bursa normally is inverted within the posterior body cavity of males. The muscular Saeftigen's pouch contains liquid that is pumped into the bursa, forcing the bursa out into the form of a cuplike structure that covers the posterior of females and aligns the penis with the vagina. Cement is then released into this area to seal the two individuals in copula.

Although the exact modes of neural communications are not known, the **genital ganglion** probably controls the performance and sequences of the various genital organs. However, it is interesting that a similar organ has not been reported in females.

Life Cycles

Compared to other groups of helminths (that is, Digenea, Cestoda, Nemata, and others) a typical life cycle of acanthocephalans is relatively simple to learn—the definitive host is always a vertebrate and the intermediate host is always an

arthropod. A typical life cycle, that of *Moniliformis moniliformis*, is shown in Figure 7. Acanthocephalan life cycles are linked to trophic relationships. This means that a definitive host becomes infected by ingesting its normal food that has a larval acanthocephalan that is infective to that particular species of definitive host.

For this reason, many species of Acanthocephala have very narrow host ranges. Using the previous example, a bird that feeds on insects that eats a cockroach infected with *Moniliformis moniliformis* will not become infected. Likewise, neither will a rodent feeding on pillbugs (*Armadillidium vulgare*) infected with cystacanths of *Plagiorhynchus cylindraceus*, which normally occurs in the robin (*Turdus migratorius*) (see Coady and Nickol, 2000 for a study of this type of interaction). However, this elucidates one curiosity of acanthocephalans. In this latter case, upon ingestion, the helminths would migrate out of the intestine into the body cavity of the rodent. While there, the cystacanth does not develop further; however, it may re-encyst in the rodent where it remains, in a kind of stasis, until a proper host comes along that it can infect, which in this case is probably never, unless of course the rodent dies and an isopod feeds on the dead rodent then becoming infected, ready to transfer the infection on to the avian final host.

As mentioned above, all parasite life cycles are trophically linked and in the cases discussed here, only arthropods can function as intermediate hosts. This would preclude any species that does not eat arthropods from being infected with acanthocephalans. However, there are cases in which the definitive host (such as a hawk or an owl) does not eat arthropods but those species can become infected naturally—here enters the **paratenic host.** The paratenic host is an ecological bridge between the arthropod intermediate host and the definitive host that does not eat arthropods. Usual paratenic hosts are small, insect-eating vertebrates, or in the case of fish, small fish that eat very small aquatic crustaceans; that is, frogs, toads, small lizards, snakes, rodents, and other small fish.

One would never think of a noble eagle or hawk eating insects, but they still can be infected with acanthocephalans. An example of a life cycle of species that involves paratenic hosts is that of the owl dwelling acanthocephalan called *Centrorhynchus* (of which there are several species). Insects become infected when they ingest eggs in the feces of the definitive host (owl or hawk). Snakes, frogs, and/or toads eat the insects, and a lot of them. The cystacanths in the infected insects excyst in the intestinal lumen and migrate to the body cavity where, as mentioned above, they re-encyst. They stay there, alive but in a type of hibernation, until a predatory bird captures the paratenic host, whereupon the cystacanths excyst again and develop within the bird. As an example of the complexity of the situation, Tavares dos Santos and Amato (2010) studied a life cycle in Brazil involving a species of *Centrorhynchus* and a toad, *Rhinella fernandezae*. The definitive host has not yet been identified, but several species of *Centrorhynchus* occur in Brazilian birds.

Finally, it is important to note again that there is no development of cystacanth larvae in paratenic hosts. If the definitive host, such as an eagle or the fish mentioned above, was fed an infected insect or crustacean, respectively, it would become infected with the acanthocephalan, just as it does when it eats the paratenic host.

Before leaving life cycles, one might wonder why there is relatively little precise information on more acanthocephalan life cycles. To give an example, when J. R. Crook was a graduate student, he captured specimens of *Peromyscus maniculatus* (commonly called a deer mouse) and found them to be infected with adult acanthocephalans, *Moniliformis clarki*. Imagine the difficulty in figuring out what arthropods the mouse might be eating, particularly because it is an omnivore. Eventually, Crook discovered that the mice were catching and eating crickets, *Ceuthophilus utahensis*, the Utah camel cricket, that lived in the underground tunnels that the mice made in which to live. The mice set aside a space in the tunnel where they defecated, and the crickets would go there and eat the feces, some of which carried eggs of *Moniliformis* that were passed in the feces of the mouse. The mice would then catch and eat infected crickets, completing the life cycle. This is not the most obvious place to look to find insects on which the mouse was feeding, unless the general life cycle of Acanthocephala was known and if the natural history of the mouse itself was known (Crook and Grundmann, 1964).

Searching out the participants in a life cycle is difficult and often might be the result of luck! This points out a second problem. The parasitologist, who might be a specialist in helminths, must also be a specialist in the vertebrate species that are definitive hosts for the helminths they study, and must know where they live and what they eat. In the case of acanthocephalans, the parasitologist must also know the arthropods, where to find them, and how to identify them. Much of this is not obvious when one reads the description of a life cycle. Today, molecular techniques often are used to match up the identity of cystacanths with adult worms, which often cannot be identified using only morphological details of the cystacanth larvae. Such a study was carried out by Lorenti and colleagues (2018).

Classification and Phylogenetic Relationships

The classification of the members of the phylum Acanthocephala has been relatively stable for some time, but understanding of the phylogenetic relationships of acanthocephalans and relationship to other invertebrate taxa has been in flux significantly. This is largely because the

classification of the phylum is still grounded upon classical inductive interpretation of how acanthocephalans should be grouped based on particular characteristics. A list of classical characteristics is presented in Table 1 (Bullock, 1969). These and, of course, other characters have been thought to be indicators of similar ancestry. Thus, species with these characters were placed in the same group (classes are indicated in the table).

A phylogenetic hypothesis, on the other hand, is the result of an analysis of data without the a priori decisions that classical reasoning might give (even though the two might be consistent). It is a provisional conjecture to guide further investigation, although it can be accepted as highly probable based on sound analyses, in view of established facts (data). Instead of using similarity, the hypothesis of relationships is based on characters that are homologous. However, the same classical data might be used in a phylogenetic analysis but the methodology is completely different (see Monks, 2001, for a partial list of the type of data that are useful for this type of analysis).

Because of the different methodology, classifications are rarely 100% consistent with phylogeny, although it would be advantageous if they were consistent. However, thanks to the intellectual acuity of the classical experts who studied acanthocephalans, the classification and recent phylogenetic hypotheses for the higher taxa are relatively similar.

Several works provide complete classifications of the Acanthocephala (Amin, 1985; 2013; Golvan, 1994). Each recognizes the 3 classical classes, **Archiacanthocephala**, **Eoacanthocephala**, and **Palaeacanthocephala**, and some add a fourth class, **Polyacanthocephala** (though others view it as a part of Palaeacanthocephala). Interestingly, the 3 names are tied to early views that one or the other was the most ancient taxon. For those interested in classical classification of the phylum it would be worthwhile to consult the works of Petrochenko (1956; 1958) and (Yamaguti, 1963). The most recent compendium discussing all aspects of acanthocephalan biology, including classification, is Crompton and Nickol (1985). For a list of higher taxa and the number of species known from each at the time of publication, see Monks and Richardson (2011).

Phylogenetic hypotheses of the Acanthocephala are largely consistent with the arrangement of higher taxa, with a continuing greater resolution of relationships and changes in placement as studies have advanced. The first phylogenetic hypothesis for a partial group of genera representing the 3 classes using molecular data were Near and colleagues (1998) and García-Varela and colleagues (2000). The first hypothesis based on molecular data was Monks (2001). Despite some differences in the inclusion of taxa and the methodology, the results of the 3 are similar. In each, Eoacanthocephala and Palaeacanthocephala are designated to be monophyletic sister taxa, meaning, 2 taxa that descended from the same most recent common ancestor. Archiacanthocephala is the most basal class in both cladograms, but in one (Figure 8A) it is a monophyletic clade and the members do not form a monophyletic

Table 1. Characterization of the 3 orders in the Acanthocephala. Adapted from Bullock, 1969.

Character	Archiacanthocephala	Eoacanthocephala	Palaeacanthocephala
Body size	Mostly large	Small	Small to large
Host habitat	Terrestrial	Aquatic	Mostly aquatic
Lacunar system, main longitudinal vessels	Dorsal and ventral or dorsal only	Dorsal and ventral, at least anteriorly	Generally lateral
Cement glands	Usually (always?) 8 uninucleate	Usually 1, syncytial, with giant nuclei; distinct cement reservoir	From 2 to 8, multinucleate
Trunk spines	Absent	Present or absent	Present or absent
Subcuticular nuclei	Few, elongate or branched, or with fragments remaining close together	Very few giant nuclei	Numerous amniotic fragments or few highly branched
Proboscis receptacle	Single muscle layer, often modified by ventral cleft or accessory muscles	Closed sac with single muscle layer	Closed sac with 2 muscle layers, except in Polyacanthorhynchinae
Ligament sac	Dorsal and ventral, persistent, with dorsal sac attached to uterine bell	Dorsal and ventral; disappear in adult; ventral sac attached to uterine bell	Single, ruptured in mature worms; posterior attachment inside uterine bell
Nephridia	Present or absent	Absent	Absent
Embryonic membrane	Usually thick	Thin	Usually thin
Intermediate host	Insects (and millipedes)	Crustacea	Crustacea

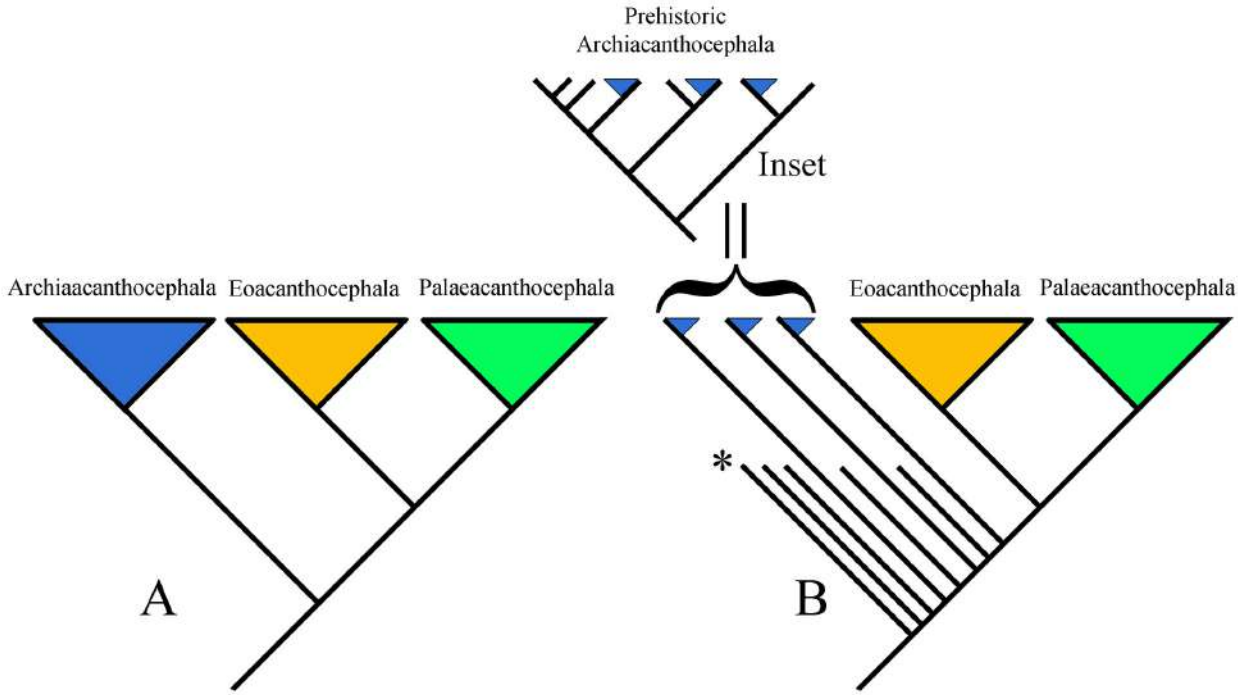

Figure 8. Hypotheses of the phylogenetic relationships of the Acanthocephala. A) Cladistic representation of the general results of 2 molecular analyses; B) Cladistic representation of the general results of another morphological analysis. Inset: Hypothetical clade of prehistoric archiacanthocephalans that in nature represented a monophyletic clade. The asterisk (*) indicates extinct taxa, represented by shorter lines. In the inset, the lines without triangles represent extinct taxa. The clade shown is a hypothetical monophyletic clade including extinct taxa. In this clade none of the extant taxa are closest relatives; the sister groups of each are extinct. Synapomorphies for this putative clade have not been identified; thus, the 3 branches with blue triangles (extant archiacanthocephalans) cannot be identified as a monophyletic clade. Sources: A) Adapted from García-Varela et al., 2000; Near et al., 1998; B) adapted from Monks, 2001.

group (Figure 8B) (see the cladograms in García-Varela et al. (2000), Near et al. (1998), and Monks (2001), respectively).

The failure of the methodology to recognize the class Archiacanthocephala as a monophyletic group was interpreted by Monks (2001) as an artifact caused by the very old origin of acanthocephalans. Monks suggested that present day taxa (Figure 8B) are only a relict of the original species in the group (Brooks and Bandoni, 1988); that is, many of the original species (and their hosts) are extinct (Figure 8B, inset) and their absence from the analysis hindered the ability of the methodology to identify synapomorphies for the clade.

Interestingly there are studies that have been interpreted that indicate that acanthocephalans are a part of the phylum Rotifera. One of the first was by Herlyn and colleagues (2003). To continue to explore this interpretation, refer to that study and the subsequent works, both pro and con, which cite this study. Earlier studies (Conway Morris and Crompton, 1982) postulated the phylum Priapulida as a sister group to Acanthocephala, but this idea mainly was based on similarity of fossil priapulids with present-day acanthocephalans.

This summary is far from providing a complete picture of this fascinating group. For more information there are numerous published papers available on the internet or in university libraries, only a very few of which were cited here. Many of these are descriptive taxonomic works, but there are also studies on physiology, behavior, ecology, and more on the subjects mentioned above.

The information and interpretations presented here are based on a phylogenetic perspective. For more information on phylogenetics, terminology, and methodology, a great source is The Compleat Cladist (Wiley et al., 1991; available as a free PDF download at https://kuscholarworks.ku.edu/handle/1808/24957). For further information on phylogenetic hypotheses of different groups of helminth parasites, see Brooks and McLennan (1993; 2002). For sources which bring ecology, behavior, biogeography, and other areas of biology together in a phylogenetic perspective, see Brooks and McLennan (1991). Searching in the Web of Science or Google Scholar for sources that cite these works will provide more recent sources of information.

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PENTASTOMIDS

59

Pentastomida

Pentastomida: Endoparasitic Arthropods

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Chapter 59

Pentastomida: Endoparasitic Arthropods

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Introduction

The name Pentastomida comes from the Greek: **pente** (five), and **stoma** (mouth), so chosen due to the 5 protuberances that are found on the anterior end of the body; however, only 1 of which is a mouth (also called the snout) (Bush et al., 2001). This cosmopolitan phylum encompasses a homogeneous and distinctive systematic assemblage of over 130 taxa of worm-like dioecious obligate endoparasites that, as adults, inhabit the respiratory tract (bronchi, lungs, and nasal passages) and coelomic cavity of various freshwater and terrestrial vertebrates, with the overwhelming majority (~ 90%) maturing in reptiles (Riley, 1986).

Although there appears to be some disagreement surrounding the classification of pentastomids (Abele et al., 1989), many researchers are content to designate Pentastomida as its own phylum, whereas others (Kelehear et al., 2014) consider it a class. For the purposes of this chapter, pentastomids (also known as tongue worms or linguatulids) will be considered to belong to the phylum Crustacea, subphylum Pentastomida Huxley, 1863, and class Eupentastomida Waloszek, Repetski, and Maas, 2006.

Fossil Record

Pentastomids are the oldest metazoan endoparasites known to science (Kelehear et al., 2014). Fossils occur in the Cambrian and Ordovician marine strata of Canada and Sweden. There are at least 8 Paleozoic fossil species, including species allocated to 4 genera as follows: *Aengapentastomum* Waloszek, Repetksi, and Maas 2006, *Boeckelericambria* Walossek and Müller 1994, *Haffnericambria* Walossek and Müller 1994, and *Heymonsicambria* Walossek and Müller 1994. A fifth genus, *Invavita* Siveter, Briggs, Siveter, and Sutton, 2015, is from Silurian-aged marine strata of England (Siveter et al., 2015) and fossil specimens of *Invavita* were found firmly attached to their ostracod (class Crustacea: order Ostracoda) hosts (*Nymphatelina gravida*). It is probable that these ancient pentastomids have been associated with their hosts since the Mesozoic Era. Prehistoric larvae closely resembling extant primary larvae appeared in the fossil record approximately 100 million years prior to the vertebrates they now parasitize (Riley, 1996), but the identity of the fossil pentastomids' hosts remains an enigma. Today, the higher vertebrates which are infected by tongue worms were basically not present in the early Ordovician period (500 Ma = million years ago) and while the limestone strata were formed in ancient seas in which the fossils were found, modern pentastomids occur only in freshwater or terrestrial vertebrates. However, one possible explanation is that ancient pentastomids attached themselves as ectoparasites to the gills of some of the large marine arthropods which were common in the Ordovician. These include the trilobites, and a lesser-known group called the anomalocarids, voracious predators which could grow up to 2 m-long. Riley and colleagues (1978) suggested that the pentastomids must have made the hurdle from marine invertebrates to freshwater and terrestrial vertebrates, and so were able to survive when their former hosts became extinct.

Extraordinarily well-preserved, 3-dimensional and phosphatized fossils from the Cambrian–Ordovician boundary of Canada and the Upper Cambrian Orsten fauna of Sweden, have been identified by Walosek and colleagues (2006) as pentastomids. These fossils suggest that pentastomids evolved very early and raise doubts about whether these organisms were actually true parasites at that time, and if so, on which hosts. A possible host in this venue is the Conodont (an extinct agnathan chordate).

Evolution

Evolutionarily speaking, knowledge of the relationships of pentastomids are in a state of flux as they share characters with the phylum Annelida, but most evidence suggests that they are more closely related to members of the phylum Arthropoda. As introduced above, some researchers have even proposed that the Pentastomida be regarded as an order of the crustacean class Brachyura, while others (Wingstrand, 1972; Riley et al., 1978; Abele et al., 1989) essentially agree that pentastomids be deemed a subclass of Crustacea (Pancrustacea), closely allied with the Brachyura. In addition, analyses of the mtDNA gene arrangements and sequences have indicated unambiguously that pentastomids are a group of modified crustaceans, probably related to brachyuran crustaceans (Lavrov et al., 2004). Using morphological characters as well as molecular techniques (such as 18S rRNA sequences) some advocates retain the Pentastomida as a separate phylum (Abele et al., 1989), although others recommend supporting their inclusion in the Crustacea. Therefore, pentastomids may

be most closely related to brachyuran lice, which are ectoparasitic on fish. For the purposes of this chapter, the designation of the species is retained in this group at the phylum level (the Pentastomida).

Geographic Range

Pentastomids are considered cosmopolitan in distribution, but as a rule, occur more commonly in hosts found in subtropical and tropical regions of the world. Some geographical hotspots for potential hosts include those from equatorial Africa, Australia, the Middle East, and Southeast Asia; they occur less often in vertebrates of the Americas and southeastern Europe. In addition, only 4 species have been reported from the Iberian Peninsula and Macaronesian Islands (Christoffersen and de Assis, 2015).

History of Pentastomid Research

Almeida and Christoffersen (1999) provided a summary of the history of pentastomid research and here additional information is added to their account. Evidently, the first to report a genuine pentastomid was the French veterinarian Philibert Chabert (1737–1814). In 1787, he discovered what he called a worm, which he mistook for the tapeworm *Ténia lancéolé* (now referred to as *Drepanidotaenia lanceolata* (Bloch, 1782) which is actually a member of the Hymelolepididae) in the nasal cavities of dogs and horses. Pioneer descriptions and further efforts to understand pentastomids were made during the next century by Josef Aloys von Frölich (1789), Alexander von Humboldt (1812), Pierre-Joseph van Beneden (1849), and Karl Moritz Diesing (1850), culminating with the account of the *Linguatula* (in 1860) by the German zoologist, C. G. F. Rudolph Leuckart (1822–1898). More recently, meaningful works were written by Richard Heymons (1867–1943) (see Figure 1) of the Berlin Museum, Konstantin von Haffner (1895–1985) of the University of Hamburg, and J. Teague Self (1906–1995) of the University of Oklahoma. One of the most prolific writers of all time on pentastomid biology was John Riley of the University of Dundee, Scotland, United Kingdom.

Chief Morphological Characters

The simple body design of pentastomids is surprisingly conservative. All possess an elongate and vermiform-cuticular (chitinous) and porous body (Figures 2A–E), often with a conspicuous abdomen showing distinct annulations (annuli = external segmentation). These are usually strongly united with a rounded cephalothorax possessing, on its ventral surface, a small sucking-type mouth region lacking jaws but bordered by 2 pairs of sclerotized hooks (Figures 2D–E), that can be retracted by specialized locomotor muscles into cuticular pockets (Paré, 2008). In some species, these hooks articulate against a basal fulcrum and are controlled by strong muscles used to tear and embed their mouth into host tissues. Males are generally smaller than females and possess copulatory spicules (Figures 2B–C). The conical-shaped pentastomid body, which can range from 2 to 130 mm (0.8 to 5.1 in) in total length, depending on the species, is divided into an anterior forebody and posterior hindbody, which, in some, is bifurcated at its tip. The cuticle of some species is covered with a dense network of chitinous spikes.

Figure 1. Historical specimen of a female *Armillifer armillatus* Wyman, 1848 (4 cm-long) collected from an African rock python *Python sebae* from an unknown site. Source: R. Heymons; specimen deposited in the Museum für Naturkunde, Berlin, Germany. Photographer: José Grau de Puerto Montt, 2008. License: CC BY-SA 3.0 Unported.

Integumentary System

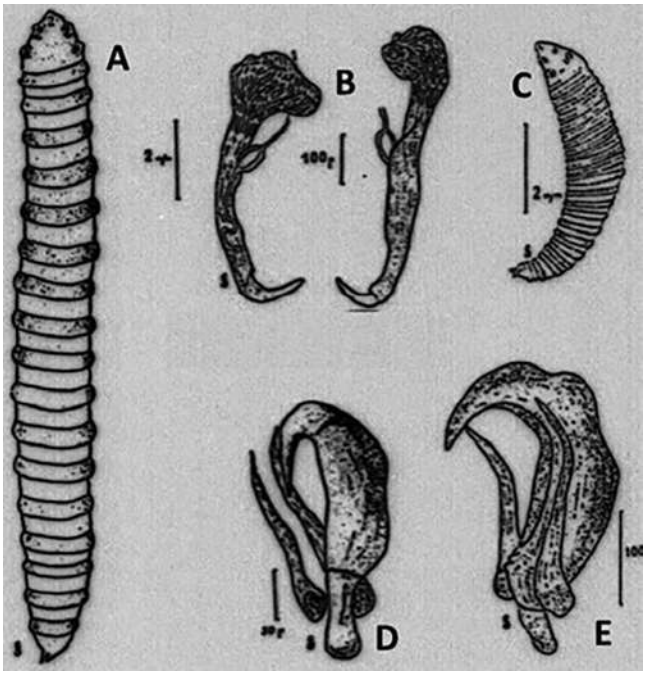
The cuticle of pentastomids is thin and similar to that of other arthropods. It consists largely of 3 layers (subcuticle, endocuticle, and epicuticle) (Riley and Banaja, 1975).

Muscular System

Longitudinal and circular muscles of pentastomids are arthropodan biologically and are cross striated and segmentally arranged.

Digestive System

The gut of pentastomids is a simple straight tube, with the anus opening at the posterior end of the abdomen. Their mouth is held open permanently by a sclerotized lining, the circular, ovoid, or U-shaped cadre; this structure is a significant taxonomic character. Adult pentastomids are hematophagous feeders, breaking lung capillaries and ingesting tissue fluids and blood cells of their hosts.



Figures 2A–E. *Raillietiella chamaelionis* Gretillat and Brygoo, 1959, from *Chamaeleo* sp. from Madagascar showing general characters of pentastomids. A) Female; B) male copulatory spicules; C) male; D) female anterior hooks; E) female posterior hooks. Source: Gretillat and Brygoo, 1959. License: CC BY.

Circulatory, Excretory, and Respiratory Systems

Although the body of pentastomids possess a hemocoel containing blood or hemolymph (Paré, 2008), there are no definitive circulatory, excretory, or respiratory organs.

Nervous System

The nervous system of Pentastomida is similar to that of other arthropods (Doucet, 1965). Their sensory organs are arranged in a definitive pattern and appear to be very simple structurally (Heymons, 1935), which may be due to their parasitic mode of life. The nervous system includes a ventral nerve cord with ganglia in each segment. Mechanosensitive sensilla are present throughout larval development on the anterior head region and positioned in characteristic patterns, increasing in number to the infective stage; the majority of anterior sensilla are located on sensory papillae (Storch and Böckeler, 1979; Winch and Riley, 1986). A subterminal or terminal anus might be flanked by a pair of terminal papillae (Haffner, 1977).

Reproductive System

Riley (1983) provided an excellent review of the reproductive biology of pentastomids. They are dioecious (having males and females) and exhibit distinct sexual dimorphism, with females usually being larger than males (Junker, 2002). Males have a single, tubular testis; however, there are 2 present in the genus *Linguatula*. The testis is continual with a seminal vesicle, which, in turn, connects to a pair of ejaculatory organs. The male genital pore is mid-ventral on the anterior abdominal segment, close to the mouth. Female pentastomids possess a single ovary that extends almost the entire length of the body cavity and may bifurcate at its distal end to become 2 oviducts that unite to form the uterus (Nørrevang, 1983). The uterus terminates as a short vagina that opens through the female gonopore. Fertilization is internal and females mate only once, while the males may be polygamous. Females are capable of producing several million fully embryonated eggs per day, which pass up from the lungs to the trachea of the host and are then either swallowed passed out with the feces or coughed up to the outside.

Pentastomid-Host Relationships

Insects as Hosts

Four species of pentastomids are known from intermediate host insects (3 coprophagus cockroaches and 1 coleopteran) (Lavoipierre and Lavoipierre, 1966). For example, cephalobaenid pentastomids *Raillietiella frenatus* and *R. gehyrae* employ geckos as definitive hosts and cockroaches as intermediate hosts (Ali and Riley, 1983). For some other raillietiellid definitive hosts that do not ingest insects, intermediate hosts may be amphibians, lizards, or snakes (Ali et al., 1982).

Fishes as Hosts

Despite the fact that pentastomids are potentially important endoparasites of subtropical and tropical fishes, comparatively little is known about the occurrence and distribution of pentastomid larvae in freshwater fish and information on this particular host-parasite relationship in the scientific literature is lacking (Giesen et al., 2013). Two families of pentastomids, Sebekidae and Subtriquetridae, use various freshwater fish species as intermediate hosts (Fain, 1961; Overstreet et al., 1985; Winch and Riley, 1986; Boyce et al., 1987; Junker et al., 1998). Most of these fishes (including cichlids and barbs) are common intermediate hosts for pentastomids occurring in crocodilians and piscivorous chelonians, and rarely for some species of snakes. Nymphs (Figure 3) develop in the viscera and muscle tissue of various fishes. Sebekiid and subtriquetrid pentastome larvae have been recovered from the body cavity or swim bladder of several fish species from various localities in South Africa (Luus-Powell et al., 2008). For example, 3 genera found in crocodilians as adults are found in

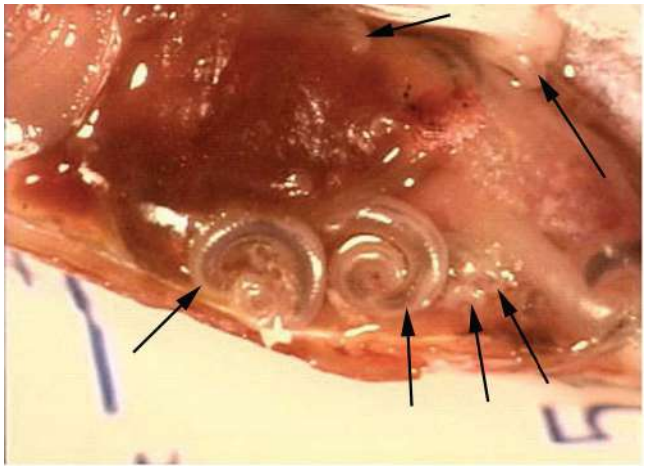


Figure 3. Pentastomid larvae (nymphs; arrows) in the body cavity of a swordtail *Xiphophorus helleri*. Note the varying sizes and locations of the pentastomids. Source: R. P. E. Yanong, 2019. Public domain.

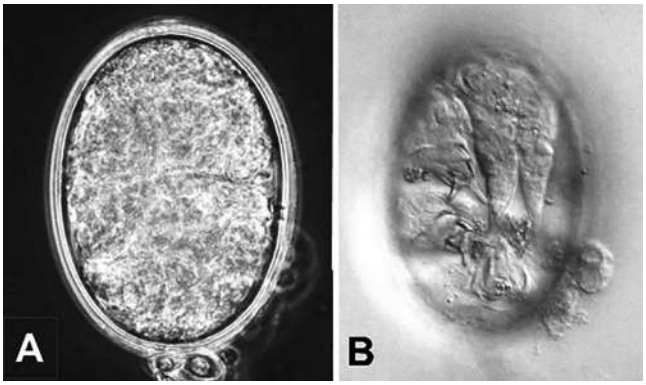


Figure 4. *Raillietiella teagueselfi* eggs from feces of Mediterranean geckos *Hemidactylus turcicus* from Texas, United States. A) Phase contrast microscopy of unembryonated egg; B) Nomarsky-interference contrast microscopy of fully embryonated egg. This pentastomid was described as a new species by Riley and colleagues, 1988. Source: S. J. Upton. License: CC BY-NC-SA 4.0.

intermediate host fish that they eat. To date, several fish species belonging to a number of families worldwide have been recorded as intermediate hosts of sebekiids, namely *Sebekia oxycephala* in cichlids and *Sebekia mississippiensis* in North American *Amia calva*, *Gambusia affinis*, *Fundulus grandis*, *Lepomis gibbous*, *L. macrochirus*, *L. megalotis*, *L. microlophis*, *Micropogonias undulatus*, *Micropterus salmoides*, *Pimephales promelas*, *Pomoxis nigromaculatus*, *Ameiurus natalis*, and *Xiphophorus helleri* (Hoffman, 1999; Luus-Powell et al., 2008). Fain (1961) and Reichenbach-Klinke and Landolt (1973) list *Alestes macrophthalmus*, *Bathybates ferox*, *Chrysichthys brachynema*, *C. mabusi*, *Lates microlepis*, *L. niloticus*, *Mastacembelus* sp., and *Oreochromis niloticus* as intermediate hosts of *Leiperia cincinnalis* in Central Africa.

Experimental transmissions conducted by Riley (1989) with *Subtriquetra* of small fishes (30 to 50 mm-long) caused deaths even before parasite larval development was completed (around 30 to 40 days after infection). However, larger fish (*Aequidens* sp., 70 mm-long) survived infections with 7 (2.5 mm-long larvae), which were already infective.

Amphibians as Hosts

Gedoelst (1921) was the first to report a cephalobaenid pentastomid, *Raillietiella indica*, in the lungs of an amphibian (Asian spined toad *Duttaphrynus melanostictus*; earlier referred to as *Bufo melanostictus*). A few years later, Larrousse (1925) reported a larval linguatulid from the Berber toad (*B. mauritanicus*). Since then, there have been other reports of amphibians as hosts including *Raillietiella bufonis* from Puerto Rican crested toad (*Peltophryne* [= *Bufo*] *lemur*) in Puerto Rico, United States (Ali et al., 1982), cane toad *Rhinella marina* from Hawaii, United States (Barton and Riley, 2004), and *Raillietiella rileyi* from *D. melanostictus* from Malaysia (Krishnasamy et al., 1995). Transmission of pentastomids to amphibian hosts has been reported by Nadakal and Nayar (1968) and Ramachandran (1977). To date, there are apparently no reports of pentastomids from salamanders (Caudata) or caecilians (Gymnophiona).

Reptiles as Hosts

About 90% of adult pentastomids are known from carnivorous reptiles, including lizards, turtles, snakes, and crocodilians (Kelehear et al., 2014). Reptiles become infected by ingesting an intermediate host containing nymphal stages and then pass the eggs (Figure 4) in feces to the environment or the infected reptiles are eaten by another host. The most common genera of reptilian pentastomids are *Armillifer* (Figure 1), *Kiricephalus* (Figure 5), *Porocephalus*, *Raillietiella* (Figure 4), *Sebekia*, and *Waddycephalus* with the majority found as adults in the buccal cavity, trachea, bronchi, and lungs of snakes, lizards, and crocodilians. They also can occur in the heart or the brain of these hosts. Of all reptiles, snakes appear to be the most common hosts, and as Kelehear and colleagues (2014) reported in a survey of tropical Australian snakes, 59% of the specimens they surveyed were infected with at least 1 species of pentastomid. Pentastomids of the genera *Raillietiella* and *Waddycephalus* infect a suite of host taxa, including 7 snake taxa from 3 snake families (Colubridae, Elapidae, and Pythonidae).

A study by Miller and colleagues (2017) revealed that invasive Burmese pythons (*Python vittatus*) in Florida, United States, host 2 species of pentastomids, *Raillietiella orientalis* and *Porocephalus crotali*. Both species also infect some

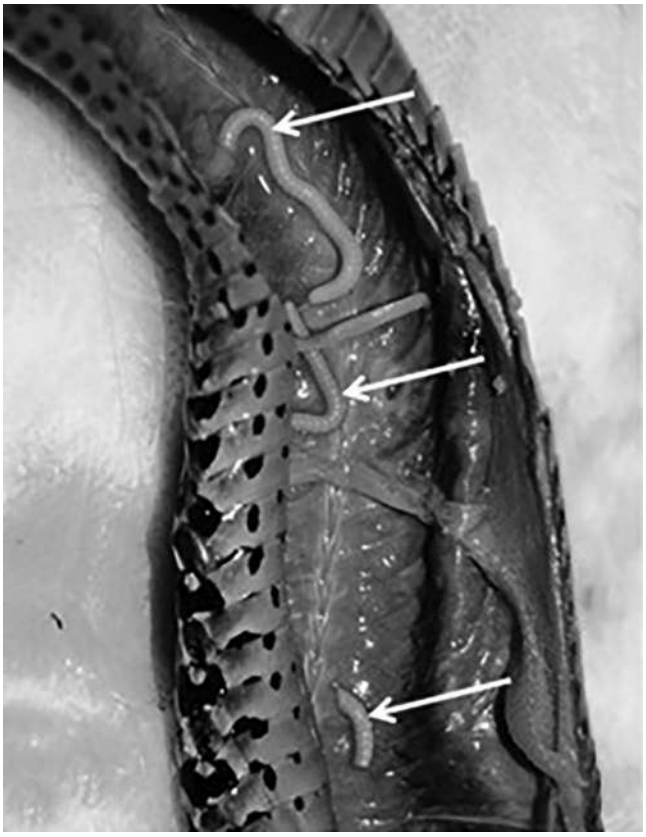


Figure 5. *Kiricephalus* spp. Macroscopic view of in situ *K. coarctatus* (arrows) from an eastern garter snake *Thamnophis sirtalis* from Arkansas, United States. This represents the first report of a reptilian pentastomid from Arkansas. Source: C. T. McAllister. License: CC BY-NC-SA 4.0.

native snakes in Florida, United States. These researchers determined that the former parasite (whose native range is Southeast Asia and Australia) did not originate in Florida but had arrived as a fugitive in the lungs of Burmese pythons. In addition, wherever *Python vittatus* occurs, native snakes in the surrounding areas of the state are also infected with *R. orientalis*.

The following examples of pentastomids in reptiles are provided by Reichenbach-Klinke and Elkan (1965): *Cephalobaena tetrapoda* in South American snakes of the genera *Bothrops*, *Lachesis*, and *Leptophis*; *Raillietiella* spp. in agamid, gekkonid, and varanid lizards, and colubrid and elapid snakes from both the Old World and New World; *Sebekia* spp. in African, South American, and North American crocodiles; *Diesingia megastomum* in Geoffroy's side-necked turtle *Phrynops geoffroanus* from South America; *Alofia platycephalum* from South American crocodiles; *A. indica* from crocodilians from India; *Leiperia* spp. from South American crocodiles and Nile crocodile *Crocodilus niloticus*; *Subtriquetra* spp. in South American and Indian crocodiles; *Elenia australis* in Australian varanids; *Waddycephalus* spp. in Asian, Australian, and Indonesian tree and ground-dwelling snakes and Asian house geckos (*Hemidactylus frenatus*) in Australia; *Porocephalus* spp. in North American and African boid and viperid snakes; *Kiricephalus* spp. in North American, Indian, Madagascan, and Australian snakes; *Armillifer* spp., in Asian and Australian boid, colubrid, and viperid snakes; *Cubirea annulata* in African snakes; and *Gigliolella brumpti* in Madagascan snakes. To date, there are no reports of pentastomids from amphisbaenians (suborder Amphisbaenia).

Birds as Hosts

There are 2 species of pentastomids found in the air sacs (Figures 6A–B) of sea birds (guillemots, gulls, puffins, skuas, and terns) and another in the trachea of white-backed vultures. The majority are found in hosts from the subpolar and polar latitudes in the Holarctic (Nicoli and Nicoli, 1966). For example, the larid pentastomid *Reighardia sternae* occurs in the body cavity and air sacs of about 13 species of gulls and terns and is the only pentastomid species known to use these avian waterfowl as hosts (Riley, 1973). The life cycle of *R. sternae* is unique among pentastomids as it includes an obligate (monoxenous) life cycle with 1-host parasite and an intermediate host phase in the egg (Thomas et al., 1999). The hatching stage directly infects the respiratory system of the avian definitive host.

Non-Human Mammals as Hosts

Adult and/or nymphal pentastomids have been reported to infect captive and natural populations of marsupials, canines, felines, rabbits and hares, antelopes, reindeer calves, camels, cattle, sheep, goats, monkeys, rodents, and many others (Spratt, 2003; Paré, 2008). Those species found in felids and canids typically occur in the nasopharynx. Most of these reports concern *Armillifer armillatus* or *Linguatula serrata*. Dechkajorn and colleagues (2016) reported a case of visceral pentastomiasis in a captive striped hyena (*Hyaena hyaena*) in Thailand.

Humans as Hosts

Humans are rarely infected by adult pentastomids and represent dead-end hosts, meaning that they play no role in the natural cycle of this parasite. However, visceral pentastomiasis caused by nymphal specimens is an emerging zoonotic infection (meaning that they can be passed between humans and animals) and is sometimes observed in individuals in rural western and central Africa and some parts of Asia. African pythons (Pythonidae) and large vipers (*Bitis* spp.) act as definitive hosts for *Armillifer armillatus*

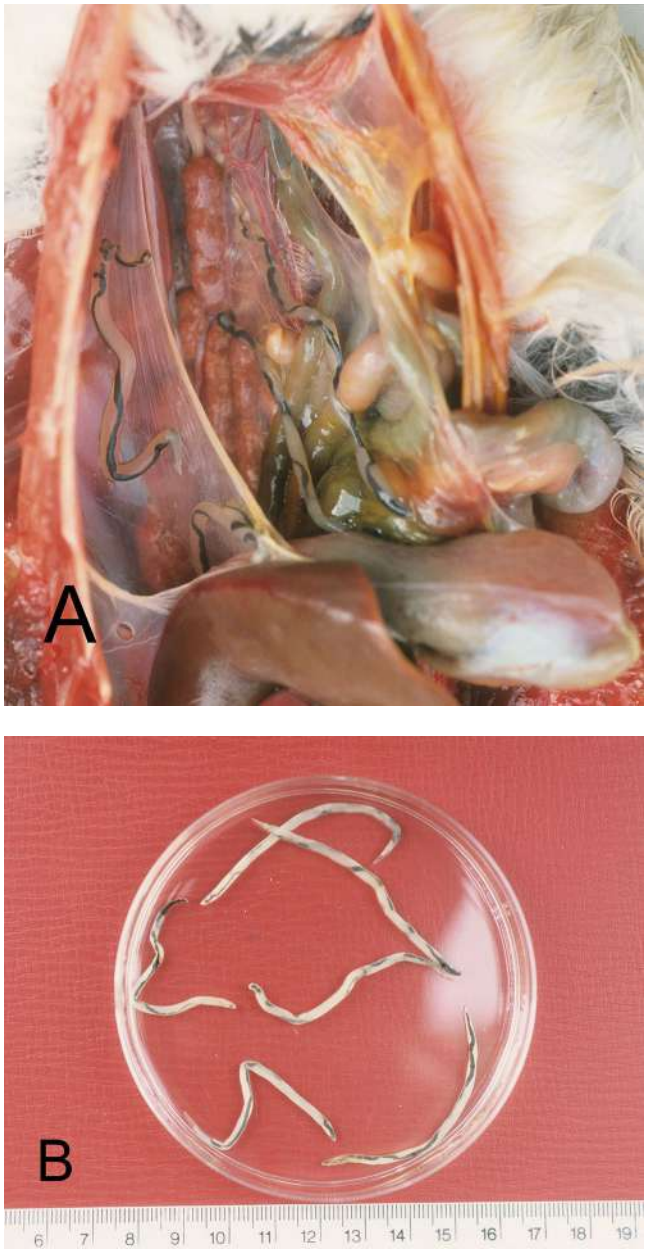


Figure 6A–B. Pentastomids (*Reighardia* sp.) from airsacs of an unknown species of guillemot. A) Worms (arrows) in situ; B) worms removed from airsacs. Source: T. Pennycott, 2016. License: CC BY.

and *A. grandis* in the Congo Basin. Snakes in the bushmeat market have gradually increased over the years and human pentastomiasis has become an important emerging zoonotic disease (Hardi et al., 2017). The mucus from the lungs of infected reptiles (especially snakes) and carnivorous mammals can also cause infection in humans. Humans can also be infected via food or water contaminated with host feces containing pentastomid eggs, by consumption of undercooked snake flesh (including the prized gallbladder), or indirectly through contaminated hands, kitchen tools, or washing water (Fain, 1975; Yapo Ette et al., 2003; Lai et al., 2010; Ibinaiye et al., 2011; Hardi et al., 2013; 2017). Several human infections have been reported from Cameroon, the Democratic Republic of the Congo, and Nigeria (Vanhecke et al., 2016). The majority (99%) of human infections are caused by 2 species, *Linguatula serrata* or *Armillifer armillatus* (Paré, 2008). The adult worm is found in the nasal passages of dogs and sheep, and goats are infected by ova from infected dogs. A syndrome known as Halzoun (also known as nasopharyngeal linguatulosis, present in the eastern Mediterranean) is the common name for the infection of *L. serrata* of the buccopharyngeal mucosa and nasopharyngeal tract of humans (Cannon, 1942; Dabick, 1987; Yagi et al., 1996). This hypersensitivity disease is also known as **Marrara syndrome** in Sudan, named after a dish of raw stomach, lung, trachea, rumen, and liver of sheep, goats, or camels, infected with larvae of *L. serrata*, which is often responsible for the transmission of the parasite to humans. Interestingly, a nymph of *Leiperia cincinnalis* (which usually is found as adults in the lungs of African crocodiles) was found in the feces of a woman in Zaire (Fain, 1960; 1961). This patient was likely infected by larvae from eating some type of fish (Fain, 1975). The possibility of a carcinogenic action of pentastomids has also been suggested (Fain, 1975); however, the arguments for this association are unsubstantiated.

Although pentastomiasis is mostly asymptomatic in humans and usually not a primary health threat, the clinical presentation is quite varied and depends on infected tissues. Nymphs are often located in the abdominal cavity, including the liver and thoracic cavity (including the lungs and pleura) and abdominal emergencies from severe systemic symptoms have been reported; infections of the eyes (ocular pentastomiasis) are rare (Sulyok et al., 2014).

Diagnostic delays are inevitable, and diagnosis focuses on the patient's lifestyle and living environment. It is mainly based on the morphological description of the parasite's calcified cuticle, the site of the lesion, and the parasite's region of origin. Those patients who present symptoms have fever, abdominal pain, diarrhea, and weight loss. When blood samples are obtained, eosinophilia, anemia, and an elevated serum immunoglobulin (IgE) level is sometimes present. Ultrasound, conventional X-ray, computerized tomography (CT) and magnetic resonance imaging (MRI) scans, and a laparoscopic approach might also be helpful for the diagnosis of pentastomiasis. Deworming treatments using praziquantel (Biltricide) and mebendazole (Emverm) are often prescribed for patients infected with certain types of worms causing pentastomiasis. However, most patients do not require any major or invasive treatment.

Human infections have been confirmed for the following pentastomids, including *Linguatula serrata*, *Armillifer agkistrodontis*, *A. armillatus*, *A. grandis*, *A. moniliformis*, *Leiperia cincinnalis*, *Porocephalus crotali* (syn. *A. moniliformis*), and *P. taiwana* (Fain, 1975; Tappe et al., 2009; 2016; Sulyok et al., 2014; Mehlhorn, 2015). In addition, adults of *A. grandis* have frequently been observed in the lungs of rhinoceros vipers (*Bitis nasicornis*) and nymphal infections in humans have been reported in Africa from this species. The nymphs encyst in the omentum, the mesenteries, and even the eyelid of humans (Fain and Salvo, 1966).

Effect on the Host

In the most common hosts (reptiles), pentastomes are hematophagous (meaning that they feed on blood), but even in heavily infected lizards or snakes, anemia has not yet been documented. Host death is often associated with larval and nymphal migration and molting, and by pathological damage caused to the pulmonary lining by the hooks and mouths of feeding adults, which often leads to secondary bacterial infection or fungal pneumonia. Mortality associated with progressive pneumonia from infection with adult *Raillietiella* has been reported in wild geckos from Nigeria, suggesting that pentastomes may act as regulators of wild reptile populations. Imported wild-caught reptiles in private collections and zoos may also develop overt disease from pentastome infection.

Larval Development

Larval and nymphal pentastomid development ranges from indirect, with up to 10 stages, to direct. Development of the embryo ceases with a pre-hatching within the eggshell, which is now termed the primary larva or nymph. Larval migration occurs through the body cavity and after several molts, the larvae become infective in the respiratory tract of the definitive host (Riley, 1986; Buckle et al., 1997).

Life Cycles

A typical pentastomid indirect life cycle (Figure 7) begins when eggs are ingested by a suitable intermediate host and develop into minute larvae or nymphs that penetrate the definitive host's intestinal tract and migrate casually to multiple tissues (usually those of the respiratory tract). These suitable intermediate hosts bridge diverse taxa, including mammals, reptiles, amphibians, fish, and insects. However, for most species the intermediate host has yet to be discovered (Riley, 1986; Paré, 2008). Interestingly, the life cycle of *Raillietiella sternae* involves an obligate 1-host parasite that has shifted its intermediate host phase into the egg. Therefore, the hatching stage directly infects the respiratory system of the definitive host, which is unique among pentastomids.

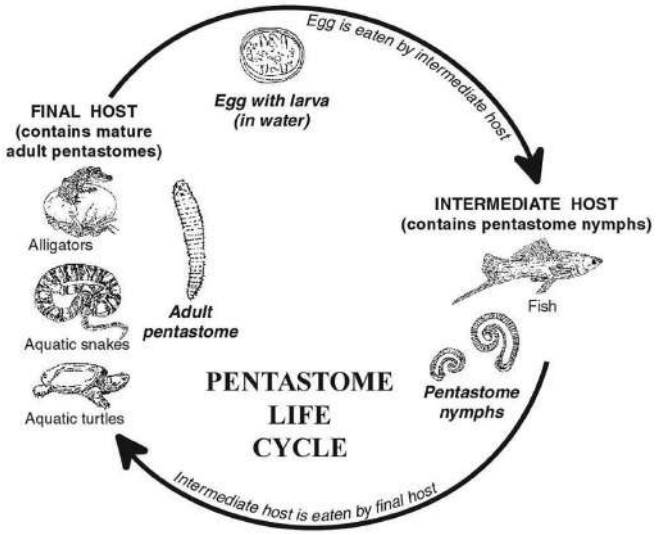


Figure 7. Generalized life cycle of pentastomids. Source: R. P. E. Yanong, 2019. Public domain.

Pentastomid larvae infect the definitive host when the host ingests a suitable intermediate host. These larvae burrow out of the host's digestive system and through to the lungs, where they can cause lesions and scars along their migration path (Jacobson, 2007). In intermediate or accidental hosts, these larvae can establish widespread visceral infections (Brookins et al., 2009; Haddadzadeh et al., 2010; Mätz-Rensing et al., 2012; Yakhchali and Tehrani, 2012). The adult pentastomids feed primarily on blood from host capillary beds in the lungs and are capable of causing severe pathologies resulting in death (Paré, 2008). Large adult pentastomids (up to 15 cm-long) can physically occlude the respiratory passages and induce suffocation. The 2 pairs of hooks they use for attaching to lung tissue can cause perforations and hemorrhaging and disintegrating molted cuticles shed into the lumen of lungs by maturing pentastomids can induce putrescent pneumonia (Jacobson, 2007).

When the life cycle involves a reptilian definitive host, ingestion of an intermediate host containing nymphs are then infective to the definitive host by penetrating the wall of the stomach or intestinal tract using its hooks. The nymphs migrate into the lungs and air passages, where they mature into tongue-shaped adults that can be up to 160 mm-long. In addition, pentastomid adults containing mature eggs may be expelled from the trachea and eliminated from the definitive host through oral expulsion. These adults may also be swallowed, resulting in eggs appearing in the feces. Autoinfection can also occur in some species.

Ecology

Adult pentastomids are mostly restricted to the respiratory tracts of tetrapods, primarily reptiles; larval stages may occur in fish. Several species cause visceral and respiratory infections termed **pentastomiasis** in vertebrates.

As is common with many other parasites, pentastomids may serve an ecological role as regulators of community size and may be considered regulators of host populations. They may even be found in threatened and endangered species and could represent a problem in conservation efforts of those hosts. Since they are often recovered from captive animals in zoos during necropsies, they may also be considered a health problem. In some cases, the death of any given host may be directly or indirectly attributable to an infection with pentastomids. For humans, pentastomids typically infect those people living in impoverished parts of developing countries, such as some in the arid Middle East, Southeast Asia, and Latin America (Riley, 1986). Here, people rely on native reptiles as a food source which can enable transmission of infective nymphs (Almeida and Christoffersen, 2002). However, despite an occasionally high number of nymphal individuals that cause visceral pentastomiasis in humans, most infections are asymptomatic and are often only diagnosed incidentally during surgery or postmortem.

Taxonomic Study

The pentastomids are sometimes classified in their own phylum and class, referred to as phylum Pentastomida Huxley, 1869, class Eupentastomida Walosek, Repetski, and Maas, 2006. In this chapter, pentastomids are considered to belong to the phylum Crustacea, subphylum Pentastomida Huxley, 1863, and class Eupentastomida Waloszek, Repetski, and Maas, 2006.

Descriptions of new species of pentastomids traditionally have been based on various morphological features of the adult worm, with emphasis placed on body size, number of body annuli, and morphology and measurements of the 2 pairs of retractile hooks, the buccal cadre, and the male copulatory spicules, the latter particularly helpful in determination of raillietiellids (Riley, 1986). However, due to 5 main factors and a high level of intraspecific variation, there is potential for misidentifications, including: 1) The small numbers of specimens generally recovered, 2) method of fixation used on those specimens, 3) state of the preserved type or voucher specimens, 4) whether there are both males and females present, and 5) intraspecific variation in the previously mentioned morphological traits. Some species erroneously described as new were eventually categorized as species inquirenda, as in the case of *Raillietiella frenatus* (= *R. frenata*) from alien Mediterranean geckos (*Hemidactylus turcicus*) from Texas, United States (Pence and Selcer, 1988). However, based on the morphological study of Sakla and colleagues (2019), *R. frenata* was discovered actually to be *R. indica* and the host range included a native species, the green anole (*Anolis carolinensis*). Interestingly, morphological features used in pentastomid taxonomy vary as the parasite goes through different developmental stages in the definitive host, especially the morphology of the hooks, which can transition strikingly and progressively. Indeed, data on hooks can be meaningful only when compared between fully mature specimens and much of the morphological variation in hook measurements, the primary diagnostic traits of raillietiellid pentastomids, is due to development or instar stage (Kelehear et al., 2011; Sakla et al., 2019). In addition, type specimens in museum collections are usually fixed as permanent mounts in 10% formalin and/or without using DNA-grade (methanol-free) ethanol as a preservative. In this respect, specimens cannot be described using molecular techniques, a major concern in resolving the taxonomic status of already-described pentastomid species. In the future, it is suggested that taxonomic work should involve a combination of morphological techniques, integrating a consideration of body size, and a quantitative measurement of hook bluntness with complementary molecular techniques (Mätz-Rensing et al., 2012) to assist in the authentication of descriptions of new pentastomid taxa. In general, one needs to account for developmental- and host-induced morphological variation to accurately identify pentastomid species (Sakla et al., 2019).

The classification of the Pentastomida given here follows the works of Almeida and Christoffersen (1999), Junker (2002), Poore (2012), and Christoffersen and de Assis (2013) as well as the more recent classification of Walldorf (2015). There are 7 families within 4 orders, **Cephalobaenida** (1 family), **Raillietiellida** (1 family), **Reighardiida** (1 family), and **Porocephalida** (4 families).

Order Cephalobaenida Heymans, 1935

Cephalobaenid pentastomids possess an anterior mouth with hooks that lack a fulcrum and females possess a vulva at the anterior end of their abdomen. Hosts in this order include amphibians and reptiles (lizards and snakes). There is a single species of Cephalobaenida Heymons, 1922 within the family, Cephalobaenidae Heymons, 1922.

Order Raillietiellida Almeida and Christoffersen, 1999

This order includes 44 species and subspecies of parasites of amphibians, lizards, and snakes, including 1 family (Raillietiellidae Sambon, 1922) and 2 genera, *Raillietiella* Sambon, in Vaney and Sambon, 1910 with 43 species, and *Yelirella* Spratt, 2010 (monotypic). Poore (2012) provides an excellent discussion on the taxonomy of the genus *Raillietiella*.

Order Reighardiida Almeida and Christoffersen, 1999

Species of this order lack abdominal annuli and the poorly developed hook-bearing podia. They are parasites of marine birds and include a single family, Reighardiidae Heymons and Vitzhum, 1936, including the genera *Reighardia* Ward, 1899 (with 2 valid species) and *Hispania* J. Martínez et al., 2004 (monotypic).

Order Porocephalida Heymans, 1935

The Porocephalida is the largest order with 4 families, 11 genera, and 84 species. They have a mouth between or below the level of the anterior hooks with fulcrum (hooks bifurcate in the larvae), a single lamina in the adults, a spirally coiled abdomen in the females, with comparatively large radial coils, and a vulva near the posterior end of the body (Rego, 1984; Riley and Huchzermeyer, 1996; Junker et al., 2000). The number of annuli is usually a consistent and reliable diagnostic criterion in differentiating porocephalid genera (Riley and Self, 1979; 1980; 1981).

The families included in the order Porocephalida Heymans, 1935 include the following: **Linguatulidae** Leuckart, 1860 (with 6 species) are parasites of mammals, with 2 genera, **Linguatula** Frölich, 1789 (with 5 species), and **Neolinguatula** Haffner (in Haffner, Rack and Sachs, 1969) (monotypic). **Subtriquetridae** Fain, 1961 (with 4 species) are parasites of crocodilians with a single genus, *Subtriquetra* Sambon, 1922 (with 4 species). **Sebekidae** Sambon, 1922 (with 34 species) are parasites of chelonians and crocodilians and include 8 genera as follows: *Agema* Riley, Hill and Huchzermeyer, 1997 (monotypic); *Alofia* (Giglioli in Sambon), 1922 (with 7 species); *Diesingia* Heymons, 1935 (2 species); *Leiperia* Sambon, 1922 (with 3 species); *Pelonia* Junker and Boomker, 2002 (monotypic); *Sambonia* Noc and Giglioli, 1922 (with 4 species); *Sebekia* Sambon, 1922 (with 12 species); and *Selfia* Riley, 1994 (monotypic). **Porocephalidae** Sambon, 1922 (with 41 species) are parasites of snakes, with 8 genera as follows: *Armillifer* Sambon, 1922 (with 11 species); *Cubirea* Kishida, 1928 (with 2 species); *Elenia* Heymons, 1932 (monotypic); *Gigliolella* Chabaud and Choquet, 1954 (monotypic); *Kiricephalus* Sambon, 1922 (with 5 species); *Parasambonia* Stunkard and Gandal, 1968 (with 2 species); *Porocephalus* Humboldt, 1812 (with 9 species); and *Waddycephalus* Sambon, 1922 (with 10 species).

Pentastomid Clades (Apomorphies)

It is usually not too challenging, with some practice, to place a given unknown pentastomid specimen within a certain genus; however, identification to the specific level can be rather problematic as well as frustrating. Because many pentastomid taxa generally do not have very good diagnostic morphological benchmarks, it renders them frustratingly difficult to identify. There have only been 2 phylogenetic analyses of the group (Almeida and Christoffersen, 1999; Junker, 2002) as well as some other traditional diagnoses.

Figure 8A–B. Nymphal and adult pentastomids. A) Excised nodule on the pleural surface of the lung showing nymphal *Linguatula serrata*; B) coiled nymphs of *Armillifer* sp. in simian omentum; C) adult female *L. serrata*; D) adult male (small) and female (large) of *Armillifer* sp.; E) adult *Porocephalus crotali* in snake lung; F) anterior part of *Armillifer* with central mouth and 4 oral hooks. Source: D. Tappe and D. W. Büttner, 2009. License: CC BY.

Some Interesting Pentastomids

Porocephalus crotali (Humboldt, 1811) was originally described from a Venezuelan rattlesnake, *Crotalus durissus terrificus*, by Von Humboldt (1808). Since then, adults have been reported from the lungs (Figure 8E), trachea, and nasal passages of various North American rattlesnakes (*Crotalus* spp.), cottonmouths (*Agkistrodon piscivorus*), and Burmese pythons (*Python bivittatus*), with nymphs in the viscera of rodents that act as intermediate hosts (Penn, 1942; Riley

and Self, 1979; Paré, 2008; Yabsley et al., 2015; Miller et al., 2017). It has also been reported from the Indian rat snake (*Ptyas mucosus*) in India (Bino Sundar et al., 2015). Adults have a cylindrical, segmented body with hooks arranged in the form of an arc or a trapeze. The internal organs occupy the entire abdomen. The nymphs are about 8–14 mm in length with a cylindrical and smooth annulated body. There are 2 unequal pairs of hooks that are located at the anterior ventral end around the mouth (Soulsby, 1982).

The life cycle of *Porocephalus crotali* was demonstrated experimentally by Esslinger (1962) and further studied by Riley (1981). When passed by the female parasite in the lung of a snake, eggs are fully developed and infective to the intermediate host. They are subsequently carried to the pharynx, swallowed, and passed out in the feces. Infection is typically without pathology in lung tissues or other tissues generally, either in the snake or the intermediate host, but very heavy infections may lead to death of the definitive host.

The adult *Linguatula serrata* (Fröhlich, 1789) (Figure 8B) is an unusual type of cosmopolitan pentastomid, as it is restricted to mammals and lives in the nasal passages, frontal sinuses, and tympanic cavity of meat-eating definitive hosts, such as domestic and wild canids and felids (such as, dogs, cats, foxes, and wolves) and other carnivores. Most herbivores, including domestic ruminants, serve as intermediate hosts. It has become cosmopolitan and has been recorded from humans in Africa, Europe, the Middle East, North America, South America, and some Caribbean islands. Rarely, severe nasopharyngeal linguatulosis appears in the Middle East when people ingest the nymphs of *L. serrata* in undercooked liver or lymph nodes from goats or sheep. The parasite then attempts to attach in the person's throat or nasopharynx resulting in halzoun or Marrara syndrome (Schacher et al., 1969). Some minor complications from this infection include frontal headaches, pain in the ears, nasal discharges, sneezing, and coughing; major physical difficulties include auditory canal abscesses, breathing difficulty, hemorrhages, facial paralysis and swelling, and occasionally asphyxiation and even death (Roberts and Janovy, 2012). In North America, nymphs of *L. serrata* have also been recovered from human mesenteric lymph nodes, the brain, lungs, and in the anterior chamber of the eye (Hunter and Higgins, 1960; Rendtorff et al., 1962).

Female *Linguatula serrata* can potentially grow up to 13 cm, while males only reach 2 cm (Figure 8C). They attach to the wall of the respiratory system by means of their mouth hooks. Females excrete thousands of eggs per day, up to 5 million (Hobmeier and Hobmeier, 1940). Certain stages are infectious for humans, where the larvae migrate into different organs away from the intestine. If the larvae are eaten by the final host, the larvae invade the nasal system and reach maturity within 6 to 7 months and live for about 15 months (patency period).

In the Old World, eating undercooked goat or sheep liver and mesenteric lymph nodes or the visceral organs of sheep, goats, cattle, and camels is the usual causation of the infection. Many of the snakes sold for human consumption at the rural bush meat markets in the Democratic Republic of the Congo are hosts of *A. armillatus* (Hardi et al., 2017).

Visceral pentastomiasis results when eggs are eaten and nymphs develop in visceral organs, causing pathology such as hepatic granuloma (Gardiner et al., 1984; Baird et al., 1988). Other complications include abscesses in the auditory canals, facial swelling, paralysis, and even asphyxiation and death.

Another unusual infection in humans of tropical Africa occurs when ***Armillifer armillatis*** Wyman, 1848 (Figure 1) infects visceral organs. Its typical definitive hosts are reptiles, mostly pythons, such as the reticulated python *Python reticulatus* and African rock python *P. sebae* while rodents are presumed to act as intermediate hosts (Christoffersen and de Assis, 2013; Murvanidze et al., 2015). Humans may become accidentally infected by the eggs, particularly if consuming (or otherwise contacting) infected snakes. Ingested eggs develop into nymphs that invade different visceral organs (especially the liver) causing a disease called porocephalosis. Most human infections are asymptomatic, whereas some can be debilitating, causing mechanical damage or hemorrhage (Boyce and Kazacos, 1991) or (though rarely) can even be lethal.

Main Sources of Information

A great deal of information on the Pentastomida can be found in the works of Heymons (1935), Hill (1948), Self and Kuntz (1966), Self (1969), Riley (1983; 1986), Almeida and Christoffersen (1999), Junker (2002), Kelehear and colleagues (2011; 2014), Poore (2012), and Christoffersen and de Assis (2013; 2015). These papers formed the basis of this chapter.

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Two very important people, the late J. Teague Self and John Riley were instrumental in mentoring the author and helping him understand the wonders of the Pentastomida. All of Self's specimens were sent to the American Museum of Natural History (New York, New York, United States). The literature in Self's library was sent to the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, United States.

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Part V

ECTOPARASITES

60

Platyhelminthes

Monogenea (Class)

Griselda Pulido-Flores

Phylum Platyhelminthes

Class Monogenea

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Chapter 60

Monogenea (Class)

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Introduction

The phylum Platyhelminthes, known as flatworms, includes the class Monogenea, mainly ectoparasites of the skin, fins, gills, and urinary bladder of fishes, amphibians, and some reptiles (Kearn, 2014). However, there is one species that is a parasite of mammals, *Oculotrema hippopotami* Stunkard, 1924, from the eye of the African hippopotamus *Hippotamus amphibius* (see Stunkard, 1924; Yamaguti, 1963). There also are a few species of monogeneans that infect cephalopods (Rohde, 2011). *Isancistrum loliginis* has been reported from squids, (*Loligo* spp.) and *Polystoma loliginum* has been reported and collected from other cephalopods (Overstreet and Hoschberg, 1975). Sometimes, instead of living as ectoparasites as is usual, a few monogeneans may be found living within the stomodeum, proctodeum, bladder, or diverticula of a host (Roberts and Janovy, 2008).

Classification: Historical Review

Entobdella hippoglossi was the first species of Monogenea described. Müller described it as *Epibdella hippoglossi*, a parasite from the skin of the Atlantic halibut *Hippoglossus hippoglossus*. In the original descriptions of this monogenean, it was mistaken for a leech and the author named it *Hirudo hippoglossi* (see Kearn, 2014).

There is controversy about whether the name that refers to this group of Platyhelminthes should be "Monogenea" or "Monogenoidea." The Latin term Monogenea derives from van Beneden's (1858) use of the French term "monogénèses" in French (cited in Carus, 1863) and is now the generally-used term for this group (Carus, 1863; Wheeler and Chisholm, 1995). Monogenoidea sensu Bychowsky (1937) is not the correct name because its use predates use of the term Monogenea. In addition, the ending of -oidea in animal taxonomy always refers to superfamily designations. Some have argued for the use of Monogenoidea as the valid name of the class; however, this is based on erroneous assumptions of authorship, priority, and rank as defined in the International Code of Zoological Nomenclature (ICZN, 2012). The resolutions adopted at the Fourth International Congress of Parasitology (ICOPA IV) in Warsaw, Poland in 1978 during the Round Table "Monogenea: Problems of Systematics, Biology, and Ecology" resulted in an agreement supported by all participants to adopt Monogenea as the name of the class rather than Monogenoidea. For more information on this process, see Wheeler and Chisholm (1995).

The Monogenea have been divided into 2 major subgroups: **Polyopisthocotylea** (which means, in adults, possession of a more complex opisthaptor) and **Monopisthocotylea** (which means possession of a single opisthaptor). The morphology of the adult's attachment organs is what distinguishes these subgroups. The morphology of the attachment organ in the larval forms is what distinguishes the **Oligonchoinea** (**oligo** = few; Greek) and **Polyonchoinea** (**poly** = many; Greek) (Justine, 1998). The groups do not overlap because of the position of the polystomatids and sphyranurids.

A phylogenetic analysis using morphological data, the ultrastructure of spermiogenesis, and spermatozoa of the taxon Rhabdocoela (Platyhelminthes) produces a hypothesis that Monogenea is a monophyletic group that is more closely related to tapeworms than other platyhelminths (Justine, 1991; Zamparo et al., 2001); however, analyses of molecular data (18S or 28S rDNA sequences) do not support the monophyly of the Monogenea (Mollaret et al., 1997). The analyses conducted by Mollaret and colleagues (1997) suggest that Monogenea is a paraphyletic group, although the monophyly of Monopisthocotylea and Polyopisthocotylea were suggested (Mollaret et al., 2000). The molecular data agree with studies of the ultrastructure of spermiogenesis of Polyopisthocotylea, all of which share the synapomorphy of having lateral microtubules present in the principal region of the spermatozoon. In the monopisthocotyleans, dorsal and ventral microtubules are absent from the principal region of the spermatozoon (Justine, 1991). However, an analysis with both the morphological and molecular data of 18S rDNA analyses supports the monophyly of the group as Monogenea (Mollaret et al., 2000).

Current Classification

The current classification of Monogenea divides the class into 3 subclasses: **Polyonchoinea**, **Oligonchoinea**, and **Polystomatoinea** (Boeger and Kritsky, 1993). The monophyly of Monogenea as a class is supported by the following morphological synapomorphic (shared derived) characteristics: Adult and oncomiracidium possessing 2 pairs of eyespots, 16 marginal hooks in the haptor, a haptor with a single ventral

pair of hamuli (= anchors; Boeger and Kritsky, 1993), and an oncomiracidium with 3 rows of ciliary epidermal bands present (Brooks, 1989; Boeger and Kritsky, 1993).

The monophyly of Polyonchoinea is supported by the mouth being on the ventral surface, the reduced numbers of subsurface sperm microtubules, the oncomiracidium, and adults having 14 marginal hooks and 2 central hooks in the haptor (Boeger and Kritsky, 1993). The monophyly of Oligonchoinea is supported by having a crochet en fléau present that is hook-like (the crochet en fléau is the form of the termination of the central part of the clamp of the haptoral sclerite), and the presence of a single pair of lateral sclerites, 4 pairs of haptoral suckers, and diverticula in the walls of the intestine (Boeger and Kritsky, 1993).

The monophyly of subfamily Polystomatoinea is supported by the absence of egg filaments (Boeger and Kritsky, 1993). Polystomatoinea is the sister group of Oligonchoinea. The relationship is supported by 6 shared synapomorphies, namely: Having more than 2 testes; the presence of a gastrointestinal canal; the presence of haptoral suckers in the adults; the presence of hooks in the adults' haptoral sucker; that there are 3 parts of the haptoral suckers; and the presence of 2 lateral vaginal ducts (Boeger and Kritsky, 1993). The clade formed by Oligonchoinea + Polystomatoinea is the sister group of Polyonchoinea (see Figure 1) (Boeger and Kritsky, 1993).

Brabec et al. (2023) show 2 different arrangements of the phylogenetic relationships of the flatworms. They elevated the Monopisthocotylea and Polyopisthocotylea to the level of class. For additional clarification see the modified trees given in the introduction to the Platyhelminthes in this book as well the paper by Brabec and colleagues (2023).

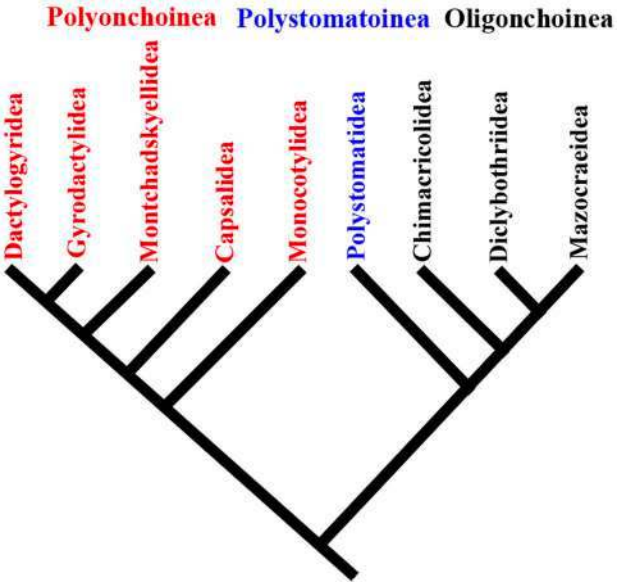

Figure 1. Relationships of the orders of Monogenea; synapomorphies of each other. Source: Adapted from morphologies in Boeger and Kristky, 1993. License: CC BY-NC-SA 4.0.

Body Wall

The monogeneans, like the digeneans (trematodes/flukes) and cestodes (tapeworms), possess an external layer called a tegument. The surface of this is a syncytial stratum laden with vesicles and mitochondria. This layer is enclosed externally by a plasma membrane and glycocalyx and internally by a membrane and basal lamina. This stratum is the distal cytoplasm and it is connected by trabeculae (internuncial processes) to the cell bodies, or cytons (perikarya), located inside a layer of superficial muscle. Often, the outer surface of the tegument has scattered short microvilli. In some species the microvilli are absent and in their place shallow pits occur (Roberts and Janovy, 2008).

The tegument is the site of the exchange by diffusion of gases and nitrogenous waste between the body and the environment. Some nutrients in the form of amino acids are taken in by pinocytosis or the cellular mechanism of taking liquids through the cellular membrane and forming a vesicle (Brusca and Brusca, 2003).

Life Cycles of Monogeneans

All monogeneans have a direct life cycle, which means that they do not have an intermediate host. They have tiny, free-swimming ciliated larvae called oncomiracidia (singular: miracidium) that hatch directly from an egg. Some life cycles have been studied, particularly those of *Dactylogyrus*, *Polystoma, Diplozoon*, *Benedenia*, and *Microcotyle* (see Bychowsky, 1957). For example, *Polystoma nearcticum*, a parasite of North American hylid frogs, lives in the urinary bladder of adult frogs and tadpoles of *Hyla versicolor* (= urinary bladder generation) and on the gills of their tadpoles (= branchial generation) (Bentz et al., 2006). In the urinary bladder of toads, the adults of the bladder generation release embryonated eggs into the urinary bladder and are voided with urine. The development of the eggs begins in the water and fully developed larvae enter the gill chambers of the tadpoles, thereby ending the urinary bladder generation and initiating the branchial (gill) generation. These larvae attach to gills of tadpoles and mature in about 22 days (see Figure 2) (Olsen, 1962).

The life cycle of monogeneans has been shown to be influenced by water temperature. For example, in *Neobenedenia girellae* infections, parasite growth, egg production, and emerging second generations stay on the same host. Infection levels and growth change on the skin corresponding with

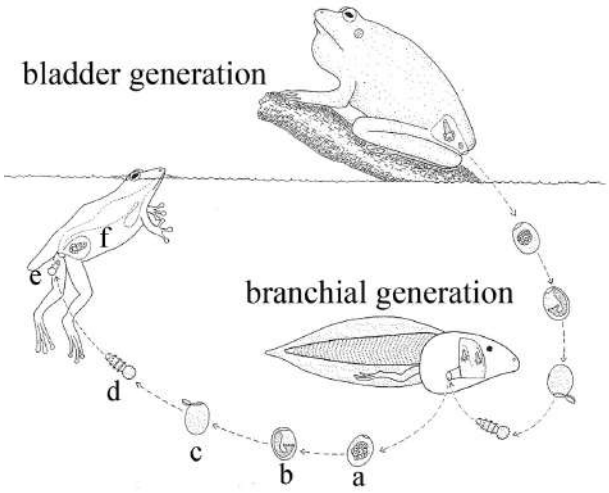

Figure 2. Life cycle of *Polystoma nearcticum* showing 2 generations. Note: a) unembryonated eggs laid on gills of tadpoles are washed into the water; b) fully developed larva, identical to those from the bladder generation; c) empty egg shell; d) the larva free in the water; e) the larva enters the cloaca of the metamorphosing toad eventually ending up in the urinary bladder; f) developing monogeneans enter the bladder and initiate the urinary bladder generation, reaching sexual maturity simultaneously with the toad. Source: Adapted from Olsen, 1962. License: CC BY-NC-SA 4.0.

differences in water temperatures. At 30 °C, the body length of worms is significantly greater than worms from fish reared at 20 °C or 25 °C. In the same manner, the number of eggs produced by adults is greater at 30° C than 20 °C or 25 °C (Hirazawa et al., 2010).

In most species of monogeneans, new hosts are infected directly by the oncomiracidia, the tiny, free-swimming ciliated larva (the adults are oviparous). The exceptions to this involve members of the Gyrodactylidae, most of which are viviparous; that is, small, unciliated larval individuals, similar to the parent, are produced within the body of the parent. After they have developed sufficiently, these young worms spread to new hosts by contagion. They use the substrate of the water body as a staging post where feeding fish may pick up the parasites. In some species the young worms float in the water until they come in contact with fish. When an infected fish dies, its parasites will infect a new host that comes close to the dead fish. Adult members of *Gyrodactylus* have several generations of embryos (young worms) within them, and each embryo has another embryo inside, even before it is released from the adult. In this manner, each adult worm produces fully developed offspring that may attach to either the same or a different host. This produces exponential population growth, which proves to be particularly problematic in freshwater fish farms (see Figure 3) (Cable and Harris, 2002).

Body Form

Monogeneans are flatworms, more or less dorsoventrally flattened, with bilateral symmetry and small sizes. The majority of them are tiny, but some species have larger bodies. In general, size range of the body is from 0.2 mm to 10.0 mm, but sometimes can be even larger. Usually, they are lanceolate, elliptical, or discoid in outline shape. The body may be clear to whitish or gray, depending upon the species, and the **eggs** generally are yellowish. The body is subdivided into

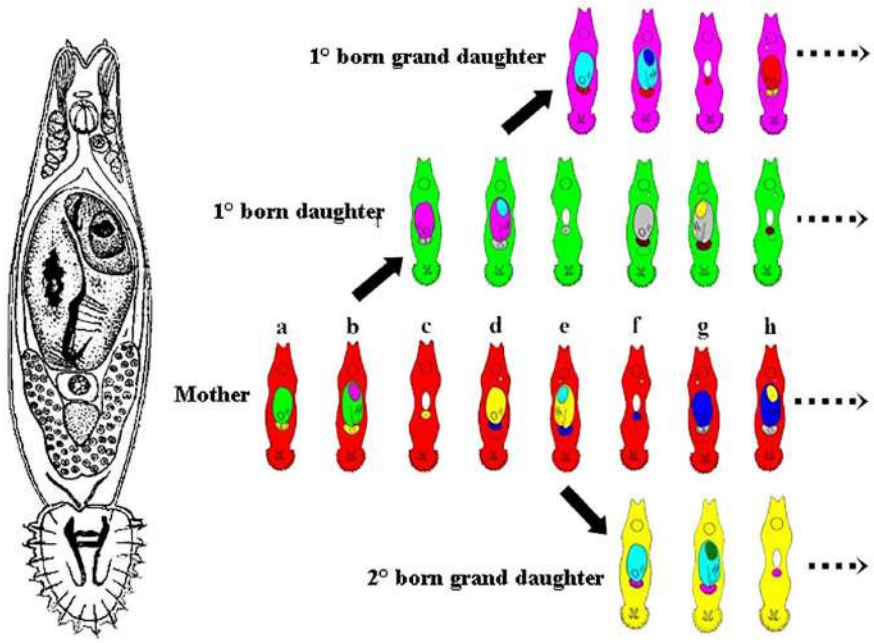

Figure 3. Life cycle of *Gyrodactylus* sp. Source: Adapted from Cable and Harris, 2002. License: CC BY-NC-SA 4.0.

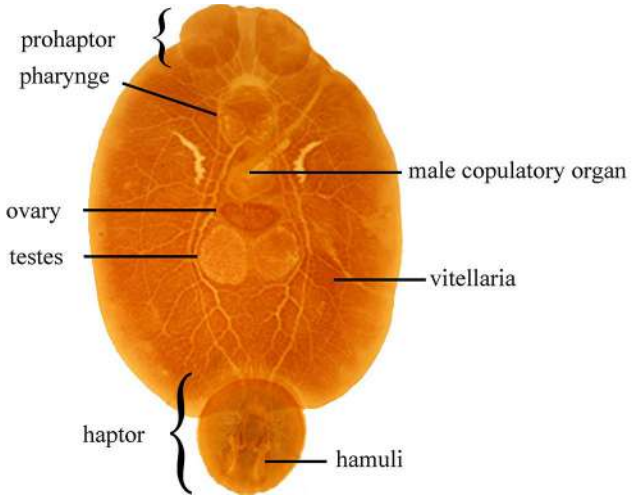

Figure 4. Subclass Polyopisthocotylea *Protomicrocotyle manteri* Bravo-Hollis, 1966, parasite of the Crevalle jack *Caranx hippos* from Campeche, Mexico. Source: G. Pulido-Flores. License: CC BY-NC-SA 4.0.

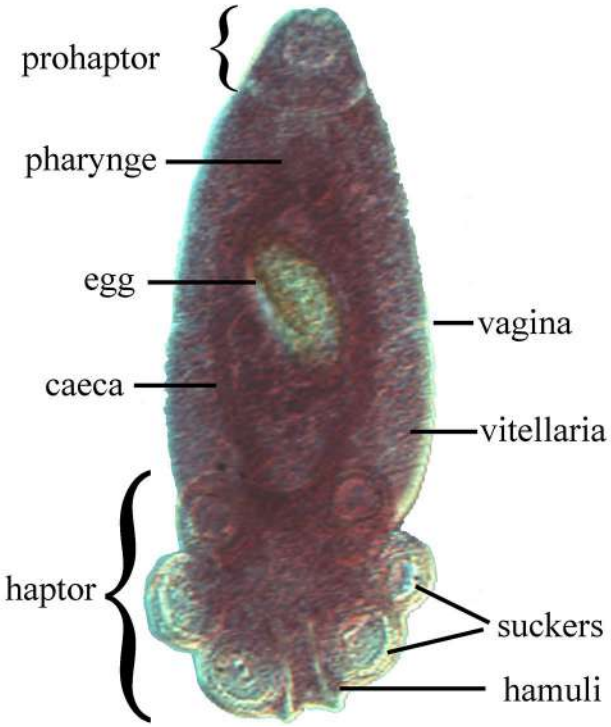

Figure 5. *Benedeniella posterocolpa* (Hargis, 1955) Yamaguti, 1963 (subclass Polyonchoinea), parasite of *Rhinoptera bonasus* from Ciudad del Carmen, Campeche, Mexico. Source: G. Pulido-Flores. License: CC BY-NC-SA 4.0.

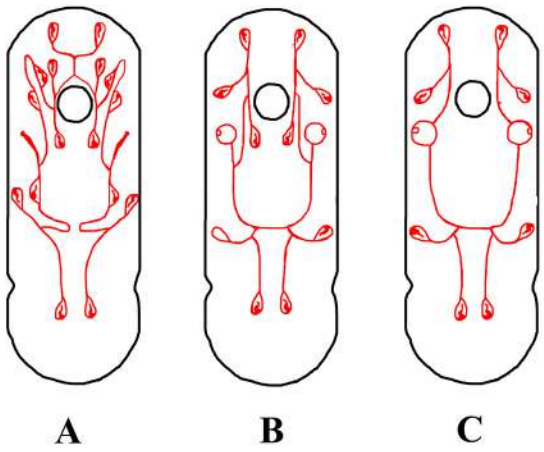

Figure 6. *Polystomoidella oblongum* (Wright, 1879) (subclass Polystomatoinea), parasite of *Kinosternon hirtipes* from Tezontepec de Aldama, Hidalgo, Mexico. Source: G. Pulido-Flores. License: CC BY-NC-SA 4.0.

3 regions: The **cephalic region** (anterior to the **pharynx**), the **trunk** (body proper), and the **haptor** (sometimes called the **opisthaptor**; the organ used to attach to the host).

Cephalic Region

The anterior end of the body, usually called the **prohaptor**, includes the feeding and adhesive organs. Sometimes the prohaptor structures are called **head lappets**, **cephalic glands**, **head organs**, and/or **pre-oral suckers**. For example, in *Protomicrocotyle manteri* and *Benedeniella posterocolpa*, the prohaptor is formed by 2 large suckers (Figures 4 and 5), and in *Polystomoidella oblongum*, the prohaptor has an oral sucker (Figure 6).

Haptor

The haptor of monogeneans is the posterior attachment organ. In the past, the majority of the papers referred to the attachment organ as an opisthaptor (meaning posterior haptor). Malmberg (1990) called the attachment organ of the oncomiracidium a haptor and he referred to the organ in adults as an opisthaptor. In most of the recent literature, the authors refer to the attachment organ as a haptor without regard to the developmental state.

The haptor of adults may be a single unit forming a simple muscular disc or a muscular sucker with 1 or 2 pairs of **hamuli** (Figure 5) and may have 1 or 2 transverse bars. Or they may have a complex attachment organ consisting of 2 or more muscular **suckers** or **clamps**. In some taxa, the haptor also has a **haptoral appendix** and the suckers are armed with **sclerites** (Figures 4 and 6) (Yamaguti, 1963; Schell, 1970; Malmberg, 1990). For example, *Denarycotyle gardneri* has a haptor with a central loculus, an additional loculus on either side of the central loculus, and 10 peripheral loculi. There are 2 accessory structures (for which the func-

tion is unknown) on the dorsal surface of the haptor and on each hamulus is a sclerotized accessory piece. The margin of the haptor has 14 hooklets (for a visual depiction, see Figure 1A from Pulido-Flores et al., 2015). *Neonchocotyle violantei* has an asymmetrical haptor with 3 paired sucker-sclerite complexes with the longitudinal axis of the haptor forming an angle of approximately 45° from the midline of the body and a dorsal haptoral appendix with pairs of microhooks (for a visual depiction, see Figures 1 and 4 from Quiterio-Rendon et al., 2018).

For all monogeneans, the haptor is the principal attachment organ. Even a larva has a tiny haptor when it hatches from an egg. It might be armed with sclerotized unhinged or hinged marginal hooks or spines that give it a strong capacity for attachment. This structure is retained in adults in the majority of the species and, as it grows, it expands into the characteristic haptor of the adult.

The total number of marginal hooks on the haptor differs among species. Some species have unhinged marginal hooks that number 10, 14, 16, or 18. They present in a symmetrical manner, such as, in species with 10 hooks, they are arranged with 5 hooks on each side of the hamuli; that is, 5 lateral + 5 lateral = 10 total hooks. In species with hinged marginal hooks, the number is either 10 or 16. The details of how these patterns of hooks were defined can be seen in Malmberg (1990). Generally, the unhinged and hinged marginal hooks retain their shape during ontogeny, but certain marginal hooks can move from their original position or sometimes even disappear (for more information, see Malmberg, 1990). The various patterns of marginal hooks are consistent among each different group of monogeneans.

Osmoregulatory System

The osmoregulatory system in monogeneans is similar to that of other Platyhelminthes and composed of **flame cells** interconnected by tubular **ducts**. Malmberg (1990) described 3 types in monogeneans and related them to 3 groups characterized by the different patterns of marginal **hooks**. Members of group A have 10 marginal hooks and a type of **spermatozoa** that is in taxa more basal in the cladogram of Monogenea. Members of group B (called the intermediate type) also have 10 marginal hooks but the spermatozoa is more derived than those of group A. Members of group C, called the Dactylogyrid type, are those with other patterns of marginal hooks (not 5 + 5 = 10) (see detailed characterizations in Malmberg, 1990).

Group A has the most simple type of osmoregulatory system, consisting of an anterior and a posterior protonephridial arrangement in the body of the oncomiracidium that has few flame bulbs, both arrangements opened laterally, either separately or by a common bladder. Members of group B, the intermediate type (also with 5 + 5 = 10 haptoral hooks), has an osmoregulatory system consisting of an anterior protonephridial arrangement (which extends through one half of the body) that opens into the posterior arrangement. The members of group C, the Dactylogyrid type, have an osmoregulatory system consisting of propulsive flame cells in the anterior and the posterior main canals (see the figure in Malmberg, 1990).

It is interesting to reflect on how the patterns of haptoral hooks and the patterns of the osmoregulatory systems are consistent with each other. Of course, that is the type of evolutionary pattern that one should expect—groups of characteristics/features that show patterns of evolution that are the same. This subject cannot be dealt with here, but it is sufficient to note that this type of similar pattern of characters (character evolution) is the basis of modern hypotheses of the phylogenetic relationships of the taxa (natural groups) of organisms. For those interested in the evolution of species and the methodology used to discover patterns of character evolution, see Brooks and McLennan (1991; 1993; 2002), as well as the studies cited within those, and those who have cited these works.

Digestive System

In general, in most species of monogeneans the digestive system is incomplete (they do not have an anus). Often, the **mouth** is surrounded by an **oral sucker** that opens in a short **prepharynx**, which connects to the muscular, glandular **pharynx**. In turn, the pharynx connects to the **esophagus**, which leads to the **intestine**. The intestine is divided into 2 **cecae** in most species; however, some species have an intestine composed of only a single cecum. Species of the genera *Tetraonchus* and *Udonella* are examples of those with only a single cecum (Schell, 1970). The cecae may be branched or unbranched, and they may end blindly or they may anastomose (connect) posteriorly.

Nervous System

The nervous system in monogeneans is ganglionic; that is, it is formed by 2 **cerebral ganglia** located in the anterior region of the body that are united by a transverse commissure. From each node arise 2 **nerves**: 1 dorsolateral and 1 ventrolateral, that run toward the posterior end of the body. From these, numerous secondary branches lead from the lateral nerves then anastomose with each other, forming a complex, ladder-like network. Also, some anterior nerves run out from the cerebral ganglia, in particular, those associated with the **sense organs**, such as ocelli, which are located in the anterior region. Many larval or juvenile forms have **ocelli** (**eyespots**) that provide orientation using light. The adults of

some taxonomic groups retain the larval ocelli and others lose them, sometimes leaving fragments of retinal pigment where they were.

Male Reproductive System

Monogeneans are hermaphroditic, but cross-fertilize. In general, the male and female **gonopores** are located some distance from each other, making self-fertilization difficult to impossible, although in some taxa they are located close together. The male reproductive system consists of 1 to several **testes**, which are located anterior or posterior to the single **ovary**. A **vas efferent duct** (**vas efferens**) runs out from each testis, if there is more than one; the vasa efferentia join together to form a single duct, the **vas deferens** that connects to the **seminal vesicle**. That in turn is connected to the male copulatory organ. Sometimes the **genital atrium** (that is, the area where the male and female gonopores can be found) may be present or absent. The **male copulatory organ** (called a **cirrus**) can be armed or unarmed, is sometimes sclerotized, and extends out of the common genital pore, which usually opens ventrally. Sometimes **prostatic glands** are present. For example, *Denarycotyle gardneri* has 1 testis with the vas deferens arising from the left side of the testis. The vas deferens is enlarged to form a spherical reservoir to hold sperm, and it leads to a smaller reservoir that is curved toward the left side of the body. The vas deferens is a loosely coiled, narrow duct that ascends dorsally, posterior to the genital pore, to connect to a seminal vesicle, then to the ejaculatory bulb and the male copulatory organ. In this species, the male copulatory organ is a short, sclerotized tube (for a visual depiction, see Figure 1B from Pulido-Flores et al., 2015). *Neonchocotyle violantei* has 8 testes. Its seminal vesicle is elongate, extending anteriorly to the proximal male copulatory organ, which is located within a pouch that is longer than the male copulatory organ (for a visual depiction, see Figure 1A from Quiterio-Rendon et al., 2018).

Female Reproductive System

The female reproductive system consists of 1 **ovary** of variable shape and position among the different species. The **oviduct** connects the ovary with the **ootype** and the **vitelline duct**, and the **vagino**- and **genitointestinal ducts** also open out. Associated with these structures is the **Mehlis' gland**, a duct that runs from the ootype and ends in the **genital pore**. Monogeneans usually have 1 **vagina**, but some groups have 2 vaginas that usually are connected to the **seminal receptacle**.

Denarycotyle gardneri has an ovary that is elongate, V-shaped, with the lateral arm of the "V" encircling the right intestinal cecum dorsoventrally, and then it narrows to form the oviduct. The oviduct, the seminal receptacle, and the common vitelline duct all join at the ootype. In this species, the vagina is muscular, unsclerotized, and sac-like. The seminal receptacle is present and the vitellaria (yolk-producing glands) extend from the level of the posterior portion of the pharynx to the posterior of the body proper (for a visual depiction, see Figure 1B from Pulido-Flores et al., 2015).

Neonchocotyle violantei has 2 vaginae that run parallel in the proximal portion and non-parallel in the distal portion. The proximal region, connected to the vitelline reservoir, is glandular and the muscular distal region connects to the vaginal pore (female gonopore). The vaginal pores open ventrally. The ovary of this species is tubular, with deep lobes and ascending and descending branches that reach to the region of the oviduct. The descending branch is coiled and connects posteriorly to the ootype. The ootype is dorsal to the ovary, but ventral to the vas deferens, and it leads to the uterus and the seminal receptacle (for a visual depiction, see Figures 1A and 2, and the detailed description in Quiterio-Rendon et al., 2018).

In *Neonchocotyle violantei*, the vitellaria are abundant, follicular, and they are arranged laterally along the entire body, and sometimes into the haptor. An efferent duct extends from the vitellaria and fuses to form the vitelline duct in close proximity to the oviduct. Near this point, they form a vitelline reservoir. In this species, the transverse vitelline ducts are dorsal, forming a Y-shaped reservoir; the proximal region of the vaginae are connected to the anterior branches of the reservoir and the posterior region of the reservoir is joined to the oviduct (for a visual depiction, see Figures 1A and 2 from Quiterio-Rendon et al., 2018).

Fertilization of the ova occurs in the ootype. Fully developed eggs are operculated and they have 2 polar filaments (some species have a single filament, others have none). The number of eggs is variable among the species; these are released to the outside through the genital pore.

The structural details of the various species of Monogenea are complex and sometimes difficult to envision. Studying the descriptions of several different species will provide a better understanding of this complexity.

Taxonomic Classification

The taxonomic classification of the Class Monogenea follows the phylogenetic analysis of Boeger and Kritsky (1993).

Class Monogenea van Beneden, 1858

Subclass Polyonchoinea Bychowsky, 1937

Order Monocotylidea Lebedev, 1988

Family Monocotylidae Taschenberg, 1879

Family Loimoidae Price, 1936

- Order Capsalidea Lebedev, 1988
  - Family Acanthocotylidae Price, 1936
  - Family Capsalidae Baird, 1853
  - Family Dionchidae Johsnton & Tiegs, 1922
- Order Montchadskyellidea Lebedev, 1988
  - Family Montchadskyellidae Bychowsky, Korotajeva & Gusev, 1970
- Order Gyrodactylidea Bychowsky, 1937
  - Family Gyrodactylidae Van Beneden & Hesse, 1863
  - Family Anoplodiscidae Tagliani, 1912
  - Family Bothitrematidae Price, 1936
  - Family Tetraonchoididae Bychowsky, 1951
- Order Dactylogyridea Bychowsky, 1937
  - Suborder Calceostomatinea Gusev, 1977
    - Family Calceostomatidae Parona & Perugia, 1890
  - Suborder Neodactylodiscidae Kamegai, 1972
    - Family Neodactylodiscidae Kamegai, 1972
  - Suborder Amphibdellatinea Boeger & Kritsky, 1993
    - Family Amphibdellatidae Carus, 1885
  - Suborder Tetraonchinea Bychowsky, 1937
    - Family Tetraonchidae Monticelli, 1903
    - Family Neotetraonchidae Bravo-Hollis, 1968
  - Suborder Dactylogyrinea Bychowsky, 1937
    - Family Dactylogyridae Bychowsky, 1933
    - Family Pseudomurraytrematidae Krtisky, Mizelle, & Bilqees, 1978
    - Family Diplectanidae Monticelli, 1903
- Subclass Polystomatoinea Lebedev, 1986
  - Order Polystomatidea Lebedev, 1988
    - Family Polystomatidae Gamble, 1896
    - Family Sphyranuridae Poche, 1926
- Subclass Oligonchoinea Bychowsky, 1937
  - Order Chimaericolidea Bychowsky, 1957
    - Family Chimaeridolidae Brinkmann, 1942
  - Order Diclybothriidea Bychowsky, 1957
    - Family Diclybothriidae Price, 1936
    - Family Hexabothriidae Price, 1942
  - Order Mazocraeidea Bychowsky, 1957
    - Suborder Mazocraeinea Bychowsky, 1957
      - Family Plectanocotylidae Monticelli, 1903
      - Family Mazoplectidae Mamaev & Splipchenki, 1975
      - Family Mazocraeidae Price, 1936
    - Suborder Gastrocotylinea Lebedev, 1972 sedis mutabilis
      - Infraorder Anthocotylina Boeger & Kritsky, 1993
        - FamilyAnthocotylidae Price, 1936
      - Infraorder Gastrocotylina Lebedev, 1972
        - Family Pseudodiclidophoridae Yamaguti, 1965 incertae sedis
        - Superfamily Protocomicrocotyloidea Johnston & Tiegs, 1922 sedis mutabilis
          - Family Protomicrocotylidae Johnston & Tiegs, 1922
          - Family Allodiscocotylidae Tripathi, 1959
          - Family Pseudomazocraeidae Lebedev, 1972
          - Family Chauhaneidae Euzet & Trilles, 1960
        - Superfamily Gastrocotyloidea Price, 1943 sedis mutabilis
          - Family Bychowskycotylidae Lebedev, 1969
          - Family Gastrocotylidae Price, 1943
          - Family Neothoracocotylidae Lebedev, 1969
          - Family Gotocotylidae Yamaguti, 1963
      - Suborder Discocotylinea Bychowsky, 1957 sedis mutabilis
        - Family Discocotylidae Price, 1936
        - Family Diplozoidae Tripathi, 1959
        - Family Octomacridae Yamaguti, 1963
      - Suborder Hexostomatinea Boeger & Kritsky, 1993
        - Family Hexostomatidae Price, 1936
      - Suborder Microcotylinea Lebedev, 1972
        - Superfamily Microcotyloidea Taschenber, 1879
          - Family Axinidae Monticelli, 1903
          - Family Diplasiocotylidae Hargis & Dillon, 1965, sedis mutabilis
          - Family Heteraxinidae Unnithan, 1957, sedis mutabilis
          - Family Microcotylidae Taschenberg, 1879, sedis mutabilis
        - Superfamily Diclidophoroidea Cerfontaine, 1895, sedis mutabilis
          - Family Diclidophoridae Cerfontaine, 1895
          - Family Pyragraphoroidea Yamaguti, 1963, sedis mutabilis
          - Family Pterinotrematidae Caballero y Caballero & Bravo-Hollis, 1955
          - Family Rhinecotylidae Lebedev, 1979, sedis mutabilis
          - Family Pyragraphoridae Yamaguit, 1963, sedis mutalibis
          - Family Heteromicrocotylidae Unnithan, 1961, sedis mutabilis

Taxa incertae sedis*:* Sudanonchidae Malmberg, 1990 [Polyonchoinea]; Iagotrematidae Mañé-Garzón & Gil, 1962 [Polyonchoinea]; Microbothriidae Price, 1936 [Monogenea].

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Platyhelminthes

Transversotrematidae (Family): Ectoparasitic Trematodes

Scott C. Cutmore and Thomas H. Cribb

Phylum Platyhelminthes

Class Trematoda

Subclass Digenea

Order Plagiorchiida

Suborder Transversotremata

Superfamily Transversotrematoidea Witenberg, 1944

Family Transversotrematidae

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Chapter 61

Transversotrematidae (Family): Ectoparasitic Trematodes

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Introduction

The suborder Transversotremata is a small but biologically significant group of plagiorchiid digenean trematodes. There are just 1 superfamily, 1 family (family Transversotrematidae), 4 genera, and about 30 species known at present. It seems likely that the family Transversotrematidae is far richer than presently realized given that only a few workers have looked for them actively. All species are known from marine fishes of the Indo-West Pacific region or from freshwater fishes from the surrounding land masses. They are of particular interest because of the site of infection of the sexually adult worms. Species of this family live under the trailing edge of the scales of a wide range of marine and freshwater bony fishes. They are described as ectoparasites in the title to this chapter, but it is true that, when removed from the fish, they survive better in physiological saline than in either fresh or sea water; thus, they are evidently well sealed off from the external environment. No other trematodes are known to occupy this niche.

Perhaps because of the unusual site of infection, transversotrematids were recognized relatively late. The first described species, *Transversotrema patialense* (Soparkar, 1924), was actually first described as a cercaria. It was not until 1944 that the first sexual adult, *Transversotrema haasi* Witenberg, 1944, was reported and, even then, the host and site of infection was not really known as the specimens were found in basin of preserved fishes. Crusz and his colleagues (Crusz and Sathananthan, 1960; Crusz et al., 1964) first realized that the distinctive cercarial type of *Cercaria patialense* matched with adult worms from the skin of freshwater fishes.

Identifying Transversotrematids

Transversotrematids can perhaps be first suspected as such by the site that they infect. Work in our laboratory suggests that they are most easily detected by simply soaking the body of the dead (potential host) fish in 0.85% saline solution for 30–60 minutes. The worms emerge from under the scales and fall to the bottom of the container where they can be easily collected by inspecting the sediment with a stereo microscope.

All transversotrematids are at least partly transversely elongate (from which the type-genus name is derived) and exceptionally flat and thin, consistent with their subscale niche (Figure 1). The largest known species, *Transversotrema gigantica* Hunter et al., 2010, has been reported as reaching just over 8 mm in width (always greater than length) but most species are closer to 2 mm-wide. Most species lack an oral sucker, but the 2 known species of *Prototransversotrema* Angel, 1969 possess what might be either a true **oral sucker** or an analogous structure (Figure 1C). All species have a **ventral sucker**, a **pharynx**, and a **cyclocoel gut**. The **gonads** (2 **testes** and an **ovary**) are enclosed by the cyclocoel. **Vitelline follicles** are usually extensive but in the single described species of *Crusziella* Cribb, Bray & Barker, 1992 (Figure 1B) they are highly reduced and, in apparent association, the **eggs** embryonate in utero and will hatch to active **miracidia** as soon as they are laid. Importantly, members of the specious genus *Transversotrema* Witenberg, 1944 (Figure 1A) are now considered to be largely morphologically cryptic; although some species of *Transversotrema* are morphologically distinct, most have overlapping metric features and can only be definitively distinguished using genetic data.

Life Cycles and Host Range

The life cycle of transversotrematids is highly distinctive and specialized (Figure 2). Notably, although far more marine than freshwater species are known, all knowledge of the life cycle relates to freshwater species; nothing at all is known with respect to marine life cycles. However, it can be predicted that the life cycle does not vary greatly except perhaps with respect to the gastropod intermediate hosts infected.

Eggs embryonate and hatch as unremarkable miracidia. These actively seek and penetrate gastropod intermediate hosts (families Tateidae and Thiaridae known at present), in which the miracidium develops to a mother sporocyst. This has been described only once (Cribb, 1988) and in that case the sporocyst appears to produce only a single redia which in turn produces another generation of rediae, which then

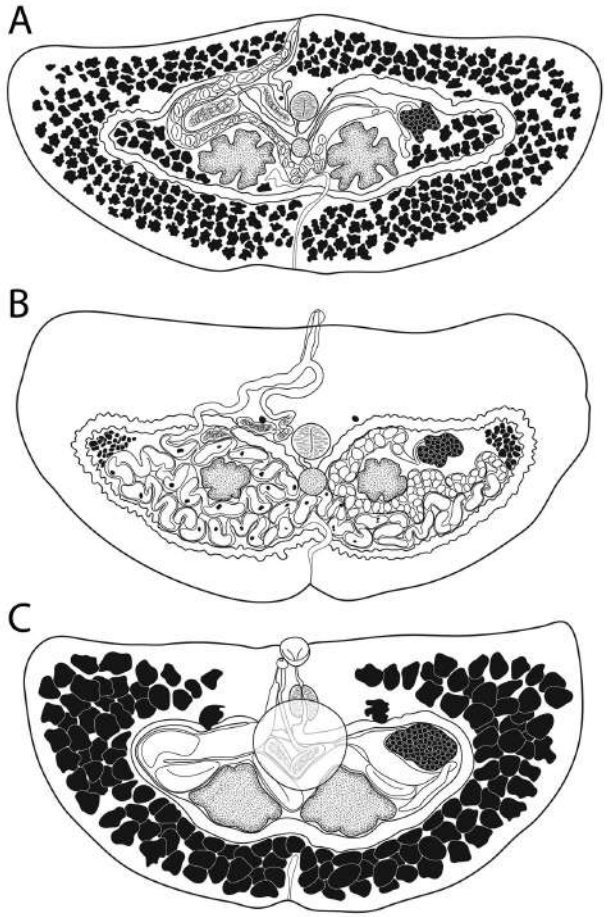

Figure 1. Transversotrematid morphology, showing cross-sections of:. A) *Transversotrema* sp.; B) *Crusziella* sp.; C) *Prototransversotrema* sp. Source: S. C. Cutmore and T. H. Cribb. License: CC BY-NC-SA 4.0.

produce cercariae. The cercaria is relatively enormous. The cercarial body is up to 0.5 mm wide, there is a pair of large eyespots, and the gonads and gut are essentially fully developed. The reproductive system may be so well developed that there is sperm in the seminal vesicle. The cercarial tail is unique among the Digenea. It is large and forked and has arm processes arising from the base of the tail. On their ends these arm processes have distinctive pads which have been shown to be concentrations of sensilla and are critical in host recognition (Whitfield et al., 1975).

Transversotrematid cercariae are highly active although relatively short-lived swimmers. They swim tail-first with the cercarial body wrapped around the tail-stem (Whitfield et al., 1975). When the cercaria bumps into a suitable fish it will recognize it as such with the pads on the arm processes, the cercarial body immediately slips under a scale and the tail detaches and swims away. Development to egg-producing adults is very quick, taking as few as 4 days (Cribb, 1988).

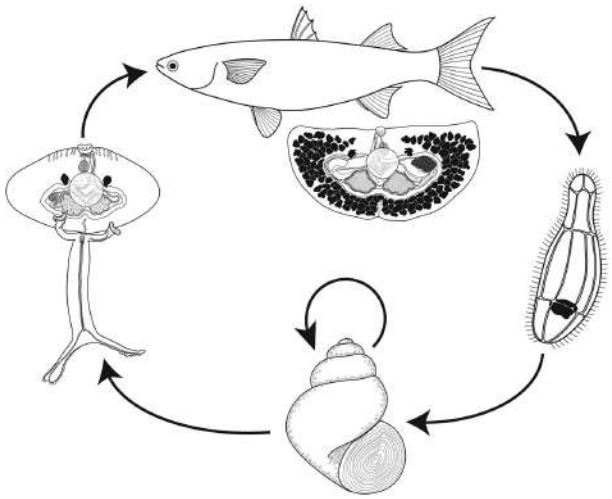

Figure 2. The generalized life cycle of transversotrematids. Source: S. C. Cutmore and T. H. Cribb. License: CC BY-NC-SA 4.0.

Interestingly, members of the Transversotrematidae exhibit a range of host specificities. Although a few species have been found to be **oioxenous** (infecting a single fish species), the overwhelming trend is for **stenoxenous** (infecting more than 1 species of a single fish family) and **euryxenous** (infecting more than 1 fish family) specificity (Hunter and Cribb, 2012; Cribb et al., 2014). Notably, *Transversotrema licinum* Manter, 1970 has been shown, using molecular data, to infect fishes of at least 8 families and 3 orders (Cutmore et al., 2016). It is likely that more extensive host sampling will show that all species of this group are either stenoxenous or euryxenous.

Significance of the Tranversotrematids

The main significance of the Transversotrematidae is in the combination of their evolutionary position and their biology. In the phylogeny of Olson and colleagues (2003), the Transversotrematidae fell unambiguously in the Plagiorchiida, sister to all other taxa except for the Bivesiculidae, the most basal taxon in the Plagiorchiida. In this context, the life cycle of the Transversotrematidae is highly intriguing. Apart from being relatively simple as a 2-host life cycle, there is little apparent connection with the life cycle of the Bivesiculidae in which the cercaria is eaten. Brooks and colleagues (1985) interpreted the ectoparasitic position of the Transversotrematidae as having occurred as the result of a secondary shift and Brooks and colleagues (1989) argued that the life cycle was secondarily reduced from a 3-host life cycle (so that perhaps the present sexual adult was once a metacercaria and the adult has been lost). These interpretations were made prior to what it now understood about the phylogenetic posi-

tion of the Transversotrematidae. Cribb and collagues (2003) suggested that, if the 2-host life cycle of transversotrematids is not a secondary condition, it might be consistent with multiple adoptions of vertebrate parasitism by the Digenea. These matters cannot yet be considered resolved, and thus the Transversotremata is a small group that should not be overlooked in the overall understanding of the evolution of the Trematoda.

The Special Case of *Transervotrema patialense*

An interesting aspect of transversotrematid biology is that 1 species, *Transervotrema patialense*, appears to be invasive. It has been reported from several countries outside its apparent native range (see Womble et al., 2015). It is transmitted by several thiarid gastropods, but especially by *Melanoides tuberculata*, which is itself a seriously invasive species. There is no evidence that *T. patialense* poses any real threat to native fish species outside of its natural range. Rather, these reports are testament to the simplicity of the life cycle.

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Hirudinia

Hirudinia (Class): Parasitic Leeches

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Phylum Annelida

Class Hirudinia

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Chapter 62

Hirudinia (Class): Parasitic Leeches

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Leeches as Parasites

Some of the most charismatic and well-known leeches are blood-feeding species that rely on vertebrates, yet some species feed on the hemolymph of invertebrates, while others are strictly predatory, while scavengers in the leech world are rare (Siddall et al., 2011). In this section, only the leeches that feed on vertebrate blood will be covered (for other species, see Govedich and Moser, 2015).

Leeches are considered temporary, mostly ectoparasites of vertebrates, feeding only for short periods of time, from a few minutes or hours as in the case of *Hirudo medicinalis* or species of *Haementeria,* to days or weeks in the case of species of family Praobdellidae (*Limnobdella, Tyrannobdella, Praobdella*, or *Limnatis*) that feed from the nasal passages of mammals, including humans (Sawyer, 1986; Phillips et al., 2010). Some leeches, such as those of the genus *Placobdella* are semi-permanent parasites mainly of freshwater turtles, but some species feed on salamanders or birds (Bolek and Janovy, 2005; McCallum et al., 2011; Oceguera-Figueroa et al., 2010). Species of the genus *Theromyzon* are also semi-permanent parasites of the nasal passages of aquatic birds, such as waterfowl. One of the most extreme cases of parasitism in leeches is represented by species of the genus *Ozobranchus*, which are permanent parasites of both marine and freshwater turtles, spending their whole life attached to their host and even lay their eggs onto the body surface of their hosts (Sawyer, 1986; Nakano et al., 2017). Notably, *Placobdelloides jaegerskioeldi* is only known from the rectal tissues of African hippopotamuses (Oosthuizen and Davies, 2011). Most blood-feeding leeches are generalists in terms of the number of species of hosts that can be parasitized, and many instances of blood-feeding species supplementing their diet with fish or amphibian eggs have been documented (Light et al., 2005; Romano and Di Cerbo, 2007).

General Morphology

Several morphological characteristics distinguish Hirudinida from other annelids, including their possession of a fixed number of 34 **somites** superficially subdivided into **annuli**, a reduced or fully absent coelom, the absence of chaeta in adult stages, and the presence of 2 **suckers**, 1 at the most anterior part of the body with the mouth laying inside (**oral** or **anterior sucker**) and 1 at the most posterior part of the body (**anal** or **posterior sucker**) (Govedich and Moser, 2015; Sawyer, 1986).

Leeches are, in general, elongated with parallel body sides, without regionalized body parts, and are slightly dorsoventrally flattened (that is, *Hirudo* and *Macrobdella* species); however, this general pattern is somewhat variable (see Figures 1–3). Some fish parasites (such as those in the family Piscicolidae) are circular in cross-section and may have distinct body regions such as the slender anterior **trachelosome** and the posterior, wider **urosome**. Species of Glossiphoniformes are, in general, foliaceous and dorsoventrally flattened. At least 2 groups of parasitic leeches, *Branchellion* and *Ozobranchus,* have developed lateral projections of the body walls forming membranous branchiae (Sawyer, 1986; also see Figure 4).

The most conspicuous morphological characteristic of leeches, in addition to the annulated body, is the presence of suckers located at the anterior and posterior ends of the body. Suckers are rather large and muscularized organs mainly used for locomotion and attachment to their host and prey (Sawyer, 1986). In general, the posterior sucker is larger than the anterior and, in some species, like the members of the family Praobdellidae, the former can be considerably wider than the width of the main body (Phillips et al., 2010). In general, 2 main types of feeding apparatuses are recognizable for blood-feeding leeches: The **proboscis** and **jaws**. The proboscis is an eversible muscular organ used to penetrate the skin of the leech prey, whereas the jaw is armed with sclerotized denticles that pierce the skin.

Reproduction

Leeches are hermaphroditic worms that perform cross-fertilization during copulation; some species have developed complex reproductive systems with a penis and vagina, such as the species of *Hirudo* and *Macrobdella*, whereas others have a simpler reproductive system with testisacs and ovisacs

Figure 1. Dorsal view of *Macrobdella decora* (family Macrobdellidae; collected from Buckingham, Gatineau, British Columbia, Canada) representing the morphological variation within the subclass Hirudinea. Source: C. Grenier, 2015. License: CC0.

Figure 2. Dorsal view of *Placobdella parasitica* (family Glossiphoniidae; collected from Ingleside, Maryland, United States) representing the morphological variation within the subclass Hirudinea. Source: SERC Fisheries Conservation Laboratory, 2022. License: CC BY-NC.

connecting to their respective gonopores through relatively simple tubes, such as the species *Placobdella* and *Haementeria*. Fertilization is internal. In species with complex reproductive systems, the penis is inserted into the vagina to discharge the spermatozoids. In species with simple reproductive systems, the sperm transfer occurs through the implantation of spermatophores on the epidermis of the recipient leech (Salas-Montiel et al., 2017). Eggs are produced and enveloped by a proteinaceous membrane secreted by the clitellum (glandular area of the reproductive somites). In most of the species, this membrane hardens and forms a protective cocoon or case where the eggs develop; all the members of Glossiphoniformes keep the eggs within a thin and flexible membrane attached to the ventral surface where the eggs develop into young leeches that remain attached to their parent, representing an uncommon case of parental care within the Annelida (Sawyer, 1986). Their ontogeny is direct, without larval stages (Sket and Trontelj, 2008).

Figure 3. Dorsal view of *Haementeria officinalis* (family Glossiphoniidae) representing the morphological variation within the subclass Hirudinea. Source: E. Caballero y Caballero and C. Loyola. License: CC BY-NC-SA 4.0.

Leeches as Vectors and Hosts

Leeches, like many blood-feeding invertebrates, may transmit bacteria or other microorganisms between hosts during the feeding process. PCR-based (Polymerase Chain Reaction-based) techniques have been used to detect bacterial communities in the digestive tract of leeches with relevant findings of *Bartonella* spp. in *Haemadipsa rjukjuana* from Korea, representing a human health concern (Kang et al., 2016). Recently, an unidentified blood-feeding leech has been implicated in the transmission of *Rickettsia* to humans (Slesak et al., 2015); however, the detailed mechanisms of the transmission patterns and frequencies need to be investigated in more detail. Leeches are occasionally vectors of *Trypanosoma* spp. and hemogregarines, particularly among fish, frogs, and turtles (Siddall and Desser, 1991; 1992).

Marine leeches of the genus *Ozobranchus*, which are permanent parasites of marine turtles, have been discussed as possible vectors of the chelonid fibropapilloma-associated herpesvirus (CCFPHV) due to the presence of relatively large loads of this virus in their body (Greenblatt et al., 2014). However, more experiments are needed to finally determine the role of leeches as vectors in these systems.

Leeches have also been recorded as intermediate hosts of cestodes (Regel, 2010), digeneans (McCarthy, 1990), and nematodes (Riggs and Ulmer, 1983). Macrophagous and blood-feeding leeches, such as *Haemopis* spp. and *Macrob-*

della spp., respectively, are definitive hosts for digeneans of the genus *Alloglossidium* that reach their adult stage in the leech intestine (Schmidt and Chaloupka, 1969; Beckerdite et al., 1974).

Recently, blood-feeding leeches (*Haemadipsa* spp.) have been successfully used to screen mammal diversity in Vietnam and southern Asia (Bangladesh, Cambodia, and China). PCR-amplification of the DNA (ingested DNA or iDNA) stored in the blood meal inside the crop of the leeches collected in the field revealed the presence of a wide diversity of mammal blood, such that a broad scope of host preference can be inferred for the leeches. In total, mammals of 6 orders (Artiodactyla, Carnivora, Chiroptera, Lagomorpha, Primates, and Scandentia) and 4 species of Aves were detected using this method. Amplifiable mitochondrial DNA was recovered from the gut content up to 140 days after blood ingestion; making leeches a promising candidate to uncover hidden vertebrate diversity (Schell et al., 2012; 2015; Tessler et al., 2018b).

Proboscis-bearing leeches that feed exclusively on vertebrate blood, such as species of *Placobdella*, *Placobdelloides*, and *Haementeria,* as well as species of Oceanobdelliformes (of the genera *Ozobranchus*, *Piscicola*, *Pontobdella*, *Branchellion*, and *Myzobdella,* among others) have established extreme symbiotic associations with bacteria, mainly Proteobacteria. Leeches of these groups house bacteria in specialized cells (bacteriocytes) that form specialized organs (bacteriomes) connected to the digestive system. It has been suggested that bacteria might complement the diet of these monophagous blood-feeding leeches, given the lack of, or low proportion of, vitamin B in vertebrate blood (Perkins et al., 2005: Kvist et al., 2011; Manzano et al., 2015). Associations between nutrient-supplying bacteria and their diet-restricted eukaryotic hosts have been heavily studied in various insect groups but poorly studied outside Arthropoda (see, for example, Aksoy, 1995; Douglas, 1998). Through genomic analyses of symbiotic bacteria, it has been demonstrated that the symbiont of the leech *Haementeria officinalis* has a much-reduced genome in terms of size, with high A + T content, and a reduced set of metabolic capabilities, all of which are a common characteristics of ancient obligate endosymbionts of arthropods. The genome of the *H. officinalis*-symbiotic bacterium*, Providencia siddalli*, has retained many pathways related to the biosynthesis of vitamin B, pointing towards a role in supplementing the blood-restricted diet of its host (Manzano-Marín et al., 2015).

Figure 4. General view of a leech, *Ozobranchus branchiatus* (family Ozobranchidae), displaying lateral branchiae. Source: Adapted from Lagunas-Calvo et al., 2021. License: CC BY-NC-SA 4.0.

Zoogeography

Most leeches inhabit freshwater habitat, but there are marine, brackish, and terrestrial species, too. They are distributed worldwide, and their patterns of distribution broadly correspond with the biogeographic regions described based on other zoological groups, with some recognizable transitional zones and areas of endemism (Ringuelet, 1985, Sawyer 1986; Sket and Trontelj, 2008). Each biogeographic region is characterized by species flocks or genera; in the Nearctic, parasitic leeches are represented by the genera *Macrobdella*, *Philobdella*, and *Placobdella,* whereas in the Neotropics, parasitic leeches include *Mesobdella gemmata*, *Haementeria*, and *Oxyptychus*. In the transitional zone between these 2 areas (Mesoamerica), leeches from both areas co-occur, including *Macrobdella*, *Placobdella*, *Haementeria*, and endemics, such as *Limnobdella* and *Pintobdella* (Moser et al., 2016; Ringuelet, 1985; Oceguera-Figueroa and León-Règagnon, 2014). Palearctic parasitic leech fauna is characterized by species of *Hirudo*; however, other blood-feeding leeches are distributed in the region, such as those of the genus *Limnatis* and a single species of the otherwise Nearctic genus

Placobdella [*Placobdella costata* (Müller, 1846)] (Trontelj and Utevsky, 2005; Siddall et al., 2005). The leech fauna in the Afro-Tropical region is characterized by *Parapraobdella, Placobdelloides, Aliolimnatis*, and *Oosthuizobdella* (Sawyer, 1986; Phillips et al., 2011). The leech fauna of the Indian region is characterized by species in the genera *Haemadipsa*, *Hirudinaria*, and *Poecilobdella* (Sawyer, 1986), whereas the leech fauna in the East Asia region (Sino-Japanese region) is characterized by species of *Batracobdella, Hirudinaria, Hirudo nipponia, Poecilobdella*, and *Dinobdella* (Lai and Chen, 2010; Sawyer, 1986). Australia and New Zealand have a characteristic leech fauna, mainly represented by species of the genus *Chtonobdella* (Tessler et al., 2016), and other enigmatic leeches, such as *Ornithobdella edentula* found on nests of the New Zealand penguins *Eudyptes robustus* or the leech *Euranophila central,* a parasite of the frog *Litoria gilleni* from central Australia (Sawyer, 1986).

Some species display wide geographic distributions. For example, *Theromyzon* is a cosmopolitan genus (excluding Antarctica). This unusually broad distribution is probably related to the biology of their waterfowl hosts. Marine leeches such as those in the genera *Ozobranchus*, *Pontobdella*, and *Branchellion* display a broad geographic distribution attributable to the dispersal abilities of their hosts across the oceanic basins (Sawyer, 1986).

Introduction to Hirudinea Classification

Jean Baptiste Lamarck coined the term Hirudinea in 1818 and the taxon was originally conceived of as a class within Annelida, or segmented worms, along with Polychaeta and Oligochaeta (Govedich and Moser, 2014). After 200 years of investigation, including the discovery of numerous species and groups, as well as the development of methods to better infer the phylogenetic relationships within this taxon, several changes have been proposed. These investigations have helped to reconcile taxonomic names and classification with the phylogenetics (Figure 5). It is now fully accepted that Oligochaeta is paraphyletic due to the inclusion of Hirudinea and, together, Oligochaeta, Hirudinea, and 2 small groups of leech-like worms (Branchiobdellida and Acanthobdellida) form the class Clitellata. Furthermore, phylogenetic studies have recovered Polychaeta as paraphyletic due the inclusion of Clitellata (Zrzavý et al., 2009; Struck et al., 2011; Kvist and Siddall, 2013; Weigert et al., 2014; Aguado et al., 2014). In further complicating the current conception of Annelida, Sipuncula (peanut worms), Siboglinidae, including pogonophores and vestimentiferans (deep-sea beard worms), and Myzostomida (which are parasitic on echinoderms) are now also considered to be annelids, although their morphological characteristics depart from the most common conditions of typical annelids and, interestingly, their phylogenetic position within the phylum is still unsettled (Aguado et al., 2014).

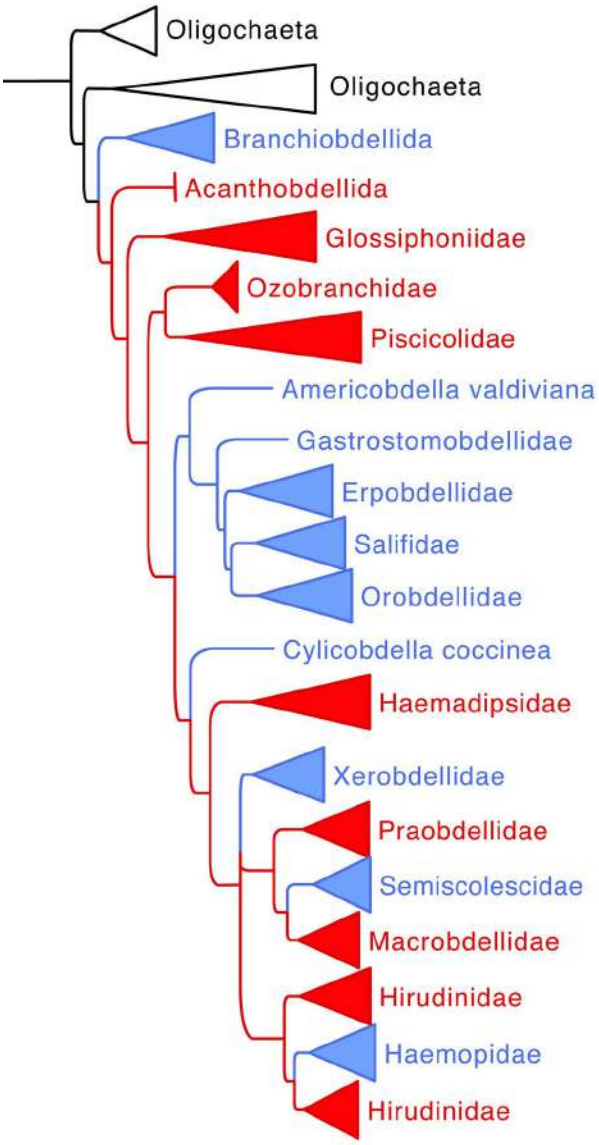

Figure 5. Composite phylogenetic diagram of the subclass Hirudinea summarizing the current knowledge of the relationships of major groups. Blood-feeding lineages are shown in red, non-blood-feeding lineages in blue. Source: A. Oceguera-Figueroa and S. Kvist. License: CC BY-NC-SA 4.0.

Order Acanthobdellida (salmonid parasites) and order Branchiobdellida (crayfish worms) were considered leech-like organisms that were thought to have developed suckers independently as an adaptation to their parasitic lifestyle. However, recent phylogenetic studies based mainly on molecular data clearly support their affinities with subclass Hirudinida (Siddall et al., 2001; Tessler et al., 2018). Both groups, Acanthobdellida and Branchiobdellida, are less speciose in comparison to Hirudinida, with only 2 species (*Acanthodella*

peledina and *Paracanthobdella livanowi*) and approximately 140 species, respectively (Gelder, 2009; Sawyer, 1986).

The number of species included in this group is still growing, with more than 680 species distributed worldwide (Sket and Trontelj, 2008).

Classification and Phylogeny

Historical classification of subclass Hirudinida recognized 2 orders, separated on the basis of the presence or absence of an eversible proboscis: Rhynchobdellida was used for proboscis-bearing leeches and Arhynchobdellida was used for species that lack such a structure (Sawyer, 1986). Recent phylogenetic studies based on molecular data failed to recover Rhynchobdellida as a monophyletic group (Apakupakul et al., 1999; Trontelj et al., 1999) and, consequently, Tessler and colleagues (2018) suppressed Rhynchobdellida and recognized 5 groups at the ordinal rank for all leeches: **Oceanobdelliformes**, including the families **Piscicolidae** (fish leeches; marine, brackish and freshwater species) and **Ozobranchidae** (turtle leeches; mainly marine, few species freshwater and brackish); **Glossiphoniformes** (blood and hemolymph feeders, freshwater species), **Americobdelliformes** (macrophagous, semi-terrestrial), **Erpobdelliformes** (macrophagous, freshwater), and **Hirudiniformes** (hematophagous and macrophagous, freshwater species).

Based on phylogenetic hypotheses and the mapping of feeding preferences onto the tree, as well as on the evidence provided by the analyses of the peptides of the saliva of some leeches (Siddall et al., 2011; Kvist et al., 2016), it has been suggested that the last common ancestor of all leeches was a blood-feeder (that is, adapted to feed on the vertebrate blood) and this feeding preference switched to macrophagy (feeding on small invertebrates and dead animals) and to liquidosomatophagy (feeding on hemolymph) on at least 6 or 7 independent occasions.

Leech Therapy: History of Medical Applications

The so-called medicinal leeches are without doubt the most charismatic and infamous members of the group. Medicinal leeches have been used for centuries ostensibly to correct imbalances of the traditionally recognized 4 humors, namely, blood, phlegm, black bile, and yellow bile (Singh, 2010; Whitaker et al., 2004), as well as a variety of other ailments including mental disorders, whooping cough, gout, tumors, epilepsy, headaches, arthritis, and obesity (Weinfeld et al., 2000; Porshinsky et al., 2011). Leeching, or hirudotherapy, became the most popular mode of bloodletting in the Old World during the 18th and 19th centuries, in particular through the application of the renowned European medicinal leech *Hirudo medicinalis*. In order to fulfill the heavy demand on the medicinal leech, local leech populations were over-harvested to the point of local extinction; as a consequence, in 1823, restrictions were implemented to manage the number of leeches being exported through Hannover, Germany and collecting seasons were instituted in Russia (Wells and Combes, 1987; Whitaker et al., 2004; Elliott and Kutschera, 2011).

Currently, surgeons use leeches to aid in the salvage of venous-congested extremities that result from an imbalance between arterial inflow and venous outflow following surgery; this includes digits (Brody et al., 1989), nipples (Güneren et al., 2000), ears (Cho and Ahn, 1999), lips (Walton et al., 1998), nasal tips (Mortenson et al., 1998), and penis (Pantuck et al., 1996). Medicinal leech therapy has enormous utility in removing stagnant blood and allowing veins to recover (Singh, 2010; Porshinsky et al, 2011) and *Hirudo medicinalis* was approved as a medical device by the United States Food and Drug Administration (US FDA) in 2004 (Rados, 2004).

Recent phylogenetic analyses have clearly demonstrated that medicinal leeches do not form a monophyletic group. Instead, and with a broad definition of the term medicinal leech, 6 different groups include species that have been used for medicinal purposes around the world: *Haementeria* spp. in South America and Mexico; *Limnobdella* spp. in Mexico; *Macrobdella*, *Philobdella*, and *Oxyptychus* in the New World; *Hirudo* spp. in the Palearctic; *Haemadipsa* spp. and *Hirudinaria* in Southeast Asia, *Chtonobdella* spp. in Australia; and *Aliolimnatis* spp. in Africa (Oceguera-Figueroa, 2012; Phillips and Siddall, 2005; 2009; Phillips et al., 2010; Tessler et al., 2018).

Preparation of Specimens

Proper fixation of leeches for morphological and molecular studies is important and necessary to understand biodiversity. To avoid morphological distortion of the specimen, it is important to narcotize or relax specimens before fixation. The main method consists of gradually adding drops of 95–100% ethanol to the water-filled container until the leeches' movements and reactions to touching stop. This process can take up to 30 minutes, depending on the specimen's size and, subsequently, the mucus produced during this operation should be removed with paper towels. Once relaxed, leeches must be straightened and placed in a container between paper towels and covered with 95–100% ethanol for 24 hours or more, depending on the size of the specimens. For molecular analyses, tissues (commonly parts of the posterior suckers, in order to avoid contaminations by potential blood meals), should be placed directly in 96% ethanol and kept at 4 °C, or colder conditions, if possible. For permanent slide preparations, in particular for small leeches, specimens should be flattened between 2 glass slides immediately after narcotization. Stain-

ing should be carried out with a mixture of Mayer's paracarmine and Ehrlich's haematoxylin and mounted on slides with Canada balsam. For histological preparations, the use of 4% paraformaldehyde, 2.5% glutaraldehyde, or instead, Fleming's or Bouin's fixatives is recommended.

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63

Arthropoda

Siphonaptera (Order): Fleas

Marcela Lareschi

Phylum Arthropoda

Class Insecta

Order Siphonaptera

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Chapter 63

Siphonaptera (Order): Fleas

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Introduction

Adult fleas (order Siphonaptera) are highly specialized holometabolous arthropods adapted to parasitic life and are morphologically very different from other insects. Fleas are parasites of birds and mammals, but their greatest specific richness is associated with rodents. There are nearly 3,000 species and subspecies placed in 19 families that are currently known worldwide (Lewis, 1998; Whiting et al., 2008).

Both male and female fleas feed exclusively on host blood. Larvae benefit from the host blood indirectly since they ingest the adult fleas' feces after the adults digest the blood (Marshall, 1981; Linardi and Guimarães, 2000; Medvedev and Krasnov, 2006).

External Morphology of the Imago (Figures 1 and 2)

Adult fleas (the **imago** is the adult or reproductive stage of the flea (Maggenti et al., 2005)) are laterally compressed, wingless insects, and are usually brownish-yellow in color. The flea body averages 4–5 mm in length, while a few giant flea species measure up to 1 cm in length and, in these species, female-biased sexual size dimorphism occurs. The body generally is covered with **bristles** angled backward that permit easy movement through the hairs or feathers of their hosts. The body is resistant, able to withstand great pressure, probably an adaptation to survive attempts of elimination by crushing or scratching by the host. The **head** is usually small, narrow, and cuneiform, and is sometimes helmet-shaped. **Eyes** may be present, vestigial, or absent. The **antennae** are short and serve as chemoreceptors. When not in use they retract back into furrows on the sides of the head. The **mouthparts** are specialized for piercing and sucking. In some species, the mouthparts are adapted to attach to the epidermal tissue of the host. Some fleas have **ctenidia**, or combs, which are rows of spines, similar to strong teeth, directed backwards and which are located on the head (frontal and genal) and in the **thorax** (pronotal and mesonotal). The ctenidia are species specific and can be used for flea identification (Figure 2). The thorax has 3 pairs of **legs** with **tarsi** with bristles, plantar spines, and a pair of long claws to cling to the host (Figures 1 and 2). The **abdomen** has 10 segments, 8 each with a pair of **spiracles**, and includes the **pygidium**, or **sensilium** (sensory organ), at the posterior end. The last segments are modified variously, for copulation in males and egg laying in females.

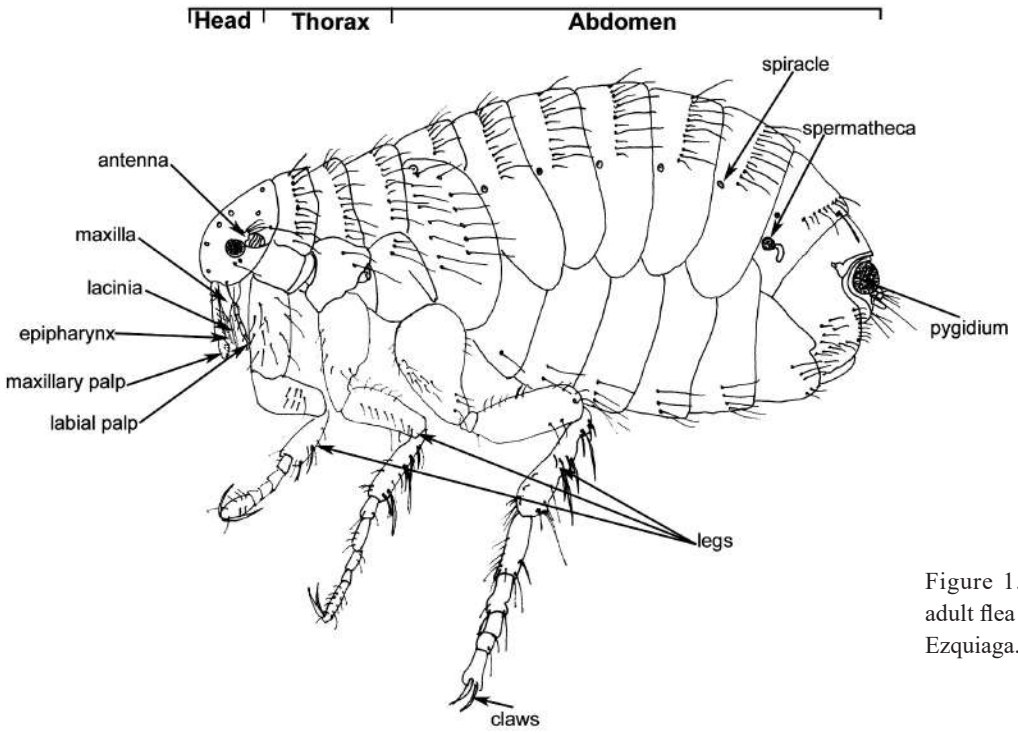

Figure 1. External morphology of an adult flea (*Pulex irritans*). Source: M. C. Ezquiaga. License: CC BY-NC-SA 4.0.

Figure 2. Details of the cteniodia (*Craneopsylla minerva*). Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

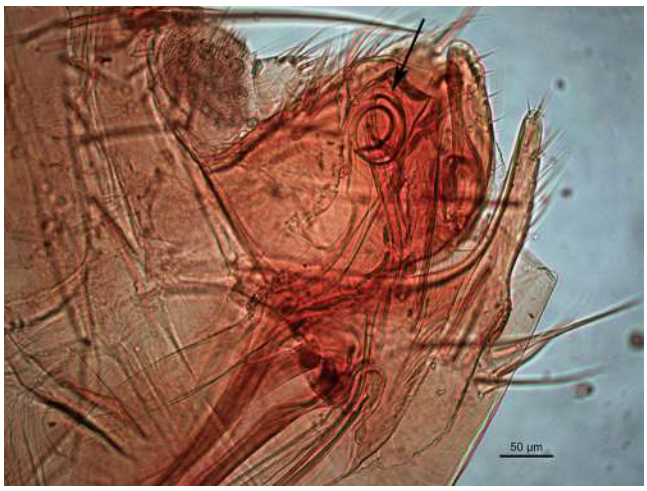

Figure 3. Details of the aedeagus (*Polygenis platensis*). Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

Figure 4. *Hectopsylla* sp. Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

Figure 5. Male *Malacopsylla grossiventris* (family Malacopsyllidae). Source: M. C. Ezquiaga and E. Soibelzon, 2021. License: CC BY-NC-SA 4.0.

Sexual dimorphism is pronounced, with females larger than males; the posterior part of females is rounded, while that of males is upturned, to accommodate the copulatory apparatus in the last segments; the males have an internal structure that is projected during copulation, called the **aedeagus** (Figure 3). The sperm receptacle in the female is called the **spermatheca** (Figures 1 and 2). Genitalia and the associated modified segments have diagnostic value at the species level (Hopkins and Rothschild, 1953; Johnson, 1957; Beaucournu and Launay, 1990; Linardi and Guimarães, 2000; Medvedev and Krasnov, 2006; Linardi, 2017).

Some fleas, most of them belonging to the genus *Tunga* (family Tungidae), are particular in that the females are the ones that penetrate the hosts' skin. The abdomen of a gravid female of these species increases up to 20 times its original size, which is referred to as **neosomy**. Neosomy is an external transformation of shape involving the formation of new cuticle during a larval stadium. The best species known is *Tunga penetrans* in which the second stage larvae do not feed, but the adult females penetrate into the toes of humans and produce eggs. Neosomy also occurs in species other than *T. penetrans*, such as those of the genus *Hectopsylla* (Figure 4) and in the family Malacopsyllidae (Figure 5). These fleas attach to the outside of the host by remarkably well-developed mouthparts (Audy et al., 1972; Marshall, 1981).

Morphological Adaptation to Parasitism

Morphological adaptation to parasitism in fleas includes the mouthparts and their jumping mechanism. Flea mouthparts (Figure 1) are adapted to obtain blood from the host. The suctorial mouthpart of fleas includes the **maxilla**, **maxillary palp**, **labial palp**, the **epipharynx**, and two **laciniae** of the maxillae, which together enclose a food channel for inbound blood. The laciniae form a smaller salivary channel for outbound saliva. These structures have an elongated stylet-like form, and each outer side of the laciniae has 2 rows of backward-pointed **teeth** which cut or saw the skin of the host and anchor the mouthparts. The length of the mouthparts and the number and development of the teeth vary among flea species (Hopkins and Rothschild, 1953; Linardi and Guimarães, 2000; Medvedev and Krasnov, 2006).

The best known locomotory characteristic of the fleas is their ability to jump, which allows these wingless insects to parasitize their hosts successfully. The legs are adapted for jumping, with the hind leg longer than the 2 prior legs. This mechanism has been studied by various authors (see the literature cited in Medvedev and Krasnov, 2006) where differences in the jumping ability between the sexes and among species is reported. For example, it has been found that male fleas jump shorter distances than female fleas and jump length varies among species (Rothschild et al., 1975; Medvedev and Krasnov, 2006).

Morphology of the Larvae and Pupae

Whereas the morphology of adult fleas is well known, the morphology of flea larvae and eggs has not been investigated so intensively. The larvae (Figure 6) of the fleas are of a grayish transparent appearance, and many segments may be covered with very fine **setae**, which may obscure their honeycomb appearance. Larvae are eyeless but possess dermal light receptors and are generally negatively phototropic. The larvae are vermiform and legless, with chewing **mouthparts**. The larvae are characterized by the presence of **anal lobes**, which play a major role in locomotion. The anal lobes possess slightly divergent fingerlike expansions on segment X providing the larva with support points on the substrate and this enables the larva to move. Three stages of larvae are recognized, with the exception of the species of Tungidae, which present only 2. The first stage is recognized by the presence of a front tooth that aids in hatching, while the remaining 2 are differentiated only by being larger than the other one. Although flea larvae are highly active, they generally remain buried in organic debris in the host's environment, and it is within this that they pupate. Prior to pupation, they empty their alimentary canal and spin a silken cocoon around them which may adhere to the substrate, and in which they come to lie in a U-shaped position prior the first pupal molt (Cotton, 1963; Marshall, 1981; Beaucournu and Launay, 1990; Pilgrim, 1992; Linardi and Guimarães, 2000; Pilgrim and Galloway, 2000; Linardi, 2017).

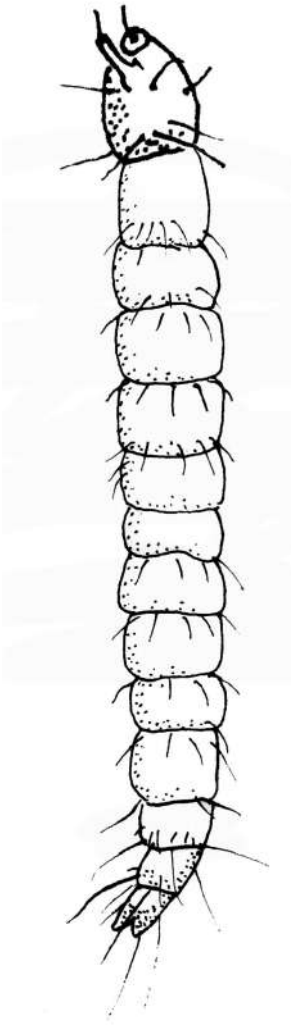

Figure 6. External morphology of the larva. Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

Morphology of the Eggs

Flea species can be identified based on the external morphological characters of their **eggs**. The posterior end of the egg has holes termed **micropyles** and the anterior end of the egg has holes termed **aeropyles**. The characters that help aid in the identification of the eggs include various distributions and combinations of reticulation on the surface, micropyles, anterior aeropyles, lateral aeropyles, and the egg's size.

A scanning electron microscope (SEM) is used to examine the flea egg **exochorion** (Figure 7). The eggs of Malacopsyllidae are large, as is the case for other large-sized fleas, such as *Sphinctopsylla ares*, and species of Hystrichopsylla. Species with relatively very large eggs never have more than 2 eggs within the **oviduct** at any one time, but in contrast with these species, malacopsyllids present neosomy and it is possible

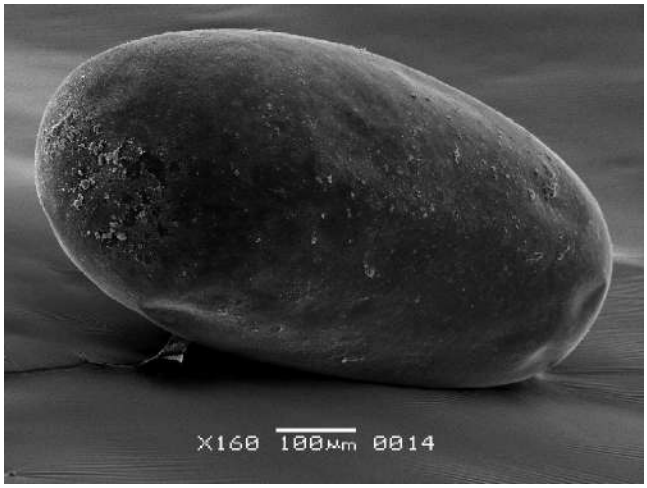

Figure 7. External morphology of the egg of *Malacopsylla grossiventris* (scanning electron microscope image). Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

Figure 8. *Polygenis* sp. (family Rhpalopsyllidae). Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

that there may be more than 2 clutches of eggs (Rothschild et al., 1986; Chen and Wang, 1993; Lynley et al., 1994; Krasnov, 2008; Ezquiaga and Lareschi, 2012).

Phylogeny, Systematics, and Taxonomy

The combination of morphological with molecular data provides compelling evidence for a sister group relationship between the winged mecopteran family Boreidae and the Siphonaptera (Rothschild, 1975; Whiting, 2002; Whiting et al., 2008). The ancestor of fleas, with detritus-feeding larvae and adults feeding upon plant material or live arthropods, was probably afirst associated with the nests of mammals. Fleas remain primarily mammal parasites, but some have secondarily moved to birds, such host-switches or ecological fitting occurring at least 16 times in the evolution of the order. Many bird fleas have arisen from the fleas of tree-climbing rodents, whereas others have moved from burrow-dwelling mammals to burrow-dwelling birds (Holland, 1964; Marshall, 1981; Whiting et al., 2008).

Hopkins and Rothschild published a 5-volume series on flea systematics based on the extensive Rothschild Flea Collection deposited at the Natural History Museum in London, United Kingdom (Hopkins and Rothschild, 1953; 1956; 1962; 1966; 1971). Subsequently, 3 additional volumes were published for the families Pygiopsyllidae (Mardon, 1981), Ceratophyllidae (Traub et al., 1983), and Malacopsyllidae and Rhopalopsyllidae (Smit, 1987).

Currently, the most accepted higher classification for Siphonaptera is based on morphological characteristics, provided by Medvedev (1998) and Lewis (1998), and which have been modified by Whiting and colleagues (2008), by analyzing flea relationships based on molecular data. Whiting and colleagues (2008) present the first formal analysis of flea relationships based on a molecular matrix. Almost 3,000 species and subspecies are known, from 238 genera and 19 families in the order Siphonaptera.

The family **Tungidae** is the most basal flea lineage, a sister group to the remainder of the extant fleas. Tungidae includes a group of fleas that have an unusual morphology, with a characteristic compression of the 3 thoracic segments, having mouthparts that are always enlarged and modified for firm attachment to the host, an eye that is reduced or absent, and no ctenidia. As noted above, they live a neosomic lifestyle. Tungidae is placed at the base of the phylogeny, as sister to the remaining flea taxa, and includes species allocated to the genera *Tunga* and *Hectopsylla* (Figure 4). Of all the fleas, females of the species *Tunga* are the only ones known to live within the host's cutaneous tissues.

The majority of the natural mammalian hosts of the genus *Tunga* are sloths and armadillos, and secondarily seem to have switched hosts via ecological fitting and diversified extensively on various species of rodents. Although humans and domestic animals are the principal hosts for *T. penetrans*, from an evolutionary standpoint, these are certainly secondary associations. *Hectopsylla* prefers caviomorph rodents, birds, and bats. The geographical distribution of its members covers the Neotropics (*Tunga* and *Hectopsylla*), Africa (*Tunga*), and East Asia (*Tunga*) (Hopkins and Rothschild, 1953; Johnson, 1957; Hastriter and Méndez, 2000; Linardi and Guimarães, 2001; Whiting et al., 2008).

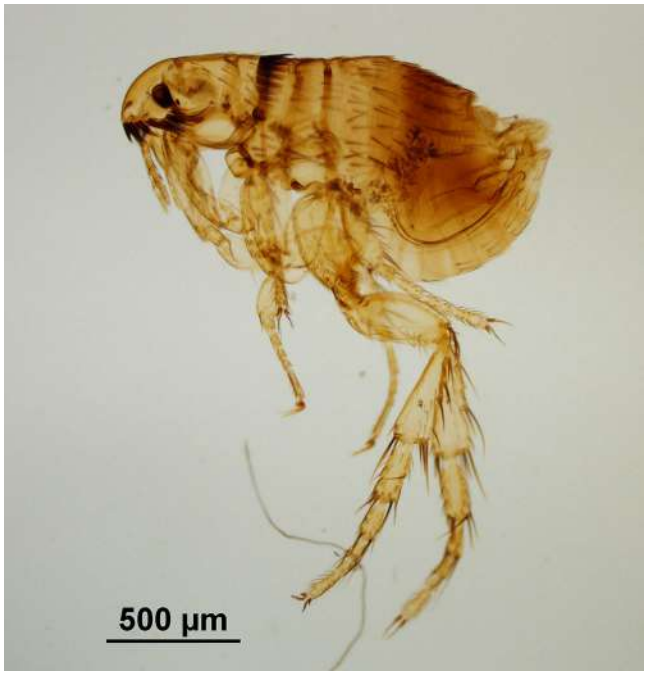

Figure 9. *Adoratopsylla intermedia intermedia* (family Ctenophthalmidae). Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

Figure 10. *Ctenocephalides* sp. (family Pulicidae). Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

Species included in the **Lycopsyllidae**, **Pygiopsyllidae**, and **Stivaliidae** families are classified in the suborder Pygiopsyllomorpha, with a sister group relationship between the latter 2 families. These 3 families each have general biogeographic differences with a few exceptions of sympatry where they have been shown to occur in the same region. Lycopsyllidae is restricted to Australia including Tasmania. The distribution of Pygiopsyllidae is far broader and includes Australia and East Asia, with 1 genus, *Ctenidiosomus*, represented in South America. Stivaliidae is mainly distributed in New Guinea. Species of the family Pygiopsyllidae usually lack genal and pronotal ctenidia, but present several abdominal terga with well-developed combs, and have an eye, though it is reduced. Pygiopsyllidae contains more than 30 genera, that are associated with metatherians in Australia and South America and with callosciurine squirrels and tree squirrels (Tupaiidae) in the Indo-Malayan subregion. Species in the genus *Ctenidiosomus* are found in cricetid rodents. In addition, some species are associated with birds in Australia (Johnson, 1957; Mardon and Dunnet, 1972; Whiting et al., 2008; Hastriter, 2012).

The families **Macropsyllidae** and **Coptopsyllidae** are sister groups. Macropsyllidae is a small family comprising 2 genera: *Macropsylla* (2 species) and the monotypic genus *Stephanopsylla*. These occur in Australia and are found on murid rodents. Morphologically, Macropsyllidae is very similar to Stephanocircidae, but differs in the single, continuous comb on the head of macropsyllids compared with 2 separate cones in Stephanocircidae. Additionally, Macropsyllidae present an abdomen with combs of long spines, and females have 2 spermathecae of unequal size (Hopkins and Rothschild, 1956; Whiting et al., 2008). Coptopsyllidae fleas are completely combless and vestigial abdominal combs or pseudosetae are absent, with antepygidial bristles. Females possess 2 spermathecae. Coptopsyllidae is also a small group (1 genus, 19 spp.) with Palearctic distribution (Hopkins and Rothschild, 1956; Whiting et al., 2008).

The family **Stephanocircidae** (Figure 2), or helmeted fleas, are unique among fleas because of the division of the forward portion of the head that forms a sort of helmet, which presents more-or-less vertical combs along the posterior margin. A second vertical comb is present along the genal margin. The helmet serves in a manner similar to that of the prow of a boat as it separates hairs as the flea moves through the pelage of its host. The family includes 2 subfamilies, Stephanocircinae, which is restricted to metatherians in the Australian region, and Craneopsyllinae, which is more speciose than Stephanocircinae and is restricted to metatherian and rodent hosts in the Neotropical region (Hopkins and Rothschild, 1956; Traub, 1980; Schramn and Lewis, 1988; Sánchez et al., 2015).

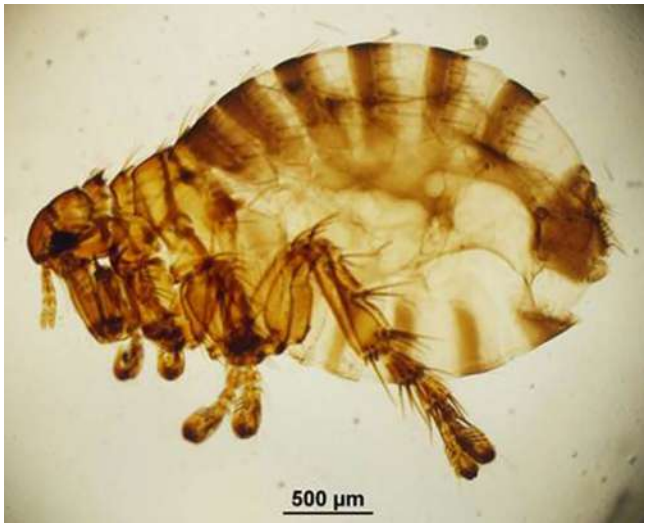

Figure 11. *Dasypsyllus* sp. (family Ceratophyllidae). Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

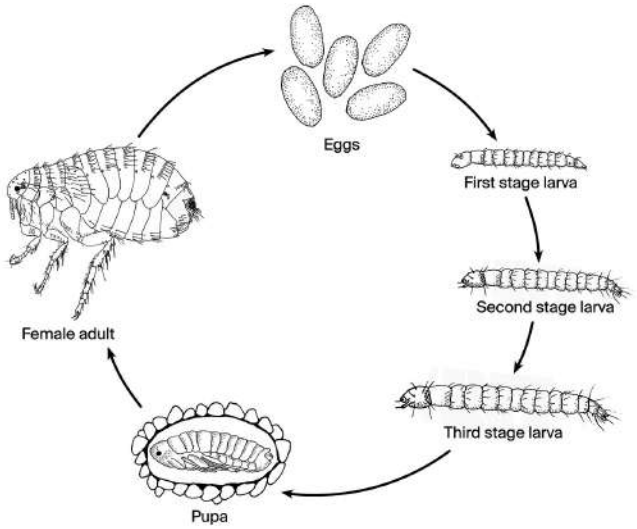

Figure 12. Life cycle of the fleas. Source: M. C. Ezquiaga. License: CC BY-NC-SA 4.0.

Vermipsyllidae is a small family comprising 3 genera and 42 species, characterized by lacking a ctenium, the absence of an anal stylet in females, the presence of a frontal tubercle, lacking antepygidial bristles, having very large spiracles, possessing reduced tergites and sternites, especially in females, and having 1 spermatheca. Vermipsyllids are found on carnivores, mustelids (Chaetopsylla), and ungulates (Hopkins and Rothschild, 1956; Whiting et al., 2008).

The family **Rhopalopsyllidae** (Figure 8) is characterized by the absence of true ctenidia, the presence of a lower haft of fronds with a well-developed large or very large somewhat trapezoid-shaped tubercle situated in a groove, a large and sinuate eye, terga with 1 or 2 (or sometimes 3) rows of setae, a complete or incomplete mesocoxal oblique break (this has importance for taxonomic purposes), a symmetrical or asymmetrical antennal club with sexual dimorphism, 4 lateral plantar bristles on the fifth segment of all tarsi, 2 heavy ventral subapical bristles, a solitary long, antepygidial bristle in both sexes, and females with 1 spermatheca. Two very speciose subfamilies are recognized, Rhopalopsyllinae, represented mainly in the Neotropical region of South America, and Parapsyllinae, which is more abundant in the Andean Patagonia region. Although Rhopalopsyllidae is almost exclusively Neotropical, it extends into the southern part of the Neartic region while 1 genus, associated with birds, is widespread on many islands in the seas surrounding Antarctica and has radiated into the Australian region. Most of the species infest cricetid rodents but a few species have host-switched to birds (Smit, 1987; Linardi and Guimarães, 2000; Beaucournu et al., 2014; Lareschi et al., 2016).

Hystrichopsyllidae, a paraphyletic family, present horizontal, oblique, or vertical genal ctenidia, but these are sometimes very reduced. If a vertical ctenidium is present, it extends far dorsally and has some spines drawn out into long, thin points. A fifth tarsal segment is present with 5 pairs of lateral plantar bristles, and females possess 2 spermathecae. Hystrichopsyllinae is composed of the tribes Ctenopariini with 1 Neotropical genus, and Hystrichopsyllini with 2 Nearctic genera and 1 Paleartic genus (Johnson, 1957; Hopkins and Rothschild, 1962; Whiting et al., 2008).

Ctenophthalmidae (Figure 9) is a paraphyletic family and is sometimes considered a subfamily within Hystrichopsillidae. It is distinguished from the Hystrichopsillidae by the presence of the fifth tarsal segment with 4 pairs of lateral plantar bristles (at times with 1 anterior plantar pair on the ventral surface) and the female possessing only 1 spermatheca (Johnson, 1957; Hopkins and Rothschild, 1966; Whiting et al., 2008).

Pulicidae (Figures 1 and 10) and **Chimaeropsyllidae** are sister groups. Both families share the following characters: A pygidium with 14 pits per side, the inner side of the hind coxa having spiniform setae, having generally 1 row of setae per tergite, and having setae that are usually fine and rather sparse. In addition, Pulicidae is characterized by well-developed eyes without an internal sinus and the female having an anal stylet. In Pulcidae, a genal and pronotal ctenidium may be present or absent in the female, and both sexes usually possess an antepigypdial seta on each side. Species of Chimaeropsyllidae are found exclusively in the Ethiopian region, in xeric environmental conditions, associated with elephant

shrews (Macroscelidae) and small rodents. Pulicidae present cosmopolitan distribution because some of its species have experienced secondary dispersal by their hosts, which are synanthropic rodents, domestic animals, and humans; therefore, some species of Pulicidae are of medical and/or veterinary importance (Linardi and Guimarães, 2000; Whiting et al., 2008).

Leptopsyllidae, **Ischnopsyllidae**, and **Ceratophyllidae** are included in Ceratophyllomorpha. The family Leptopsyllidae is characterized by the presence of a vertical, or subvertical, genal ctenidia (sometimes with at least 3 teeth oriented in a vertical position), the presence or absence of a pronotal ctenidium, and a reduced eye. Leptopsyllidae currently consists of 2 subfamilies, Amphipsyllinae and Leptopsyllinae, mostly Paleartic, with some cosmopolitan species (for example, *Leptopsylla segnis*) associated with cricetid and synanthropic rodents (Johnson, 1957; Hopkins and Rothschild, 1956; 1971).

Species included in the family **Ischnopsyllidae** are known as the bat fleas since they occur exclusively on bats. They are distinguished by the preoral placement of the genal ctenidium at the extreme anterior end of the ventral margin of the head. This ctenidium is typically composed of 2 broad, flattened spines, present in most of the species within the family. Ischnopsyllidae comprises 2 subfamilies distributed on every continent with the exception of Antarctica; with the species being highly host-specific, since the distribution of genera follow that of their hosts on which they have evidently cospeciated (Hopkins and Rothschild, 1956; Johnson, 1957; Linardi and Guimarães, 2000; Withing et al., 2008).

All species of **Ceratophyllidae** (Figure 11) are characterized by the absence of a genal ctenidium and the possession of vestigial eyes. Ceratophyllidae comprises 2 subfamilies, Ceratophyllinae and Dactylopsyllinae, mostly Paleartic, with some cosmopolitan species (for example, *Nosopsyllus fasciatus*) associated predominantly with sylvatic and synanthropic rodents, with some species parasitizing birds (Johnson, 1957; Smit, 1983: Traub et al., 1983; Withing et al., 2008).

Species allocated to the families **Xiphiopsyllidae**, **Ancistropsyllidae**, and **Malacopsyllidae** were not included in the molecular analyses by Whiting and colleagues (2008). Xiphiopsyllidae is an Ethiopian flea, without combs in the head region, with a pronotal ctenidum present, an abdomen with spinelets, and a metanotum without either spinelets or pseudosetae (Hopkins and Rothschild, 1956). Malacopsyllidae (Figure 5) are big fleas; they do not present true ctenidia, their frontal tubercle may be absent or deciduous, and they possess a main row of long setae on the pronotum shifted forward to a sub-basal position, and a hind tarsus with the fifth tarsal segment of all legs enlarged with strong claws and plantar bristles. Finally, species of Malacopsyllidae include only 2 monotypic genera, *Malacopsylla* and *Phthiropsylla*, which occur only in Argentina in association with armadillos and carnivores with carnivores probably as secondary hosts (Johnson, 1957; Smit, 1987; Lareschi et al., 2016).

Figure 13. Tungiasis: The leg of a dog infested with *Tunga penetrans.* Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

Geographic Distribution

Fleas are distributed all around the world, present in a range of habitats from equatorial deserts, distributed from the Arctic to Antarctica, through tropical rainforests to the tundra. Sometimes the distribution of fleas is a consequence of their introduction by humans and their pets and livestock. The flea fauna of the Palaearctic region has the most diverse world distribution, while the number of species in the Nearctic, Afro-Tropical, and Neotropical regions is fewer, and that in the East Asian and Australian regions is considerably less. Malacopsyllidae, Rhopalopsyllidae, and Craneopsyllinae are dominant in South America, Xiphiopsyllidae and Chimaeropsyllidae are present in Africa, and Macropsyllidae, Lycopsyllidae, and Stephanocircinae are present in Australia. In contrast, more speciose and paraphyletic flea families, such as, Hystrichopsyllidae, Ceratophyllidae, and Leptopsyllidae, inhabit the Northern Hemisphere (Medvedev and Krasnov, 2006; Whiting et al., 2008).

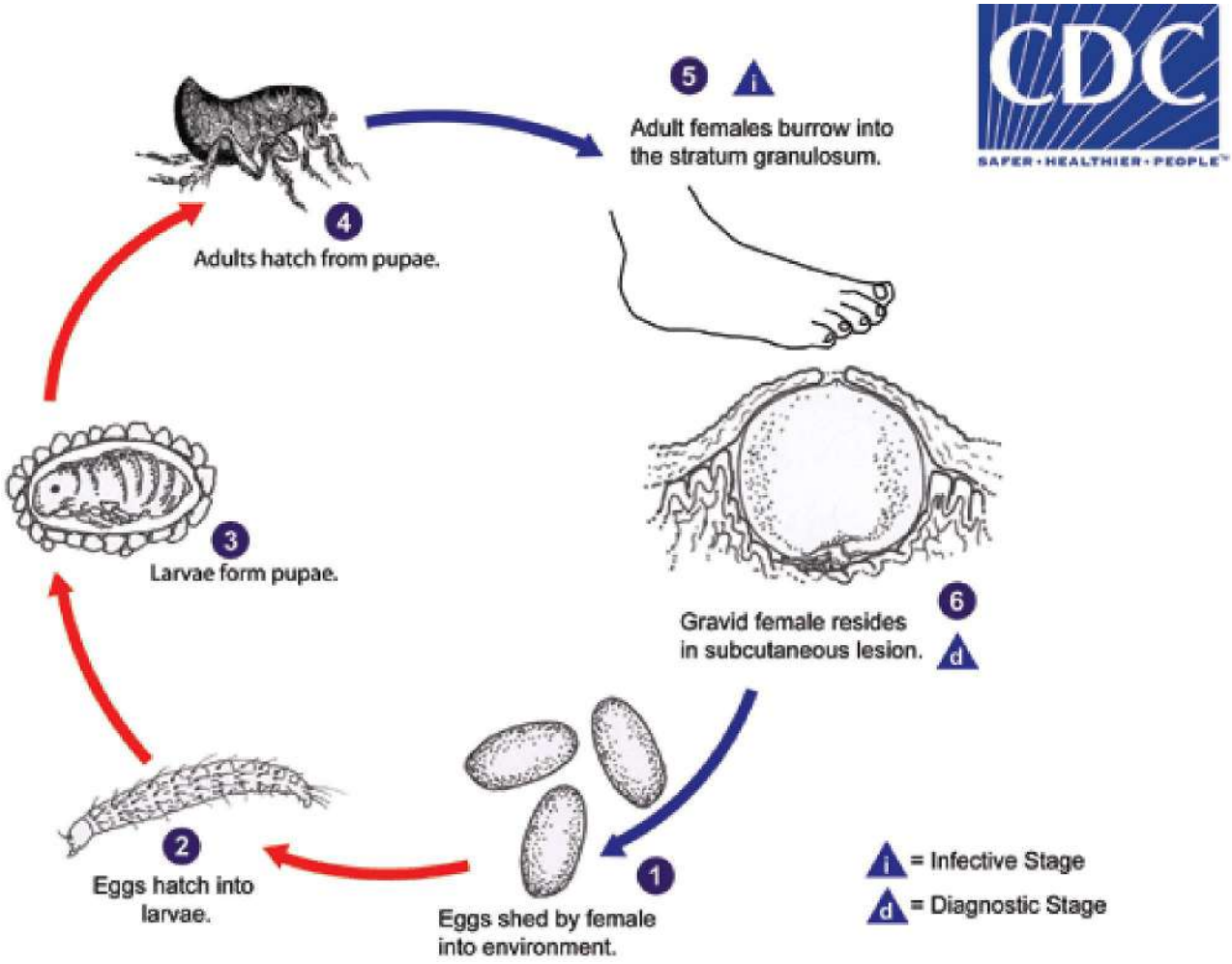

Figure 14. Tungaiasis life cycle. *Tunga penetrans* eggs are shed by the gravid female into the environment (1). Eggs hatch into larvae (2) in about 3–4 days and feed on organic debris in the environment. There are 2 larval stages before forming pupae (3). The pupae are in cocoons that are often covered with debris from the environment (sand, pebbles, etc). The larval and pupal stages take about 3–4 weeks to complete. Afterwards, adults hatch from pupae (4) and seek out a warm-blooded host for blood meals. Both males and females feed intermittently on their host, but only mated females burrow into the skin (epidermis) of the host, where they cause a nodular swelling (5). Females do not have any specialized burrowing organs, and simply claw into the epidermis after attaching with their mouthparts. After penetrating the stratum corneum, they burrow into the stratum granulosum, with only their posterior ends exposed to the environment (6). The female fleas continue to feed and their abdomens extend up to about 1 cm. Females shed about 100 eggs over a 2-week period, after which they die and are sloughed by the host's skin. Secondary bacterial infections are not uncommon with tungiasis. Source: Division of Parasitic Diseases and Malaria, United States Centers for Disease Control and Prevention, 2017. Public domain.

Host Associations

Throughout their history, fleas associated very early with mammals with 4 evidently independent shifts to birds. The majority of flea species are associated with mammal hosts, with about 74% of described species recorded from rodents. In addition, rodents comprise 82% of all specific and/or principal hosts for fleas. Primary association of fleas with rodents is observed in all parts of the world except Australia, where fleas are harbored mainly by marsupials (Marshall, 1981; Krasnov and Medvedev, 2006; Whiting et al., 2008).

Fleas vary greatly in the degree of their host specificity ranging from having a very narrow host-range (highly host specific) to being highly host-opportunistic with a wide host-range. Although Siphonaptera are rarely monoxenous at the host species level, there are clades of fleas associated with a particular host group at higher ordinal levels. For example, species of *Parapsyllus* (family Rhopalopsyllidae, subfamily Parapsyllinae) are exclusively associated with birds, fleas of the family Ischnopsyllidae are associated with bats, and fleas of the family Malacopsyllidae are associated mostly with ar-

madillos. Besides, mammals that generally have vast home ranges and do not inhabit dens for rearing their young almost always lack fleas of their own, whereas mammals or birds with dens or nests reused seasonally exhibit a more specific flea fauna (Marshall, 1981; Krasnov and Medvedev, 2006; Whiting et al., 2008; Beaucournu et al., 2014; Lareschi et al., 2016).

Biology and Reproduction

The life cycle of fleas (Figures 12 and 13), like other holometabolous insects, consists of eggs, larvae, pupae, and adults. Female fleas of some species oviposit on the host and the eggs drop off into the nest or burrow, while other species mate and oviposit both on-host and off-host (for example, *Xenopsylla cheopis*). Each female may lay 300–800 eggs per day in the soil or on the host body. Eggs then fall off the host and, depending on the species, temperature, and humidity, they hatch into first-stage larvae in about 3–4 days and feed on organic debris in the environment. Three stages of larvae are recognized (with the exception of species of *Tunga*, which presents only 2 stages). The larvae do not suck blood; they feed on feces of adult fleas that contain digested host blood, skin flakes, or the plumage of hosts, and other organic substances. The 3/2 larval stages last between 14 and 21 days. Then they stop feeding and molt to pupae, which live in cocoons that are often covered with debris from the environment (such as, sand, pebbles, etc.). The larval and pupal stages take about 3 to 4 weeks to complete. Afterwards, adults hatch from pupae and seek out a warm-blooded host for blood meals, but when the temperature is very low or in the absence of a host, the pupae remain quiescent in their cocoons for several months. The completely hematophagous adults must parasitize a host to feed themselves; if possible, they do so more than once a day and there is only development of eggs in females if they ingest blood. The cycle comprises a total of 3 to 6 weeks in optimal conditions, but often lasts several months, depending on the environmental conditions and the species. Fleas can withstand prolonged periods of desiccation (6 months or more) when the proper host is not present (Marshall, 1981; Linardi and Guimarães, 2000; Medvedev and Krasnov, 2006).

Fleas of the genus *Tunga* (Tungidae) are particular in having females that penetrate and embed in the skin of the host, while males move over the body of the host. No gravid females dig in the epidermis of the host, instead they penetrate mainly in the subungual, periungual, interdigital, and plantar areas, and once introduced, plunge their head toward the deepest part of the integument and, with their abdomen sticking out of the host's body, are fertilized by males from the outside. After embedding, the abdomen of the female

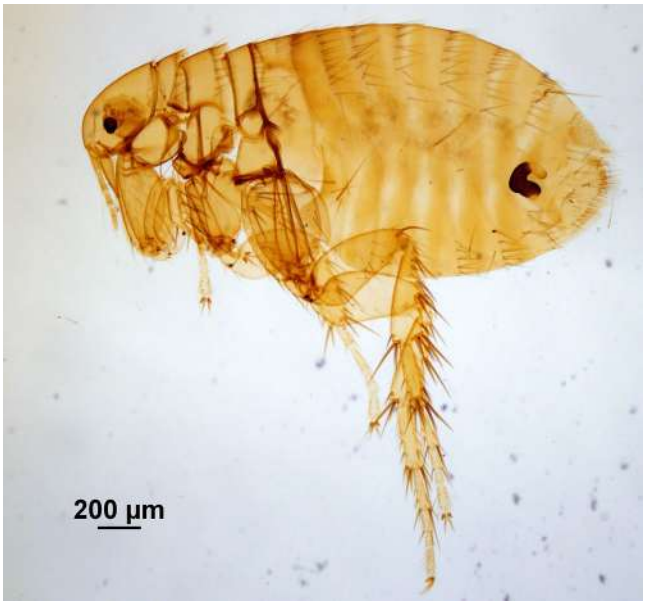

Figure 15. *Xenopsylla cheopis*. Source: J. Sánchez, M. Urdapilleta, and L. Giambelluca. License: CC BY-NC-SA 4.0.

begins to relax and the head and legs become less visible, depending on the species. This is termed neosomy. The last 2 or 3 abdominal segments are exposed on the surface and have spiracles for breathing, as well as the genital opening and the anus. The eggs mature in a week and are expelled, falling to the ground, where the 2 larval stages develop and in 10 to 14 days they change to pupae. After 1 week, the adult emerges and the female goes in search of a new host, and in this way the cycle is restarted, with a total duration of 17 to 21 days (Marshall, 1981; Linardi and Guimarães, 2000).

Although the laciniae are not heavily serrated, females of *Malacopsylla grossiventris* (Figure 5) fix their mouthparts to the skin of the venter of their armadillo hosts, clinging very firmly to the coarse hairs of these hosts. These fleas present enlarged tarsal claws, apparently modified for grasping, and copulate on the venter of their hosts (Johnson, 1957; Smit, 1987; Ezquiaga and Lareschi, 2012).

Medical and Veterinary Importance

From an epidemiological point of view, fleas are important as parasites, intermediate hosts, and vectors. Many species of fleas cause serious medical and economic problems, since flea bites on people and domestic animals are insidious, causing severe irritation and discomfort due to the formation of papules and urticarias, and they affect blood loss. The sites of bites are mainly the legs and the waist, and in allergic people the injuries can be more severe, including formation of lacerations and alopecias, and scratching can produce bacterial superinfection. Another pathology is tungiasis (Figure 14), caused by

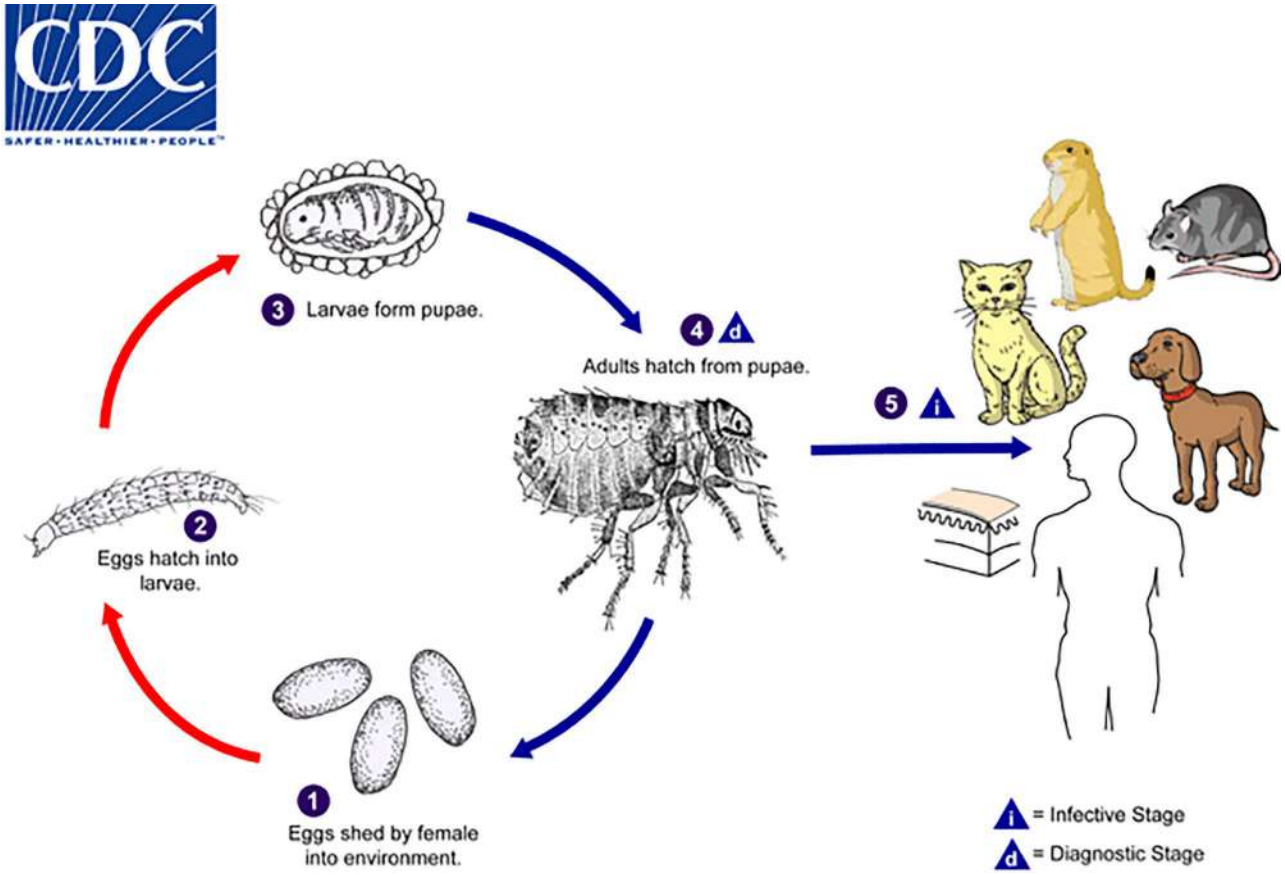

Figure 16. General flea life cycle. Fleas, like other holometabolous insects, have a 4-part life cycle consisting of eggs, larvae, pupae, and adults. Eggs are shed by the female in the enviroment (1). Eggs hatch into larvae (2) in about 3–4 days and feed on organic debris in the environment. The number of larval instars varies among the species. Larvae eventually form pupae (3), which are in cocoons that are often covered with debris from the environment (sand, pebbles, etc.). The larval and pupal stages take about 3–4 weeks to complete. Afterwards, adults hatch from pupae (4) and seek out a warm-blooded host for blood meals. The primary hosts for *Ctenocephalides felis* and *C. canis* are cats and dogs, respectively, although other mammals, including humans, may be fed upon. The primary hosts for *Xenopsylla cheopis* are rodents, especially rats. In North America, plague (*Yersinia pestis*) is cycled between *X. cheopis* and prairie dogs. Humans are the primary host for *Pulex irritans.* The chigoe flea (*Tunga penetrans*) has a different life cycle and is discussed above. Source: Division of Parasitic Diseases and Malaria, United States Centers for Disease Control and Prevention, 2017. Public domain.

Tunga penetrans (the life cycle of which is described above; see also Figure 15) that parasitizes humans, domestic animals, and wildlife in tropical areas. Tungiasis is a zoonosis which causes severe complications like deformation of digits, loss of toenails, tetanus, gangrene, and superficial lacerations prone to opportunistic infections. *Ctenocephalides canis* and *C. felis* (family Pulicidae) are intermediate hosts of helminths, such as *Dipylidium caninum* and *Hymenolepis diminuta*, respectively, parasites of carnivores and rats. The larvae of fleas ingest cestode eggs, and when the adult flea metamorphoses into an adult, the cestode cysticercoid transfers to the adult. These tapeworms can develop in humans if they inadvertently ingest an infected flea. In addition, fleas act as vectors for several disease-causing organisms, including bubonic plague (*Yersinia pestis*), murine typhus (*Rickettsia typhi*), among other species of pathogenic bacteria such as those from the genera *Bartonella* and *Rickettsia*, as well as viruses. In recent years, the flea-borne spotted fever agent *Rickettsia felis* has emerged and can be found throughout the world. Fleas have also been proven to harbor, and sometimes transmit, *Bartonella henselae*, the agent of cat-scratch disease. Flea-borne organisms are widely distributed throughout the world in endemic disease foci, where components of the enzootic cycle are present. However, flea-borne diseases may re-emerge in epidemic form because of changes in vector-host ecology due to environmental and human behavior modifications (Bitam et al., 2010; Tsai et al., 2011; Gutiérrez et al., 2015; Linardi, 2017; Abreu Yanes et al., 2018; Whiting et al., 2008).

Fleas of Medical Importance

Some species are notable for a variety of reasons. For instance, *Xenopsylla cheopis* (Figure 15) is perhaps the most notorious flea because it is the vector of the bacterium *Yersinia pestis* which causes both pneumonic and bubonic plague in humans. The plague produces an inflammation of the lymph nodes, in severe cases causing the rupture of these lymph nodes. It is fatal in almost 50% of untreated cases. Fleas contaminate by sucking infected blood from a rodent and the bacterium multiply to the point of clogging the proventriculus. When the flea returns to feed, the blood does not enter the digestive system and the contaminated blood is regurgitated at the point of the bite. *Xenopsylla cheopis* parasitizes not only rodents, but other vertebrates including humans and it is also a vector of murine typhus caused by *Rickettsia mooseri*. Transmission takes place due to the flea bite or by the contamination of wounds in the skin by the flea's feces. Primary pneumonia and primary septicemia may also ensue from interactions with infected fleas (Linardi and Guimarães, 2000; Krasnov, 2008; Linardi, 2017).

Pulex irritans (Pulicidae) (Figure 1), called the human flea since it was first described from a human, has been the most studied species within the genus *Pulex*. *Pulex irritans* has been confused with similar species for years, but recently characters of diagnostic importance to identify it have been reported. There is evidence of a long relationship between *P. irritans* and humans. Currently, *P. irritans* has cosmopolitan distribution, probably due to human transportation, but species in the genus *Pulex* appears to be Central American to South American in origin, where several congeners are known. Although this flea is presently relatively promiscuous, initial evolution is likely to have involved a single host, probably a peccary, closely associated with humans. Currently, a variety of mammals are known to serve as hosts of *P. irritans* and because of its close association with domestic mammals such as pigs and dogs, *P. irritans* can also bite humans, causing dermatitis. *Pulex irritans* is also well-able to transmit several zoonotic pathogens, includingthe flea-borne spotted-fever rickettsiosis, and it has been important in transmitting *Yersinia pestis* from human to human, and possibly from domestic animals to humans (Hopla, 1980; Marshall, 1981; Buckland and Sadler, 1989; Brouqui and Raoult, 2006; Lareschi et al., 2018).

Within the order Siphonaptera, species of the genus *Tunga* are particularly unique due to their biology and morphology. These fleas have the capacity to perforate the skin of their hosts by using their mouthparts and they all present neosomy. With the exception of *T. penetrans*, the remaining species are parasites of wild mammals, most of them rodents and armadillos (Linardi and Guimarães, 2000; Whiting et al., 2008; Pampiglione et al., 2009; De Avelar, 2012). Females of *T. perforans* are unique in perforating the osteoderms of their armadillo hosts and living inside the carapace. Osteoderms, or bony dermal scutes, are compact and are overlaid by epidermal horny scales which form a protective dorsal cover (carapace) of armadillos. Thus, these fleas have specialized mechanisms to perforate the thin skin between these plates (Ezquiaga et al., 2014). Additionally, osteoderms of piche armadillos (*Zaedyus pichiy*) with holes produced by *Tunga* were recovered at the archaeological shell midden called Las Hormigas, on the northern coast of the province of Santa Cruz in the Argentinean Patagonia (Hammond et al., 2014).

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Arthropoda

Phthiraptera (Order): Lice

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Phylum Arthropoda

Class Insecta

Order Phthiraptera

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Chapter 64

Phthiraptera (Order): Lice

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Introduction

Parasitic lice (superorder Psocodea, order Phthiraptera; also known as true lice, or lice, singular: louse) constitute the largest insect taxon (with about 5,000 known species) of permanent and obligate parasites. The taxon is subdivided into 4 suborders: Amblycera, Ischnocera, Rhynchophthirina, and Anoplura (Johnson and Clayton, 2003).

Morphology

Lice are secondarily wingless (this means that their ancestors had wings, but the current forms of lice have no wings) ectoparasites having a dorsoventrally flattened head and (to a lesser extent) flattened body. They possess reduced compound eyes (or may be eyeless), have no ocelli, and their mouthparts are either mandibulate (with mandibles for chewing) or modified for piercing the host skin and sucking blood (with stylets). The labial palps are reduced and the antennae have 3–5 segments and are either recessed into the head (as in Amblycera), filiform (as in Ischnocera), or short (as in Anoplura). The first thoracic segment is usually free, while the second and third segments may be partially fused. Their legs are relatively short and stout, the tarsi have 1 or 2 segments, and are equipped with a single or paired pretarsal claws. The tibio-tarsal claws of Anoplura are adpated for grasping host hairs. The abdomen comprises 8–11 visible segments with no cerci. The coloration of lice may vary, including shades of black, gray, brown, yellow, or white, often more-or-less matching the host's pelage or plumage (Bush et al., 2010).

Lice are small-bodied insects (adults 0.35–11 mm-long) with their body size covarying with the host's body size in at least 2 ways (Harnos et al., 2017). First, species of hosts with larger body sizes tend to harbor species of lice that also have larger body sizes (Harrison, 1915) and, second, hosts with larger body sizes also tend to harbor species of lice with more variable body sizes (Poulin, 2007). Practically, this means that only small lice can parasitize small hosts, while both small and large lice (thus, on average larger) species may occur on large-bodied host species. The optimal body size of a species of louse is a compromise between 2 opposing selection pressures; host defenses may select for smaller body size, and fertility selects for larger body size (Villa et al., 2018b). If invading markedly different-sized hosts, these selection pressures can result in different-sized louse populations with reproductive isolation emerging between them due to size incompatibility during copulation which can be considered a pre-mating isolating mechanism (Villa et al., 2018a). It is worth noting that practically all body size data on lice refer to slide-mounted individuals flattened essentially into 2-dimensions by force (Palma, 1978) so any morphometric evaluations need to take this into consideration.

Feeding

Amblyceran lice mostly consume dead fragments or living tissues of the host skin, and also partially feed on blood and other excretions. In contrast, ischnoceran lice mostly feed on non-living tissues, such as skin fragments and the fluffy microstructures of feathers (Johnson and Clayton, 2003). To a lesser extent, both of these taxa may also predate on ectoparasitic mites (Oniki and Butler, 1989; Valim, 2006) and other lice, including cannibalizing members of their own species (Nelson, 1971). Living in a relatively dry environment (such as the host's plumage or pelage), they possess sclerites between the mouthparts specialized for water vapor uptake from the air (Rudolph, 1982). Some amblycerans even drink the eye fluids of the host (Mey et al., 2006). Members of Rhynchophthirina and Anoplura lice feed exclusively on mammal blood (Durden, 2019).

In species that feed on non-living tissues like feathers, endosymbiotic bacteria belonging to the phylum Proteobacteria help digest the keratin and supply vitamins and other trace nutrients to the host. These symbionts are maternally transmitted through the oocytes and inhabit specialized cells, called bacteriocytes in the body cavity of lice (Fukatsu et al., 2007). Further, the diverse microbial community of *Acinetobacter* and *Staphylococcus* species may often accompany them (Reed and Hafner, 2002). Blood-sucking lice also carry mutualistic *Rickettsia*-like bacteria that supply lice with vitamin B and cofactor biosynthesis (Perotti et al., 2009; Rio et al., 2016).

Host Range

Most louse species are known to parasitize only 1 or very few closely related host species. Although the known range of host species may often be underestimated due to sampling bias (more sampling in countries with higher income, etc.) (Poulin, 1992), lice seem to have a more narrow host-range relative to other major taxa of ectoparasitic arthropods. A few species (or morphospecies, like *Menacanthus eurysternus*) appear to be more generalists, parasitizing several host taxa. These species may involve morphologically similar but genetically distinct species that are sometimes called cryptic species in light of the fact that they appear morphologically similar but are genetically divergent.

Host Distribution

Practically all avian families host several genera of lice (up to 20 in the family Tinamidae). Only a very few species-poor bird families (including the families Balaenicipitidae, Rhynochetidae, Picathartidae, and Todidae) are not yet known to host any lice (Price et al., 2003), probably due to inadequate research intensity. Contrarily, their occurrence is much less diverse and less prevalent on mammals. Some major taxa of mammals, such as the monotremes, pangolins (order Pholidota), bats (order Chiroptera), sea cows or sirenians (order Sirenia), tapirs (family Tapiridae), rhinoceroses (family Rhinocerotidae), and the clade Whippomorpha (which includes whales, dolphins, and hippopotamuses) are free of lice (Durden and Musser, 1994).

Lice always inhabit the integumentary structures of the outer surface of their hosts, the plumage of birds or the pelage of mammals. Only a very few taxa may slightly shift toward endoparasitism, such as *Piagetiella peralis*, which occurs inside the pouch of pelicans, or *Somaphantus lusius* and *Rediella mirabilis*, that may live within the quill (calamus) of feathers.

Life Cycle

The vast majority of lice species reproduce sexually, very few are parthenogenetic. They exhibit a hemimetabolous life cycle with all developmental phases completed on the host body surface. Their eggs, often called nits, are glued firmly to the hairs or feathers. After hatching, the nymphs develop through 3 nymphal stages to reach the adult stage (note that, being hemimetabolous insects, lice do not include a larval stage; their immature stages are called nymphs). The morphology of the nymphs resembles that of the adults, although it is much simplified, especially in chaetotaxy (that is, the arrangement of the bristles).

Sex-ratios are often female-biased in lice, or close to equal, and are rarely male-biased. Male bias may occur in host individuals with high intensity infestations (Rózsa, 1997a) or in host populations that carry highly prevalent infestations (Rózsa et al., 1996; Pap et al., 2012), where multiple infestations are more likely to occur. In contrast, female-biased sex-ratios characterize scarce infestations, for example, on the peripheries of the geographic distribution (Rózsa et al., 2015) where multiple infestations are rare and thus inbreeding may be strong.

Macroecology

From a macroecological point of view, the distribution, abundance, and richness of lice is very much determined by the host characteristics. The most prominent effect is traditionally called Eichler's rule, a hypothesis that predicts a positive covariation between host diversity and parasite diversity (Eichler, 1942; Vas et al., 2012). Past bottlenecks in host population size often result in long-lasting reductions of louse species richness; this is why birds introduced from Europe to New Zealand harbor fewer species than the same species in Europe (Paterson et al., 1999; MacLeod et al., 2010). In comparisons across species, large-bodied hosts tend to harbor more individuals than smaller ones (Rózsa, 1997b). Colonial host species, living a more social life, do not harbor more lice but the same number of parasites are distributed in a less aggregated (less biased) way than in territorial breeders (Rózsa et al., 1996; Rékási et al., 1997). Bird and mammal species that dive under water to feed tend to host species-poor communities of lice as compared to sister clades (Felső and Rózsa, 2006; 2007).

Transmission

Lice almost exclusively transfer from host to host through bodily contacts between conspecific hosts. Parent-offspring contacts that enable vertical transmission of lice are particularly important for many species. In birds, the evolutionary transitions to brood parasitism caused the loss of this transmission route and, consequently, all brood parasitic clades (for example, cuckoos) host poorer louse communities than their sister clades (Vas et al., 2013). Horizontal transmission often relies on sexual contacts (Hillgarth, 1996), aggression, or other bodily contacts between conspecifics. Some ischnoceran lice often attach to hippoboscid flies for transmission, a phenomenon called phoresy (Keirans, 1975). This is a secondary route of transmission, more often exhibited when the host is diseased or dying, and it likely plays a prominent role in creating non-specific infestations that may accidentally result in host-switches (Harbison et al., 2009).

Effects on Hosts and Role as Vectors

Although most infestations are symptomless, lice may

Figure 1. A lateral view of a female body louse *Pediculus humanus* var. *capitis* as it was obtaining a blood-meal from a human volunteer, who in this case, happened to be the photographer (J. Gathany). Note its elongated abdominal region without any processes and 3 pairs of legs, which are all equal in length and width, features displayed by *Pediculus* members. Source: J. Gathany and F. Collins, 2006. Public domain.

reduce host life expectancy in severe infestations (Brown et al., 1995), reduce avian thermoregulation (Booth et al., 1993), and decrease the sexual attractiveness of their hosts (Clayton, 1990). Lice also play a vector role for several other infections, including *Pediculus humanus humanus* (see Figure 1), transmitting at least 3 potentially lethal human bacterial infections (Raoult and Roux, 1999). Amblyceran and ischnoceran lice may also play a vector role in domestic and wild animals (Clayton et al., 2008), such as the species *Trinoton anserinum* that transmits filarioid juveniles of the heartworm of geese and swans (*Sarconema eurycerca*) (Seegar et al., 1976).

Severe infestations of chewing lice may cause irritation, resulting in restlessness and a loss of sleep. In case of extreme infestations, skin lesions may arise that become the site of secondary infections (Durden, 2019). This is not at all typical in the wild, where most infestations are practically symptomless. In domestic animals, however, such effects may incur losses of millions of US dollars (Kunz et al., 1991) to the poultry, dairy, and leather industries through the decline of egg, milk, meat, and leather production (Durden, 2019).

Host Defenses

Birds and mammals exhibit a variety of immunological, physiological, or behavioral defenses against lice (Clayton et al., 2010; Bush and Clayton, 2018). Grooming behavior, such as preening by the bill and scratching by the legs in birds, as well as scratching by the legs and oral grooming (the alternate use of both teeth and tongue) in mammals, plays a predominant role in defense against lice. Experimentally, impaired grooming not only triggers a dramatic increase in louse populations, but also increases their body size–indicating that preening exerts a strong selection pressure for small body sizes (Murray, 1987; Clayton et al., 1999). Lice exhibit morphological adaptations to resist grooming such as the tibio-tarsal claws of anoplurans and the mandibles of ischnocerans enabling a strong attachment to the hair or feather of hosts. Since birds rely on the visual detection of lice during preening, lice can evolve a camouflage coloration in response to host-imposed selection (Bush et al., 2010). On the other hand, hosts evolve adaptations to improve the efficacy of grooming. Thus the minor bill overhang on the upper mandible of several birds (Clayton et al., 2005), the pectinate claws of barn owls (Bush et al., 2012), the grooming claws (or toilet-claws) of prosimians (Soligo and Müller 1999), or the laterally mobile lower incisors (acting like tweezers) of house mice (Murray, 1987), all exemplify morphological adaptations of hosts.

Blood-sucking insects inject saliva into the wound created by their piercing mouthparts, which contains proteins that manipulate capillary blood flow and suppress host defensive responses. Such proteins provoke immune responses against anopluran lice (Mumcuoglu et al., 1997; Lehane, 2005; Rózsa and Apari, 2012) and apparently also against amblycerans (Møller and Rózsa, 2005) that feed on blood, at least partially.

Birds possess uropygial glands on the rump that secrete a sort of preening oil, and they spread this secretion throughout the plumage during preening. Experimental studies could not unambiguously verify the antiparasitic effect of preen oils in rock pigeons (Moyer et al., 2003); however, comparative studies have shown that the relative size of avian uropygial glands coevolve with the richness of amblyceran lice (Møller et al., 2010).

Contrary to conventional wisdom (see, for example, Post and Enders, 1970), molting does not reduce louse burdens in avian hosts (Moyer et al., 2002), most likely because feather lice (just like feather mites; Pap et al., 2006) avoid adjacent feathers.

Conservation

The human-induced size decline and fragmentation of several host populations necessarily drives many parasite species to extinction due to random population fluctuations (Rózsa, 1992). In spite of this, conservation biologists rarely consider issues about conserving parasite biodiversity (but see Whiteman and Parker, 2005; Tydecks et al., 2018), and this extinction crisis is mostly undocumented (Koh et al., 2004).

At least 6 species of lice (Table 2) are classified as co-extinct, that is, they were specific exclusively to hosts that already went extinct and an additional 40–41 species are

known to be critically co-endangered, parasitizing critically endangered hosts exclusively. More surprisingly, 4 louse species apparently have gone extinct due to purposeful conservation efforts, specifically, due to the administration of veterinary antiparasitic treatments during captive-breeding and translocation efforts to save endangered hosts (Table 2) (Rózsa and Vas, 2015).

Conversely, some apparently "extinct lice" anecdotes that are widespread in the conservation literature have never been verified. Thus, *Columbicola extinctus* did not go extinct with *Ectopistes migratorius* (the passenger pigeon), because it was also parasitizing *Patagioenas fasciata* (band-tailed pigeon), a bird that is still extant (Clayton and Price, 1999). *Campanulotes defectus* also did not go extinct with passenger pigeons (Price et al., 2000) as was formerly concluded from an erroneous host record. Similarly, the black-footed ferret (*Mustela nigripes*) did not host a separate species of trichodectid louse (Emerson, 1964); thus, it was not extirpated by conservationists, as had been suggested (Gompper and Williams, 1998).

Origins

Lice are phylogenetically embedded within bark lice (superorder Psocodea, order Psocoptera, suborder Troctomorpha, family Liposcelididae (or Liposcelidae)) (Lyal, 1985; Yoshizawa and Johnson, 2003; 2010; Johnson et al., 2004). Free-living bark lice are small-bodied, often wingless insects feeding on fungi, algae, and organic debris. They are not parasitic, although several species inhabit the nests of birds or mammals, including human habitations. They also feed on materials shed from mammals or birds, such as dead skin, loose hair, or feathers, and may even accidentally end up on the pelage or plumage of these animals. This nest-dwelling commensal way of life likely served as a pre-adaptation to the evolutionary shift to ectoparasitism, an event considered as a key innovation that gave rise to the original parasitic lice. Accordingly, from a taxonomic point of view, the order of bark lice is a paraphyletic taxon with respect to parasitic lice.

An early molecular phylogenetic study suggested 2 parallel switches to parasitism and thus the polyphyly of the order of parasitic lice (Johnson et al., 2004). However, more detailed subsequent analyses failed to unambiguously support this hypothesis (Yoshizawa and Johnson, 2010) and later transcriptome data reject the double origin hypothesis in favor of a single origin (Johnson et al., 2018a). The single shift to parasitism might have occurred in relation to mammal, bird, or possibly some reptile hosts (like feathered theropod dinosaurs or haired pterosaurs). The earliest known fossil representing this order is an avian louse (*Megamenopon rasnitsyni*) that dates back to only 44 Ma (= million years ago) (Wappler et al., 2004). Since parasites fossilize poorly (Leung, 2017), the actual switch to parasitism might have occurred much earlier. The major louse suborders radiated before the Cretaceous–Paleogene (K–Pg) boundary 66–65 Ma (Smith et al., 2011) and they further diversified after this boundary (Johnson et al., 2018a; 2018b).

Studies dating the origin and earliest divergences within lice have varied extensively. Using molecular data of a few mitochondrial and nuclear genes, Light and colleagues (2010) estimated the origin of the suborder Anoplura to 75 Ma, with a 95% certainty ("highest posterior density") interval 96–58 Ma.

Table 1: Anopluran lice of main veterinary importance. Adapted from Durden, 2019.

Common name	Scientific name	Host
Horse louse	*Haematopinus asini*	Equids
Short-nosed louse	*Haematopinus eurysternus*	Cattle
Cattle tail louse	*Haematopinus uadripertusus*	Cattle
Hog louse	*Haematopinus suis*	Swine
Buffalo louse	*Haematopinus uberculatus*	Asiatic buffalo, cattle
	Hoplopleura capitosa	House mice
Tropical rat louse	*Hoplopleura pacifica*	Domestic rats
African blue louse	*Linognathus africanus*	Deer, sheep, goats
Sheep face louse	*Linognathus ovillus*	Sheep
Sheep foot louse	*Linognathus pedalis*	Sheep
Dog sucking louse	*Linognathus setosus*	Canids
Goat sucking louse	*Linognathus stenopsis*	Goats
Long-nosed louse	*Linognathus vituli*	Cattle
Little blue cattle louse	*Solenopotes capillatus*	Cattle

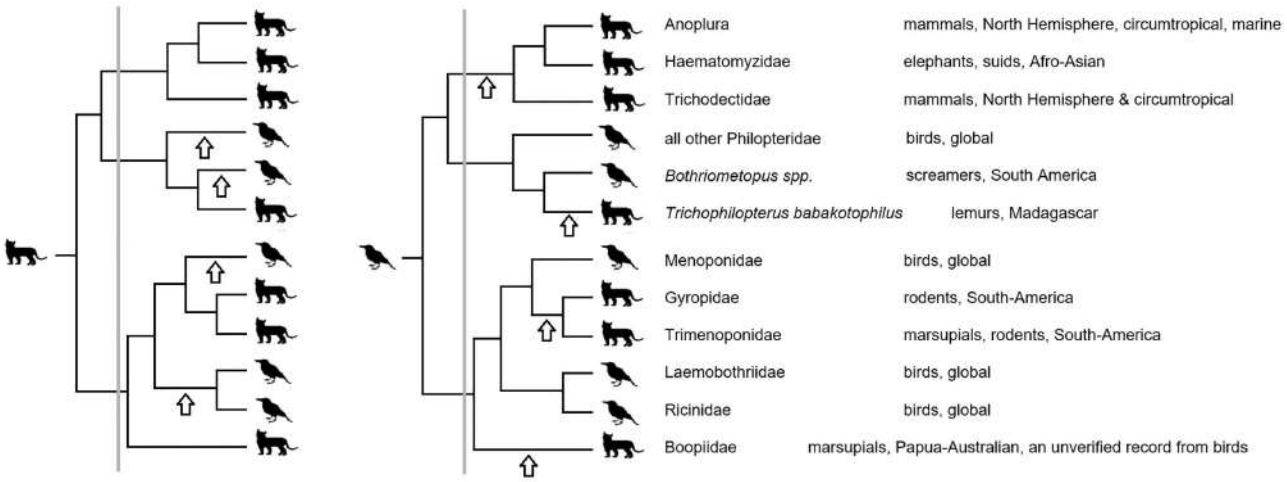

Figure 2. The most parsimonious scenarios for the major host-switches between mammals and birds illustrated along a dendogram representing a simplified phylogeny of lice. The minimally required major switches are indicated by arrows. The left scenario is based on the presumption that lice originate from a mammal host archetype, the right one is presuming that lice originate from a bird archetype. The vertical gray lines represent the Cretaceous/Paleogene (K–Pg) boundary, but otherwise the graph is not drawn to scale. Source: Adapted from Johnson et al., 2018. License: CC BY-NC-SA 4.0.

More recently, Misof and colleagues (2014) based a phylogenomic analysis on a much greater gene sampling and concluded that parasitic lice began diverging about 53 Ma, well after the emergence of their bird and mammal hosts. However, a similar analysis with many additional taxa (Johnson et al., 2018b) put this date at 171 Ma, while an analysis of genomes (Johnson et al., 2018a) places it at 93 Ma. It is worth noting that the 95% confidence intervals of many of these estimates overlap. In general, it can be reasonably assumed that liposcelid ancestors most probably switched to a parasitic way of life and thus gave rise to the order of parasitic lice sometime during the middle or late Cretaceous, possibly well after the rise of mammals or birds.

Phylogeny

Presuming that their present-day host-range also holds for ancestral lineages, it is expected that the phylogeny of lice should mirror the host phylogeny due to co-speciation events (Fahrenholz, 1913; Hafner and Nadler, 1988). However, the similarity between the 2 trees more often does not exceed the level of similarity expected by chance (see, for example, Weckstein, 2004). This is because other evolutionary events, like parasite extinction or host switching, often eliminate similarity between the 2 trees. Ecological fitting (also known as host switching) is relatively common between closely related and morphologically similar potential host species. In contrast, host switches between taxonomically distant and anatomically dissimilar hosts are very unlikely. However, the likely monophyletic origin and present host-distribution of parasitic lice necessitates at least a few relatively major switches that must have occurred between birds and mammals (Johnson et al., 2018b). Figure 2 illustrates the most parsimonious scenarios of these major switches.

Lice Nuclear Genome

The nuclear genome of lice is the smallest known in any insects, suggesting that the parasitic way of life greatly reduced the size of its genome (Pittendrigh et al., 2006; Kirkness et al., 2010); this could be tested by looking at the genome of the closest relatives of the parasitic lice. The mitochondrial genome structure is extremely variable and complex due to RNA and protein coding gene rearrangements and, particularly in mammal lice, due to subdivision into multiple minichromosomes, and the splits and mergers of these minichromosomes (Cameron et al., 2011; Shao et al., 2017; Yoshizawa et al., 2018; Song et al., 2019). Further, the human head and body louse (*Pediculus humanus*) exhibits an unusual form of meiotic drive, in which the males transmit preferentially or exclusively only their maternally-derived chromosomes (de la Filia et al., 2018). Yoshizawa and Johnson (2013) concluded that selection is more relaxed on phthirapterans and a closely related clade of free-living bark lice than on other comparable bark lice taxa, yielding a more random base composition for both the mitochondrial and nuclear genes. Overall, the inheritance characteristics of louse genomes exhibits a set of unusual and surprising molecular evolutionary processes that often confounds molecular phylogenetic analysis.

Taxonomic Classification

In traditional classifications created for the lice by systematists, these parasites were typically divided into 2 orders according to their different mouthparts, that is, the old names: chewing lice (Mallophaga) and sucking lice (Anoplura). This was practical from a veterinary point of view, but did not reflect their true phylogenetic relationships. In fact, anopluran lice are phylogenetically embedded within a group of chewing lice, the suborder Ischnocera. The numbers of known species given below are only approximate; inconsistencies may arise due to the different species concepts applied by different authors (Mey, 2003).

Suborder Amblycera

Most amblycerans possess heavily sclerotized chewing mandibles forming relatively unspecialized mouthparts, although some taxa partially feed on host blood. Their body size is variable, with adult body length ranging from 1.0 to 11.0 mm.

Family Boopiidae

The 55 extant species of boopiid lice parasitize Australian and New Guinean marsupials. There is an unverified record of a single species, *Therodoxus oweni*, possibly parasitizing a bird species, the New Guinean southern cassowary (*Casuarius casuarius*) (Clay, 1971). *Heterodoxus spiniger*, the louse of the agile wallaby (*Macropus agilis*) in North Australia has secondarily switched to the domestic dog probably in historical times, and achieved a circumtropical distribution mostly on canids and, to a lesser extent, also on other carnivores.

Family Ricinidae

Approximately 110 species of ricinid lice parasitize hummingbirds (family Trochilidae) and small-bodied passerines (order Passeriformes), occurring more scarcely on some medium-sized passerines (perching birds) like thrushes (*Turdus* spp.) and Old World orioles (*Oriolus* spp.). Their adult body size is about 1.6–5.4 mm, relatively large for the small-sized hosts. Prevalence and infestation intensity is typically lower than in menoponid and philopterid lice. Chewing mouthparts are more-or-less modified for piercing the host's skin to enable feeding from a pool of blood caused by tissue laceration (Clay, 1949).

Family Laemobothriidae

This is a small family (20 species) of very large lice, with adult body length ranging between 5.7 and 11.0 mm. Like members of family Ricinidae, *Laemobothrion* spp. lice are also telmophagous (meaning, blood pool feeders). Their host range is more broad compared to other species of lice classified in other families. *Laemobothrion tinnunculi* is widespread on falcons (*Falco* spp.), *L. maximum* on several diurnal raptors (*Accipiter* spp., *Aquila* spp., *Buteo* spp., and *Circus* spp.), and *L. vulturis* on Old World vultures (*Aegypius* spp., *Gyps* spp., etc.) and eagles (*Aquila* spp.). A few more species, forming a separate clade, parasitize mostly moorhens (rails) and coots (order Gruiformes: family Rallidae).

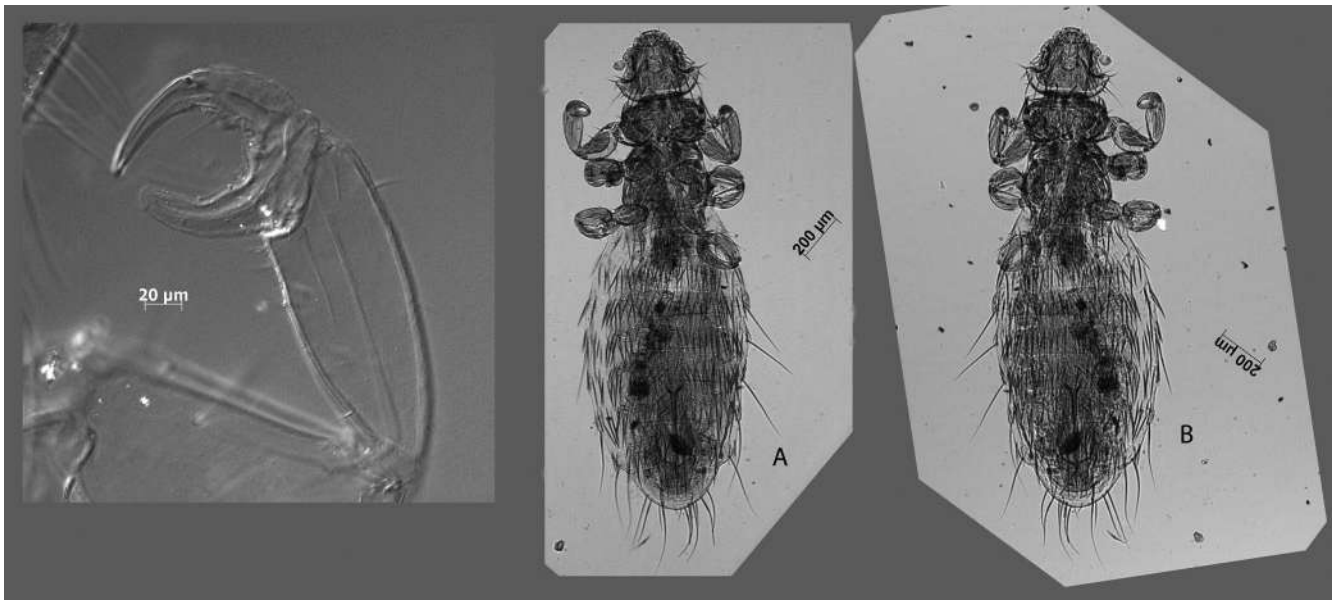

Figure 3. Chewing lice, genus *Phtheiropoios* from rodents of the genus *Ctenomys* collected in Bolivia in the 1980s. Source: S. L. Gardner, HWML. License: CC BY.

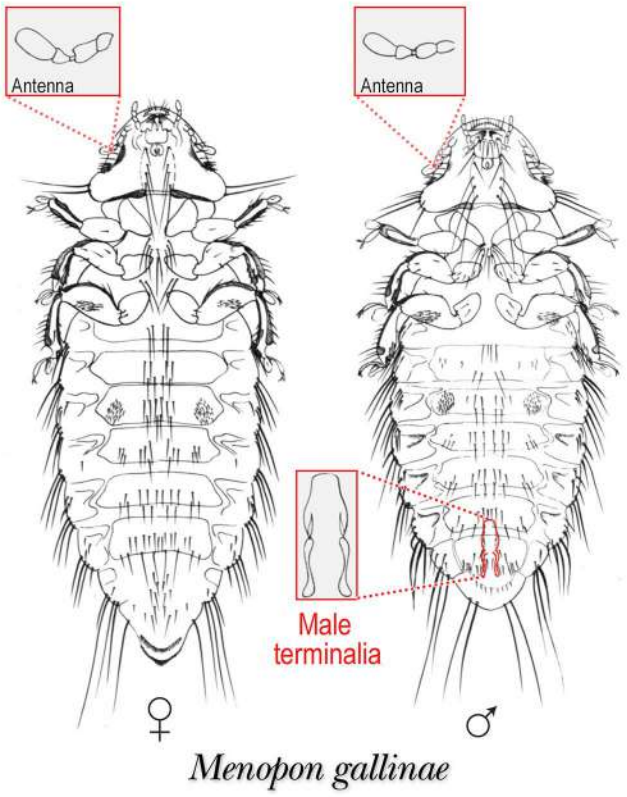

Figure 4. A female and a male *Menopon gallinae* lice revealing the insect's ventral morphology. Source: United States Centers for Disease Control and Prevention, 1975, available at the Public Health Image Library, image 5496. Public domain.

Family Trimenoponidae

Only 18 species constitute this family that parasitize rodents in South America and Central America. *Trimenopon hispidum* is known in veterinary practices as a parasite of the domestic guinea pig (*Cavia porcellus*).

Family Gyropidae

Fewer than 100 species parasitize South American and Central American rodents, with the families of guinea pigs (Caviidae) and degus (Octodontidae) being the most preferred hosts (Figure 3). Only 1 species, *Macrogyropus dicotylis,* is hosted by peccaries (family Tayassuidae). *Gyropus ovalis* and *Gliricola porcelli* are both globally widespread on domestic guinea pigs.

Family Menoponidae

Menoponids occur exclusively on birds, constituting 1 of the 2 most species-rich (> 1,050 species), most prevalent, and abundant families of avian lice (the other being Philopteridae). Several genera are known to feed partially on blood and are capable of causing economic harm to the poultry industry (for example, *Menacanthus cornutus*, *Menopon gallinae* (Figure 4), and *Trinoton querquedulae*) (see, for example, Saxena et al., 1985; 2004; Sychra et al., 2008; Mullens et al., 2010; Kumar and Kumar, 2016; Kumar et al., 2017). The diversity of species in this group appears to be correlated with host defensive capabilities, like T-cell immune responses (Møller and Rózsa, 2005) and uropygial gland size (Møller et al., 2010).

Suborder Ischnocera

The majority of Ischnoceran lice inhabit avian plumage, and only a minority of them live in the mammalian pelage.

Family Philopteridae

Philopterids occur (almost) exclusively on birds. They constitute 1 of the 2 most species-rich (around 2,750 species), most prevalent, and abundant families of avian lice (the other being Menoponidae). However, one species, *Trichophilopterus babakotophilus*, parasitizes lemurs in Madagascar. Philopterids evidently feed on non-living tissues, and when on birds, they most often are found grazing like tiny cows, on the tiny barbs and barbules of plume feathers and on non-living skin fragments. There is little evidence of cospeciation in this group and studies have shown no correlation with speciation and host physiological defenses like a T-cell immune response (Møller and Rózsa, 2005) or uropygial gland size (Møller et al., 2010). On the contrary, they appear to be more strictly affected by mechanical defenses, and preening in particular. To evade preening pressure, it appears that philopterids have evolved morphological adaptations (shape, size, and color) to particular parts of the plumage, and even to major types of feathers.

The shape variability of philopterids is approximately described by applying the guild or ecomorph concepts of ecology. Accordingly, the so-termed body lice, generalist lice, head lice, and wing lice guilds are distinguished. These categories do not represent monophyletic groups but share distinct morphological and behavioral characteristics that have evolved repeatedly along parallel and independent lineages. As indicated by their names, they exhibit characteristically different specificities to particular areas of the host body surface (Johnson et al., 2012; Bush et al., 2016; Clayton et al., 2016). Overall, anatomical site specificity and site segregation appear to be even more pronounced in this group than in other taxa of lice. For example, head lice and wing lice often attach themselves firmly to feather surfaces using their strong mandibles.

The phylogeny of philopterids has not yet been studied in detail, and their systematics is somewhat controversial.

Table 2. Amblyceran and Ischnoceran lice of economical and veterinary importance. Adapted from Durden, 2019.

Vernacular name	Scientific name	Host
Dog louse	*Heterodoxus spiniger*	Dog, other carnivores
Chicken body louse	*Menacanthus stramineus*	Domestic fowl
Domestic fowl Shaft louse	*Menopon gallinae*	Domestic fowl
Goose body louse	*Trinoton anserinum*	Goose
Large duck louse	*T. querquedulae*	Duck
Slender goose louse	*Anaticola anseris*	Goose
Slender duck louse	*A. crassicornis*	Duck
Large turkey louse	*Chelopistes meleagridis*	Turkey
Chicken head louse	*Cuclotogaster heterographus*	Domestic fowl
Fluff louse	*Goniocotes gallinae*	Domestic fowl
Brown chicken louse	*Goniodes dissimilis*	Chicken
Large chicken louse	*Goniodes gigas*	Domestic fowl
Wing louse	*Lipeurus caponis*	Domestic fowl
Slender turkey louse	*Oxylipeurus polytrapezius*	Turkey
Cattle biting louse	*Bovicola bovis*	Cattle
Goat biting louse	*B. caprae, B. limbata*	Goat
Angora goat biting louse	*B. crassipes*	Goat
Horse biting louse	*B. equi*	Horse
Donkey biting louse	*B. ocellata*	Donkey
Sheep biting louse	*B. ovis*	Sheep
Cat biting louse	*Felicola subrostrata*	Cat
Dog biting louse	*Trichodectes canis*	Dog, other canids

Smith (2000) proposed family rank for Heptapsogasteridae and Goniodidae, two putatively basal clades of philopterids that are traditionally included in this family as subfamilies. Both parasitize relatively basal clades of birds. The former is hosted by tinamous (order Tinamiformes) a group of birds that live only in the Neotropical region (from South America, north to the Isthmus of Tehuantepec in Mexico), and the latter is globally widespread on galliform (order Galliformes) birds (such as, turkeys, guinea fowl, and quails) and columbiform (order Columbiformes) birds (such as, pigeons and doves). However, most molecular systematic studies suggest these 2 groups are well embedded within the order Philopteridae (Johnson et al., 2018). Further, the Madagascan lemur louse was also suggested (Cruickshank et al., 2001) to be a representative of a monotypic family ('Trichophilopteridae'), although more recent studies show that it is rather closely related to the genus *Bothriometopus* parasitizing birds, the South American screamers (Anhimidae) (Johnson et al., 2018).

Family Trichodectidae

This family includes around 380 species exhibiting a somewhat erratic distribution across some taxa of mammals. They possess large and heavy mandibles fitted to grasp a hair shaft so as to fix the louse firmly on it. A large proportion of them belong to the genera *Gemyodoecus* and *Thomomydoecus*, within a clade that has undergone an adaptive radiation on North American and Central American pocket gophers (family Geomyidae). This host-parasite system has been serving as a model for cospeciation and coadaptation studies (Hafner and Nadler, 1988; Hafner et al., 1994; Morand et al., 2000) although a recent re-analysis of the data shows that host parasite cospeciation accounts for less than half of the association and there are no data showing recriprocal evolution in these organisms (Brooks et al., 2015). While abundant and species-rich on this particular group of American rodents, they are absent from Old World rodents (Emerson and Price, 1985). Species of several genera parasitize carnivores, hyraxes, and ungulates; some of them (like *Bovicola*) harm domestic mammals, causing considerable economic damage to the dairy and meat industries (Table 2).

Suborder Rhyncophthirina (Elephant and Suid Lice)

The preantennal region of the head bears a long rostrum armed with chewing mandibles, evidently adapted to enable the louse to pierce deeply into the thick skin of the host to feed on the blood pool (telmophagy). The elephant louse (*Haematomyzus elephantis*) is a relatively small-bodied (around 2 mm) parasite of at least 1 species of African elephant (*Loxodonta africana*, the savanna elephant) and also

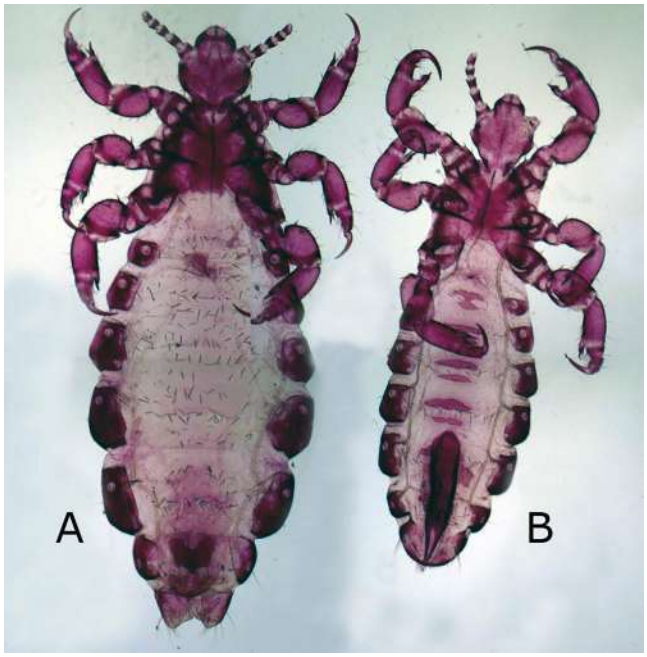

Figure 5. Sucking lice *Pediculus humanus* showing a female (A) and male (B) taken from a human host, preserved in 70% ethanol and stained in Semichon's acetic carmine and mounted in gum Damar. Source: G. Racz, HWML, 2016. License: CC BY.

occurs on *Elephas maximus*, the Asian elephant. It inhabits the hairy regions, and particularly the soft skin folds of the host body, such as the axilla, groin region, ears, neck, and the base of the tail (Sudan et al., 2015). Further, 2 species parasitize African suids (warthogs *Phacochoerus africanus* and *P. aethiopicus* and red river hogs *Potamochoerus porcus*).

Suborder Anoplura: Sucking Lice

Sucking lice occur only on mammals with around 500 known species and are much less diverse than chewing lice. They are more specialized than members of the other groups, but medically their importance and impact on human history are infinitely greater. Two species parasitize humans, *Pediculus humanus* and *Phthirus pubis*, of which *P. humanus* is the more important because it is a vector of rickettesia bacteria. The several species on domestic mammals are of considerable veterinary significance (Light et al., 2010; Kim and Ludwig, 1978).

Morphology

Sucking lice superficially resemble chewing lice, with their small, wingless, flattened bodies, but their heads are narrower than the prothorax. The sucking mouthparts are retracted into the head when the animal is not feeding. Each leg has a single tarsal segment with a large claw, an adaptation for clinging to host hairs. The first legs, with their terminal

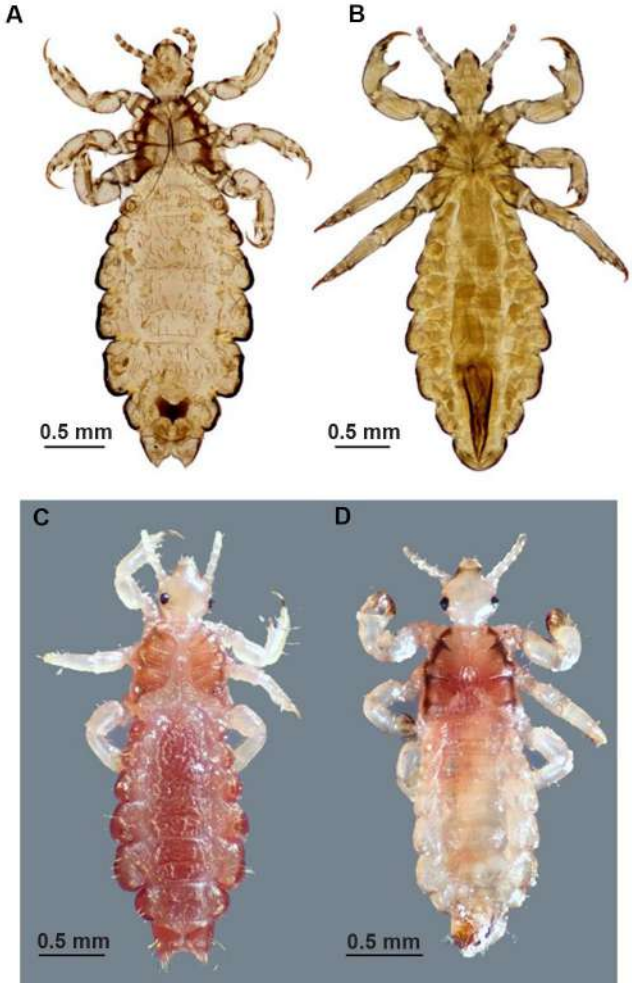

Figure 6. Adult body louse and head lice. A) Ventral view of slide-mounted female head louse; B) ventral view of slide-mounted male body louse; C) dorsal view of ethanol-preserved female head louse; D) dorsal view of ethanol-preserved male head louse. All photographs were taken using a Visionary Digital K2/SC long-distance microscope (from Infinity Photo-Optical Company, Boulder, Colorado, United States). Source: L. Beati, from Bonilla et al., 2013. Public domain.

claws, are often smaller than the other legs, and the third legs and their claws are usually largest. Eyes, if present, are small, and there are no ocelli on the head. Antennae are short, clearly visible, and composed of a scape, a pedicel, and a flagellum that is divided into 3 subsegments. All 3 flagellar subsegments bear tactile hairs, and subsegments 2 and 3 bear chemoreceptors (see Figures 5 and 6) (Bonilla et al., 2013; Slifer and Sekhon, 1980).

Mode of feeding

Lavoipierre (1965) distinguished 2 distinct feeding methods used by bloodsucking arthropods. One of these he termed

solenophagous (Greek for **pipe** + **eating**) for arthropods that introduce their mouthparts directly into a blood vessel to withdraw blood. The other he called telmophagous (Greek for **pool** + **eating**) for those whose mouthparts cut through the skin and blood vessels to produce and feed from a small pool of blood. Anoplurans are true solenophages (Lavoipierre, 1967). Their proboscis is formed from the maxillae, hypopharynx, and labium, which are produced into long, thin stylets.

The ability of lice (and fleas) to transmit prokaryotic pathogens such as louse-borne typhus caused by *Rickettsia prowazekii* may be due to the way in which they digest blood meals. In contrast to mosquitoes, lice hemolyze erythrocytes rapidly, their blood meals remain liquid, and they lack peritrophic membranes.

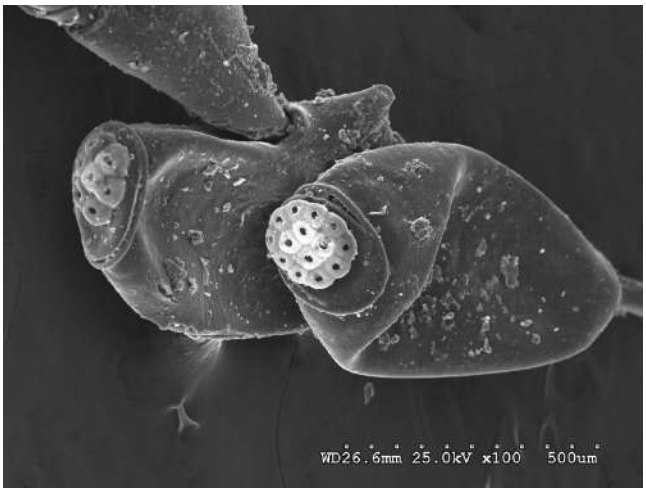

Figure 7. Sucking lice nits (lice eggs) from a mummy. High magnification view of head louse eggs from a South American mummy, 900–1200 CE. Opercula are intact and the pores can be seen. Source: N. Searcey, UNL. License: CC BY.

Pediculus humanus

Two distinct forms of *P. humanus* parasitize humans: Body lice *P. humanus humanus* and head lice *P. humanus capitis*. Body lice also have been called *P. humanus corporis* and *P. humanus vestimenti*. Common names include cooties, graybacks, and mechanized dandruff. The 2 subspecies are difficult to distinguish morphologically, although they have slight differences (see also Johnson, 2022). The subspecies will interbreed and are only slightly interfertile (Askew, 1971). It seems likely that body lice descended from ancestral head lice after humans began wearing clothes. Body lice are much more common in cooler than in warmer parts of the world; in tropical areas people who wear few clothes usually have only head lice (PAHO, 1973). This difference makes typhus a disease of cooler climates because only body lice are vectors. Curiously, however, head lice can serve as hosts for the typhus causing rickettsia and have a high potential for transmitting it (Murray and Torrey, 1975). Body lice are extremely unusual among Anoplura in that they spend most of their time in their host's clothing, visiting the host's body only during feeding. They nevertheless stay close to the body and are most commonly found in areas where clothing is in close contact. Eggs (nits) of body lice are cemented to fibers in clothes and have a cap at one end that admits air and facilitates hatching (Figure 7). Eggs hatch in about a week, and the combined 3 nymphal stages usually require 8–9 days to mature when they are close to a host's body. Lower temperature lengthens the time of a complete cycle; for example, if clothing is removed at night, the life cycle will require 2–4 weeks. If clothing is not worn for several days, the lice will die. A female can lay 9 or 10 eggs per day, up to a total of about 300 eggs in her life; therefore, she has a high reproductive potential. Fortunately, this potential is usually not realized. It is typical to find no more than 10 lice per host, although as many as a thousand have been removed from the clothes of one person (Pratt and Littig, 1973). Body lice normally do not leave their host voluntarily, but their temperature preferences are rather strict. They will depart when a host's body cools after death or if the person has a high fever. Nevertheless, they travel from one host to another fairly easily, and one can acquire them by contact with infested people in crowded locations such as buses, trains, and schools. Of course, they also may be acquired easily by donning infested clothing or occupying bedding recently vacated by a person with lice. Potential for transmission is highest when people are in crowded, institutionalized conditions, such as some prisons, where sanitation is bad and clothing cannot be changed often.

Head lice tend to be somewhat smaller than body lice: 1.0–1.5 mm for males and 1.8–2.0 mm for females, contrasted with 2–3 mm and 2–4 mm for male and female body lice, respectively (Pratt and Littig, 1973). Nits of both are about 0.8 mm × 0.3 mm. Head lice nits cement to hairs. Lice are usually most prevalent on the back of the neck and behind the ears and they do not infest eyebrows and eyelashes. They are easily transmitted by physical contact and stray hairs, even under good sanitary conditions. As in the case of body lice, however, the heaviest infestations are associated with crowded conditions and poor sanitation (Lindsey, 1993).

Infestation with lice (pediculosis) is not life threatening unless the lice carry a disease organism, but it can subject a host to considerable discomfort. The bites cause a red papule to develop that may exude lymph. Intense pruritis induces scratching, which frequently leads to dermatitis and secondary infection. Symptoms may persist for many days in sen-

sitized people. Years of infestation lead to a darkened, thickened skin, a condition at times called vagabond's disease. In untreated cases of head lice the hair becomes matted together from exudate, a fungus grows, and the mass develops a fetid odor. This condition is occasionally known as plica polonica. Large numbers of lice are found under the mat of hair. *Pediculus humanus* carries symbiotic bacteria, including *Wolbachia* sp. (Covacin and Barker, 2007), some endosymbionts occur in mycetomes, and others have been used in coevolutionary studies of primates and their lice (Allen et al., 2007).

Phthirus pubis

Origin of the common name of this insect, crabs, is evident from its appearance. These lice are 1.5–2.0 mm-long and nearly as broad as long, and the grasping tarsi on the 2 larger pairs of their legs are reminiscent of crabs' pincers. *Phthirus pubis* dwells primarily in the pubic region but it may also be found in armpits, and, more rarely, in beards, mustaches, eyebrows, and eyelashes. *Phthirus pubis* is less active than *Pediculus* spp. and it may remain in the same position for some time with its mouthparts inserted in the skin. Bites can cause an intense pruritis but fortunately do not seem to transmit disease organisms.

Nits cement to hair and the complete life cycle requires less than a month. A female deposits only about 30 eggs during her life. Infestation can occur through contact with bedding or other objects especially in crowded situations, but transmission is characteristically venereal.

Sucking lice as vectors of human disease

Three important human diseases are transmitted by *Pediculus humanus humanus*: Epidemic, or louse-borne, typhus; trench fever; and relapsing fever.

Epidemic, or louse-borne, typhus.

Typhus is caused by *Rickettsia prowazekii*. Rickettsias are bacteria that usually are obligate intracellular parasites. Various species can infect vertebrate and/or invertebrate hosts with effects ranging from symptomless to severe. Epidemic typhus has had an enormous impact on human history, detailed in Zinsser's (1934) classic book Rats, Lice and History. Typhus epidemics tend to coincide with conditions favoring heavy and widely prevalent infestations of body lice, such as pre- and postwar situations, crowding, and mass migration. Mortality rates during epidemics may approach 100%. It is not certain which or how many of the great epidemics throughout human history were caused by typhus but in historical accounts of the decimation of the Christian and Moorish armies in Spain during 1489 and 1490, the role of typhus is clear. In 1528 typhus reduced the French army besieging Naples from 25,000 to 4,000, leading to its defeat, the crowning of Charles V of Spain as Holy Roman Emperor, and the dominance of Spain among European powers for more than a century. The Thirty Years' War can be divided epidemiologically into 2 periods: 1618–1630, when the chief scourge was typhus, and 1630–1648, when the major epidemic was plague. Zinsser contends that between 1917 and 1921, there "were no less and probably more than 25 million cases of typhus in the territories controlled by the Soviet Republic, with from 2.5 to 3 million deaths" (Zinsser, 1934).

Typhus starts with a high fever (39.5 °C to 40.0 °C), which continues for about 2 weeks, and causes backache, intense headache, and often bronchitis and bronchopneumonia. There is malaise, vertigo, and loss of appetite, and the face becomes flushed. A petechial rash appears by the fifth or sixth day, first in the armpits and on the flanks and then extending to the chest, abdomen, back, and extremities. The palms, soles, and face are rarely affected (Olson, 2000). After about the second week, fever drops, and profuse sweating begins. At this point, stupor ends with clearing consciousness, which is followed either by convalescence or by an increased involvement of the central nervous system and death. The rash often remains after death, and subdermal hemorrhagic areas frequently appear.

The disease can be treated effectively with broad-spectrum antibiotics of the tetracycline group and chloramphenicol. Also, although prior vaccination with killed *Rickettsia prowazekii* does not result in complete protection, severity of disease is greatly ameliorated in vaccinated individuals.

Typhus also kills lice. When a louse contracts a rickettsial bacterium along with blood from a human host, the organisms invade the louse's gut epithelial cells and multiply so plentifully that cells become distended and rupture. After about 10 days so much damage has been done to the insect's gut that the louse dies. For several days before its demise, however, the louse's feces contain large numbers of rickettsiae. Scratching louse bites or crushing an infected louse inoculates the host human with typhus organisms from the louse's feces.

A louse's strong preference for normal body temperature causes it to leave a febrile patient and search for a new host, thus facilitating spread of the disease in epidemics. A person can also become infected with typhus by inhaling dried louse feces or getting them in the eye. *Rickettsia prowazekii* can remain viable in dried louse feces for as long as 60 days at room temperature (Harwood and James, 1979). Because infection is fatal to lice, transovarial transmission cannot occur, so humans are an important reservoir host.

Brill-Zinsser diease.

After surviving the acute phase of the disease, humans can be asymptomatic but capable of infecting lice for many years. The disease can recrudesce and produce a mild form known as Brill-Zinsser disease. Flying squirrels *Glaucomys volans* also can be a reservoir host with the infection transmitted by lice *Neohaematopinus sciuropteri* and fleas *Orchopeas howardii* (Sonenshine et al., 1978). Some cases in the United States were probably caused by contact with such animals (McDade et al., 1980). Human and possibly the animal reservoirs could provide the source for a new epidemic. As Harwood and James (1979) point out, "Current standards of living in well-developed countries have largely eliminated the disease there, but its cause lies smoldering, ready to erupt quickly and violently under conditions favorable to it."

Interesting facts: Howard Taylor Ricketts was a football player in college who went to medical school where he encountered an influential teacher, became fascinated with microbial disease transmission, and subsequently devoted his life to research. Tragically, both Ricketts and Stanislaus von Prowazek, the pioneers of typhus research, became infected with typhus and died in the course of their work (Roberts et al., 2012).

Relapsing fever.

The third important disease of humans transmitted by body lice is epidemic relapsing fever which is caused by a spirochete, *Borrelia recurrentis*. Mortality is usually low but the fatality rate can reach more than 50% in groups of undernourished people (Pratt and Littig, 1973). Lice pick up bacteria along with their blood meal, and spirochetes penetrate the insect's gut to reach the hemocoel. They multiply in hemolymph but do not invade salivary glands, gonads, or Malpighian tubules. Therefore, transmission is accomplished only when a louse is crushed by host scratching, which releases the spirochetes. Hence, infectious organisms gain entrance through abraded skin, but evidence also indicates that they can penetrate unbroken skin (Butler, 2000; Kahlig et al., 2021). Louse-borne relapsing fever apparently has disappeared from the United States, but scattered foci are in South America, Europe, Africa, and Asia (Harwood and James, 1979). Frequent epidemics occurred in Europe during the 18th and 19th centuries and major epidemics befell Russia, central Europe, and North Africa during and after World Wars I and II. During the war in Vietnam an epidemic occurred in the Democratic People's Republic of Vietnam (PAHO, 1973).

Clinically, louse-borne relapsing fever is indistinguishable from the tick-borne relapsing fevers that are caused by other species of *Borrelia*. After an incubation period of 2–10 days, the victim is struck rather suddenly by headache, dizziness, muscle pain, and a rapidly-developing fever. Transitory rash is common especially around the neck and shoulders and then extending to the chest and abdomen. The patient is severely ill for 4–5 days, when the temperature suddenly falls accompanied by profuse sweating. Considerable improvement is seen for 3–10 days, and then another acute attack occurs. The cycle may be repeated several times in untreated cases. Antibiotic treatment is effective but complicated in this disease by serious systemic reactions to the drugs. Humans are the only reservoirs and epidemics are associated with the same kind of conditions connected with louse-borne typhus epidemics. The diseases often occur together (Roberts et al., 2012).

Control of Lice

A variety of commercial preparations containing insecticides effective against lice are available. Insecticides (permethrin) may be incorporated into hair care products. In one study of 38,160 patients who used a permethrin rinse for 47,578 treatments, the delousing product proved both safe and effective (Andrews et al., 1992). But in a similar study in Israel 14 different antilouse shampoos varied in their ability to kill both lice and eggs (Mumcuoglu and Miller, 1991). An extensive literature review revealed 1% permethrin creme rinse to be the only chemical treatment virtually guaranteeing at least a 90% cure rate (Vander Stichele et al., 1995). However, permethrin resistance has been reported (Mumucuoglu et al., 1995).

Hot air also kills head lice and nits and in one study a single 30-minute treatment at temperatures slightly cooler than a standard hair dryer eradicated the parasites (Goates et al., 2006). Extensive combing and picking helps to reduce numbers of head lice. Ordinary laundering of garments, including dry cleaning of woolen and other fabrics, will help to control body lice. Devices for large-scale treatment of civilian populations, troops, and prisoners by blowing insecticide dust into clothing are effective and have controlled or prevented typhus epidemics.

Lice on pets and domestic animals can be controlled by insecticidal dusts and dips. Ear tags impregnated with cypermethrin (a synthetic pyrethroid) (James et al., 1990) and slow-release moxidectin injected subcutaneously (Webb et al., 1991) have both been used on livestock. However, acquired resistance to cypermethrin has been demonstrated in laboratory studies (Levot and Hughes, 1990). Several commercially available endectocides (primarily ivermectin, doramectin, and avermectin formulations) also are effective, depending on the dose and delivery method (Campbell et al., 2001).

Normal, healthy mammals and birds usually apply some natural louse control by grooming and preening themselves. Poorly nourished or sick animals that do not exhibit normal grooming behavior often are heavily infested with lice.

Many species of passerine birds show an interesting behavior known as anting that may represent another natural method of louse control. The bird settles on the ground near a colony of ants, allowing the ants to crawl into its plumage, or it picks up ants and applies them to the feathers. The bird uses only ant species whose workers exude or spray toxic substances in attack and defense but do not sting. Ants in 2 subfamilies of Formicidae either spray formic acid or exude droplets of a repugnant fluid from their anuses (Simmons, 1966). The worker ants liberally anoint the feathers with noxious fluids. Significant numbers of dead and dying lice have been found in the plumage of birds immediately after anting.

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65

Arthropoda

Triatominae (Subfamily): Kissing Bugs

Sue Ann Gardner, compiler

Phylum Arthropoda

Class Insecta

Order Hemiptera

Suborder Heteroptera

Family Reduviidae

Subfamily Triatominae

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Chapter 65

Triatominae (Subfamily): Kissing Bugs

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Introduction

Triatomines are insects belonging to the order Hemiptera, suborder Heteroptera, family Reduviidae, and subfamily Triatominae. All members of this subfamily are hematophagous, which is considered to be a recently derived characteristic in evolutionary terms. In relation to the taxonomy and phylogenesis of triatomines, it is interesting that the Hemiptera order has dispersed representatives throughout tropical and temperate regions. In this order more than 80,000 species are known. Traditionally, Hemiptera is divided into two suborders, Homoptera and Heteroptera. Some Homoptera and most Heteroptera are adapted to feeding on plant sap. Some insects of the Heteroptera suborder are predators on insects and on other invertebrates, sucking their hemolymph, while other Heteroptera have become hematophagous, for instance, the Triatominae subfamily (Schofield and Dolling, 1993).

Triatomine Hematophage Biology (Excerpted and adapted from Schofield, 2000a)

Relative to digestion, a whole series of physiological adaptations is required for an obligate hematophage (see Lehane, 1991). Blood is a nutritionally rich resource, but it is highly alkaline, and much of the protein is locked in the blood cells. Consequently, the Triatominae require both a hemolysin to open the blood cells and a system to acidify the blood meal before it can be digested. Species of Reduviidae are derived from plant-sucking Hemiptera which have lost the ability to secrete trypsin, the usual digestive protease, because plant sap has virtually no protein, and plant seeds have potent antitrypsins (Schofield, 1996). Thus, the Reduviidae, including Triatominae, must make use of secreted cathepsins as proteases, which are generally active only at acid pH. Blood is also generally deficient in certain vitamins, particularly folate and B vitamins, so that all obligate bloodsuckers require symbionts to assist in producing these compounds. These symbionts are so important that all other obligate blood-suckers carefully conserve them either intracellularly or in a special organ known as the mycetome. But in Triatominae these symbionts are free in the gut lumen, which is taken as additional evidence that the blood-sucking habit is a relatively recent adaptation (Schofield and Dolling, 1993).

Morphology of a Representative Species, *Triatoma sanguisuga* (Extracted verbatim from Byron and Capinera, 2019)

Eggs

The eggs of *Triatoma sanguisuga* are pearly-white, oval, and approximately 1.5 mm-long. Eggs are indiscriminately deposited individually on the substrate. Once a blood meal is taken, females begin oviposition after 4–6 days, depositing 1–5 eggs per day (Grundemann, 1947). In a study by Hays (1965), female nymphs collected in the field that developed into adults under laboratory conditions each laid an average of 711 eggs in their lifetime. However, the range of eggs laid per female was large (from 312 to 1,166) indicating a need for more research in this area.

Nymphs

According to Grundemann (1947), *Triatoma sanguisuga* goes through 8 instars, determined by measuring the head capsule (because of the swelling associated with blood-feeding, body size is an inaccurate measurement). In a laboratory setting, each instar lasted approximately 41 days. Each molt requires blood-feeding. Development time is directly linked to temperature and host availability.

Adults

Triatoma sanguisuga adults are approximately 19 mm-long, with dark brown to black, flattened bodies and elongate, cone-shaped heads (Griffith, 1947). Antennae are elbowed, with six segments. The head bears a slender beak-like structure used to administer the notorious kiss, or bite. The abdomen is wide, with sides sticking out past the wing margins, displaying 6 reddish-orange spots on each side (Drees and Jackman, 2018) (Figure 2).

History of the Subfamily Triatominae (Extracted from Tartarotti et al.., 2006)

Triatomines probably evolved from Reduviidae predator groups. The Reduviidae early on in their evolution possibly fed on soft forms of invertebrate animals that inhabited vertebrate nests, such as caterpillars, larvae, and spiders. Later they began to attempt perforating the skin of small vertebrates. It is possible that, in the first phase, hematophagy was optional, and, since the saliva of these insects had no anesthetic properties, the triatomines would have been driven to feed on newly born vertebrates, which would be attacked in a special form of predation. Later in this phase, starting with adaptations for hemolysis, the hematophagous process would have begun (Carcavallo et al., 1999).

To avoid the predatory vertebrates in the nests and burrows, it was necessary to make adaptations, such as cryptic behavior and inverse activity pattern, for feeding while the vertebrate is asleep. In predators, the saliva has a proteolytic effect, a characteristic that was lost by most of the hematophagous insects to make it possible to ingest blood by a painless bite. Hematophagy also requires a rapid compensation of the enormous amount of blood that triatomines ingest. The insect therefore excretes great amounts of water and salts immediately to reduce its weight. Another adaptation to hematophagy is the erythrocytic rupture and hemolytic process at the beginning of digestion (Carcavallo et al., 1999). Triatomines are little different from Reduviidae predators, in habitat and forms, which also corroborates the argument that this group is a recent one.

Gorla and colleagues (1997) consider that triatomines are polyphyletic in origin and they believe that hematophagy have appeared recently, associated with the evolution of vertebrate nests. The polyphyletic hypothesis suggests that the adaptative steps from free life predators to hematophagous feeding might have occurred several times, not only among different groups of Reduviidae, but also among other Hemiptera groups. Deep phylogenetic analysis should resolve this question of polyphyly.

This hypothesis may explain the close relationship between genera and species of triatomines associated with certain vertebrates. For instance, *Psammolestes* associated with bird's nests, *Dendrocolaptidae*, *Cavernicola pilosa barber* with Chiroptera, *Microtriatoma* with the biocenosis of the great Bromeliads, *Panstrongylus geniculatus* associated with the *Edentates* (see Figure 1), and some species of the *Triatoma protracta* complex associated with the *Neotoma genus*. The polyphyletic hypothesis also helps to explain most of the anatomical differences found between some tribes and their notable similarity with taxa of other Reduviidae subfamilies. For example, species of *Alberproseniini* possesses morphological characteristics of the Cetherinae and species of *Psammolestes* possesses anatomical characteristics present in the Physoderinae subfamily. Among the most convincing studies, it has been discovered that there are fundamental differences in salivary components between species of Rhodniini and Triatomini, as well as differences in sensorial patterns, suggesting different origins for these two tribes. Therefore, the Triatominae subfamily should be assumed, more correctly, to be a utilitarian group, defined on the basis of their hematophagous habits and adaptations associated to this diet, and not a phylogenetic group of individuals sharing a common ancestry (Carcavallo et al., 1999).

Figure 1. Subfamily Triatominae Jeannel, 1919. Species: *Panstrongylus geniculatus*. Locality: Montebello, Amalfi Municipality, Departmento de Antioquia, Colombia (6°55'58''N; 75°05'30'' W, 18-24 °C). Source: F. Otálora Luna, 2006. License: CC BY-SA 3.0.

Some authors, including Usinger and colleagues (1966) believe, however, that the triatomines represent a monophyletic group and that their hematophagy have appeared only once. Gaunt and Miles (2000) also postulate that the triatomines are of monophyletic origin, based on the appearance of a salivary protein (anti-thrombin).

The monophyletic hypothesis is not only difficult to support, but it also causes problems in the understanding of the insects' distribution, association with animals, source of feeding and adaptation to different habitats. The comparison between population and behavioral parameters, association with vertebrates and habitat, as well as their biogeographical characteristics support the hypothesis that triatomines probably appeared several times within the Reduviidae and that they represent species of polyphyletic origin, based on their apomorphic character with relation to hematophagy (Schofield, 1988; Lyman et al., 1999; Bargues et al., 2000, Marcilla et al., 2001).

Figure 2. Adult *Triatoma sanguisuga*, eastern blood-sucking conenose. Locality: Pryor, Mayes County, Oklahoma, United States. Source: R. Webster, 2012. License: CC BY-SA 4.0.

Biogeographic History (Extracted from Tartarotti et al.., 2006)

The New World is clearly the center of triatomine origin and diversity. Of the approximately 137 triatomine species (Galvão et al., 2003), 105 occur in this area. Of the 14 genera, 12 are found exclusively in America: *Alberprosenia*, *Belminus*, *Bolbodera*, *Cavernicola*, *Dipetalogaster*, *Eratyrus*, *Microtriatoma*, *Panstrongylus*, *Parabelmintos*, *Paratriatoma*, *Psammolestes,* and *Rhodnius*. Only 2 genera, *Linshcosteus* and *Triatoma*, occur in the Old World, and the *Triatoma* is also found in the New World. The *Linshcosteus genus*, with 5 species, is confined to the Indian subcontinent, 7 species of *Triatoma* are present in Southeast Asia, and 1 species, *T. rubrofasciata*, is cosmopolitan in the tropics. Its wide distribution can be explained by marine transport from the 17th century to the early 20th century. This species is also present in the Brazilian northeast (Schofield and Dolling, 1993). *T. rubrofasciata* is considered to be an ancestor of the other 7 *Triatoma* species in Southeast Asia (*T. amicitiae*, *T. bouvieri*, *T. cavernicola*, *T. leopoldi*, *T. migrans*, *T. pugasi*, *T. sinica*) because they share morphological characteristics and are all included in the Rubrofasciata group. Another interesting characteristic that confirms the hypothesis that *T. rubrofasciata* is an older species is related to its painful bite, considered a primitive characteristic (Schofield, 1988).

The almost total absence of triatomines in Africa, except *Triatoma rubrofasciata*, probably brought to African ports by ships, suggests that the hematophagous evolution of Reduviidae in Africa was inhibited by the evolution of the hematophagous Anthocorideos, now known as Cimicidae, which had already occupied the available niches. The high degree of morphological specialization of Cimicidae suggests that they arose prior to the triatomines and that the latter evolved independently in America after the separation of the continents. This hypothesis is better than the view that triatomines may have appeared in Africa and, subsequently, were locally extinguished (Schofield, 2000a).

The dispersion of triatomines by vertebrates was studied on *Rhodnius prolixus* in Central America. It is believed that these insects migrated from South America to Central America, transported by birds. Enzymatic and RAPD (Random Amplification of Polymorphic DNA) analyses corroborated this view, the limited genetic variability denoting the recent origin of populations from South America (Dujardin et al., 1998).

Similarly, the presence of *Rhodnius prolixus* in Mexico is associated with the migration of vertebrates. The expansion and distribution of *T. infestans*, for example, is closely related to human activity (Schofield, 1988). The species is endemic in Bolivia and has been dispersed by human action, their domiciliary invasion obeying an opportunist mechanism provided by the stimulus of shelter and feeding (Forattini, 1980).

Triotamine Phylogeny

In the triatomine group, the Rhodniini, Cavernicolini, Bolboderini, Alberproseniini, and Linshcosteini tribes appear to be monophyletic groups, that is, each tribe possesses an ancester in common, while the Triatomini tribe is considered to be polyphyletic (Lent and Wygodzinsky, 1979; Galvão et al., 2003). The recognition of Rhodiniini as a monophyletic tribe takes into account characteristics of the *Rhodnius* genus not shared with other triatomines, such as, apical antenna insertion, body forms, post-ocular callosities, male genital characteristics, egg surface architecture, and nitroforine presence in the salivary glands. Besides these characteristics, the species of both *Rhodnius* and *Psammolestes* are primarily arboreal in contrast with the terrestrial habits of most of the other triatomines (Schofield and Dujardin, 1999). In addition, studies of sequence of ribosomic RNA mitocondrial and cytochrome B genes cluster *Psammolestes coreodes* with the species *Rhodnius prolixus*, *R. robustus*, and *R. neglectus* (Lyman et al., 1999).

Currently the most widely accepted hypothesis is that triatomines are a polyphyletic group, based on their convergent apomorphic hematophagy characters which have appeared independently several times in Reduviidae. These insects are highly adaptable to different habitats created by the constant expansion by humans and other animals. The hypothesis of a

polyphyletic assemblage is corroborated by several studies on the Rhodiniini and Triatomini tribes. Analyses of sequences of mitochondrial (Stothard et al., 1998; Lyman et al., 1999) and ribosomal DNA (Bargues et al., 2000; Marcilla et al., 2002) and analysis of polymorphism length of intergenic transcribed rDNA (Tartarotti and Ceron, 2005), enzymatic studies, morphological analyses and taxonomic (Carcavallo et al., 1999), ecological studies (Schofield, 1988) show the non-monophyletic nature of this group.

Life Cycle: Triatomines as Vector for *Trypanosoma cruzi* (Extracted verbatim from DPDx, 2023)

An infected triatomine insect vector (or kissing bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound (see Figure 3, including life cycle phases numbered in the text). Trypomastigotes enter the host through the wound or through intact mucosal membranes, such as the conjunctiva (1). Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma*, *Rhodnius*, and *Panstrongylus*. Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes (2). The amastigotes multiply by binary fission (3) and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes (4). Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The kissing bug becomes infected by feeding on human or animal blood that contains circulating parasites (5). The ingested trypomastigotes transform into epimastigotes in the vector's midgut (6). The parasites multiply and differentiate in the midgut (7) and differentiate into infective metacyclic trypomastigotes in the hindgut (8).

Triatomine Behavior (Extracted verbatim from Tartarotti et al., 2006)

Primitive predatory behavior still occurs in many triatomine species, including *Triatoma rubrofasciata*, which feeds on caterpillars, *T. rubrovaria* which can feed on spiders and silkworm, and *T. circummaculata*, which feeds on vertebrates' blood and cockroach hemolymph. Young nymphs of *Eratyrus mucronatus* preferentially feed on invertebrate animals, while nymphs in more advanced stages and adults feed on vertebrates' blood. Cannibalistic behavior can be a transitional stage between predation and hematophagy. There are reports of nymphs sucking blood from other nymphs in laboratory colonies. Such cleptohematophagous behavior occurs in *Belminus herreri* which obtains blood from species of recently fed *Rhodnius*. In short, all of these observations suggest that hematophagy is a recent characteristic in triatomines and that adaptations to this habit are still occurring (Schofield, 2000b).

For mammals, the bite from Reduviidae predators tends to be very painful and can cause death, especially by anaphylactic shock in small animals. The same happens in the case of certain triatomines. For instance, the bite of *Panstrongylus geniculatus* in pigs and humans in the Amazon leaves painful lesions and, in the case of *Triatoma rubrofasciata*, there has been at least 1 report of human death (Schofield, 2000b).

Medical Importance (Excerpted and adapted from Barreto Vieira et al., 2018)

Triatominae bugs are the vectors of Chagas disease, a major concern to public health especially in Latin America, where vector-borne Chagas disease has undergone resurgence due mainly to diminished triatomine control in many endemic municipalities. Although the majority of Triatominae species occurs in the Americas, species belonging to the genus *Linshcosteus* occur in India, and species belonging to the *Triatoma rubrofasciata* complex have been also identified in Africa, the Middle East, Southeast Asia, and the Western Pacific. Not all Triatominae species have been found to be infected with *Trypanosoma cruzi*, but the possibility of establishing vector transmission to areas where Chagas disease was previously non-endemic has increased with global population mobility. Additionally, the worldwide distribution of triatomines is concerning as they are able to enter into contact and harbor other pathogens, leading to concern that they could have competence and capacity to transmit them to humans during the bite or after successful blood feeding, spreading other infectious diseases. There are reports suggesting that triatomines may be competent vectors for pathogens such as *Serratia marcescens*, *Bartonella*, and *Mycobacterium leprae*, and that triatomine infection with other microrganisms may interfere with triatomine-*T. cruzi* interactions, altering their competence and possibly their capacity to transmit Chagas disease.

The transmission of Chagas disease by species of Triatominae is very well reported in the literature. Infection with vector-borne *T. cruzi* begins when metacyclic trypomastigotes, which are motile forms of the parasite, penetrate into the vertebrate host through the triatomine feces and urine. Once in the vertebrate host, these forms, which have evolved to survive inside host cells, infect nucleated cells. Within the cell, they differentiate into amastigotes in a phagosomal compartment known as the parasitophorous vacuole, escape to the cytoplasm, and replicate asexually

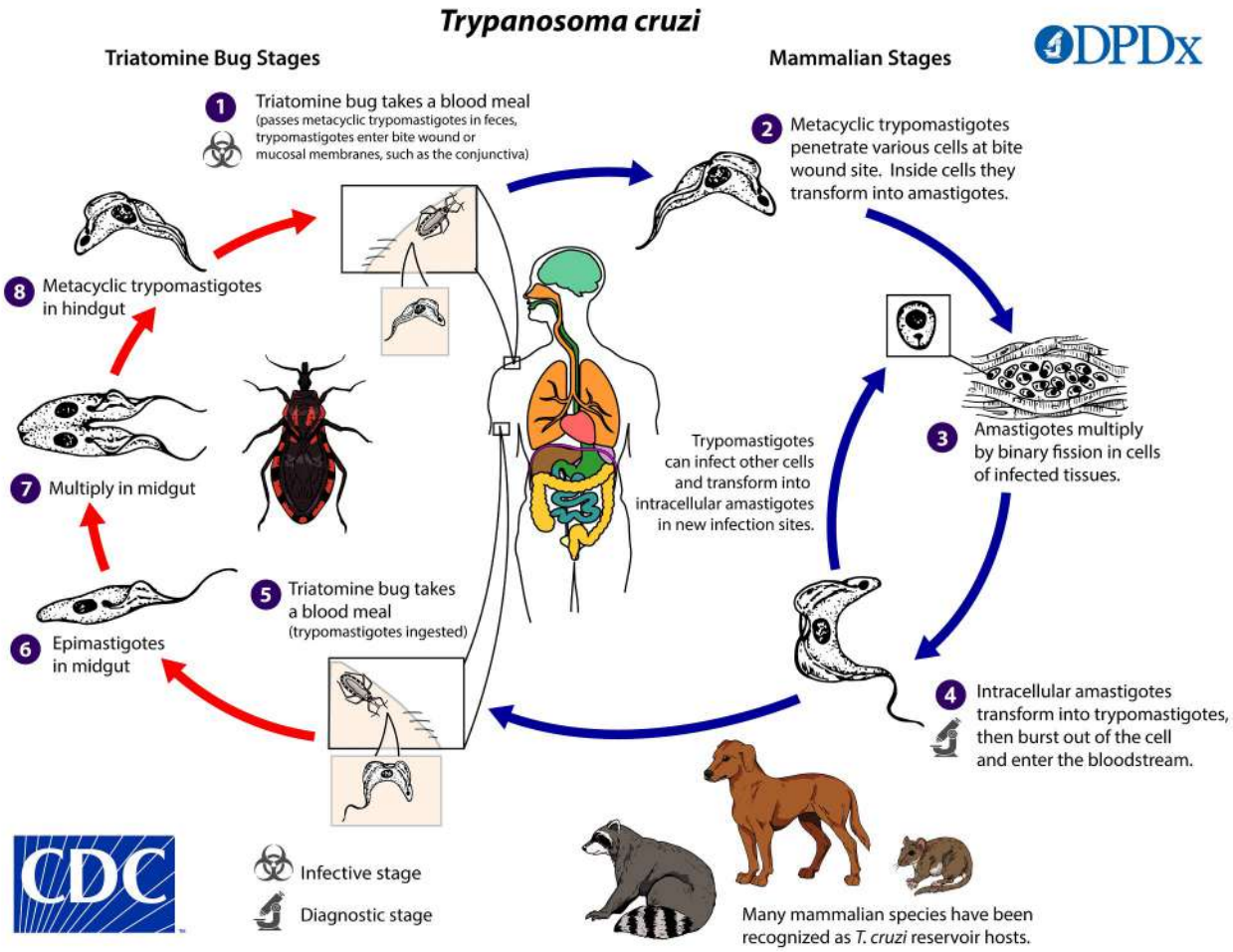

Figure 3. Life cycle of the protozoan parasite, *Trypanosoma cruzi*, the cause of Chagas disease, a zoonotic disease that can be transmitted to humans by blood-sucking triatomine bugs. Source: DPDx, 2023. Public domain.

through longitudinal binary division to form several amastigotes. As the cell becomes full of amastigotes, these convert into trypomastigotes and breach it, invading adjacent tissues and spreading to distant sites through bloodstream and lymphatics. The parasite population expands due to repeated cycles of cell invasion and replication, which lead to immune responses and can give rise to Chagas-associated pathologies (Tyler and Engman, 2001).

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Arthropoda

Acari (Order): Ticks

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Chapter 66

Acari (Order): Ticks

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Tick Biology and Life Cycles

Ticks (order Ixodida Leach, 1815) are blood-feeding ectoparasites of vertebrates representing important vectors of pathogens that cause diseases in humans and other animals (see Figure 1). They are obligate parasites at 1 or more developmental stages and may parasitize different classes of terrestrial vertebrates including mammals, birds, reptiles, and amphibians. All species of ticks have 4 stages in their life cycle, including the **embryonated egg** and 3 active stages: **Larva**, **nymph** (one or more instars), and **adult** (male and female). The tick life cycle may have many variations depending on the family and species. Depending on the number of hosts on which they feed, the tick may have a 1-host, 2-host, 3-host, or multi-host life cycle. During their off-host development phases, they can persist for long periods in the environment without feeding, particularly ticks in the family Argasidae (the soft ticks; see Sonenshine, 1991).

Ixodid ticks (family Ixodidae, the hard ticks) have a single nymphal instar and of them are **non-nidicolous**, meaning living in open and exposed habitats. In some species, only females result from the nymphal stage. These females reproduce **parthenogenetically**, that is, they do not need to mate to produce eggs (Sonenshine, 1991). Larvae and nymphs take a blood meal before molting to the next stage. Females lay several hundreds or thousands of eggs after engorging. Larvae, nymphs, and females may feed for several days, whereas males are usually intermittent feeders, taking small blood meals at each feeding and may remain on the host for long periods of time (Oliver, 1989; Sonenshine, 1991; 2013). Ixodid females have a single **gonotrophic cycle**. After completing feeding, they detach from the host to initiate oviposition in a secluded place, such as under vegetation, at the base of tree trunks, animal burrows, or even in cracks and crevices on the walls of human houses and animal sheds. Once oviposition is complete, the female dies. Males of Metastriata may stay on the hosts for long periods, mating with several females. These males usually mate on the host and need a blood meal to produce viable sperm. Males of the genus *Ixodes* (Prostriata) typically mate off the host, which is typical of nidocolous tick species. Most ixodids have a 3-host life cycle, where each stage falls to the ground after feeding. Engorged larvae detach from a host to molt in the environment, the same occurring with resulting nymphs that seek another host to feed and detach as engorged nymphs to molt into males or females, which in turn will complete the parasitic life cycle onto another host (Oliver, 1989; Sonenshine, 1991).

Other species of ixodids have a 2-host tick life cycle, characterized by larvae that feed and molt on the same host, whereas the nymphs feed and detach after engorgement. Nymphs molt in the environment and the resulting males or females attach to another host to complete the parasitic life cycle. There are a few species, including some of economic importance, that have a 1-host life cycle. They molt on the host (from larva to nymph and then to adult) and detach from the host as engorged females (Oliver, 1989; Sonenshine, 1991).

Almost all argasid species (family Argasidae, the soft ticks) are **nidicolous**, meaning living in the protected habitat of a nest of a bird or mammal, and usually have more than 1 nymphal instar in their life cycle. Many species have a multi-host life cycle, with the exception of some species, such as

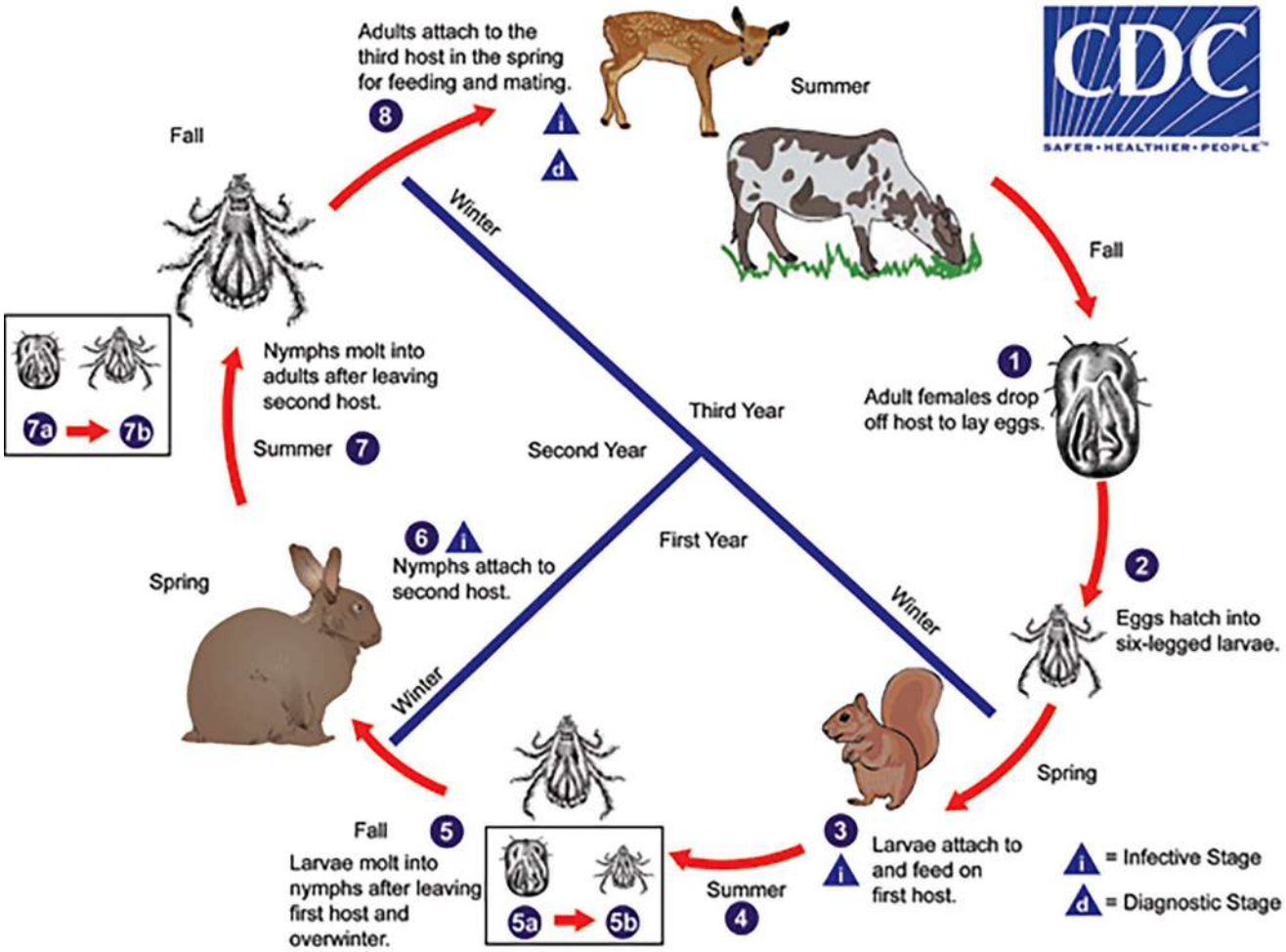

Figure 1. Life cycle of 3-host ixodid (hard) ticks. The adult is considered the diagnostic stage, as identification to the species level is best achieved with adults. Most ticks of public health importance follow this pattern, including members of the genera *Ixodes* (Lyme borreliosis, babesiosis, human granulocytic ehrlichiosis), *Amblyomma* (tularemia, ehrlichiosis, and Rocky Mountain spotted fever), *Dermacentor* (Rocky Mountain spotted fever, Colorado tick fever, tularemia, tick paralysis), and *Rhipicephalus* (Rocky Mountain spotted fever, boutonneuse fever). — Three-host ixodid ticks have a life cycle that usually spans 3 years, although some species can complete the cycle in only 2 years. Adult females drop off the third host to lay eggs after feeding (1), usually in the fall. Eggs hatch into 6-legged larvae (2) and overwinter in the larval stage. In the spring, the larvae seek out and attach to the first host, usually a small rodent (3). Later in the summer, engorged larvae leave the first host (4) and molt into nymphs (5), usually in the fall. The ticks overwinter in this stage. During the following spring, the nymphs seek out and attach to the second host (6), usually another rodent or lagomorph. The nymphs feed on the second host and drop off later in the summer (7). Nymphs molt into adults (7a–7b) off the host in the late summer or fall and overwinter in this stage. The next spring, adults seek out and attach to a third host, which is usually a larger herbivore (including cervids and bovids), carnivore, or human (8). The adults feed and mate on the third host during the summer. Females drop off the host in the fall to continue the cycle. Females may reattach and feed multiple times. The 3 hosts do not necessarily have to be different species, or even different individuals. Also, humans may serve as first, second, or third hosts. Source: United States Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, 2017. Public domain.

Ornithodoros lahorensis Neumann, 1908 that has a 2-host life cycle, and *O. megnini* that is a 1-host tick. In most argasid species, nymphs and adults are rapid feeders (generally taking around 30 to 40 minutes to complete a meal), but larvae usually remain feeding on a host for several days (Oliver, 1989; Sonenshine, 1991; 2013). Each immature stage feeds before molting to the next stage, but in some species of the genus *Ornithodoros*, such as *O. brasiliensis* Aragão, 1923, larvae molt to nymphs without feeding (Ramirez et al., 2016).

Some species of *Argas* and *Ornithodoros* may reproduce by **autogeny** (for example, *A. persicus* (Oken, 1818), *O. lahorensis*, *O. tholozani* (Laboulbène and Mégnin, 1882), *O. tartakovskyi* Olenev, 1931, and *O. parkeri* Cooley, 1936) (see Feldman-Muhsam, 1973; Oliver, 1989), and nymphs of some species (for example, *O. fonsecai* (Labruna and Venzal, 2009) and *Nothoaspis amazoniensis*) molt from the first to the second instar without feeding (Nava et al., 2010). Facultative autogeny may occur in the absence of a host. Females present multiple gonotrophic cycles and can feed many times, usually before mating and oviposition (Sonenshine, 1991). But for some species, the feeding behavior remains unknown.

Mating takes place off-host, and the female can lay a few hundred eggs after each meal, in each gonotrophic cycle. This is a survival mechanism, especially for nest dwelling species that depend on the presence, not always frequent, of their hosts. Exceptions may occur, for example, in adults of *Antricola* and *Otobius* that have vestigial mouthparts and a female may even lay eggs without feeding (this is called obligate autogeny) (Oliver, 1989; Sonenshine, 1991; 2013).

The biological life cycle involving multiple hosts is typical of argasids, which inhabit restricted environments and feed on the same individual host several times or in several hosts (of the same species or not) during their lifetime. Their habitat is intimately associated with that of their hosts. However, they can be found in remote locations far from human habitations such as loose soil, tree bark, animal burrows, caves, and in nests of wild and marine birds. Those that inhabit animal nests live in relatively stable microhabitats, feeding and reproducing continuously throughout the year. In this group, as in ixodids that inhabit nests, the development can be adapted seasonally, and a generation can take a year or more to develop in temperate climates (Oliver, 1989; Sonenshine, 1991; 2013).

The life cycle of the only species of family Nuttalliellidae is still unknown and the main hosts for each stage are uncertain. As an ixodid tick, *Nuttalliella namaqua* has a single nymphal instar and recently it has been shown that *N. namaqua* females may feed multiple times, like argasid females (Latif et al., 2012; Mans et al., 2012). Potential hosts already described for *N. namaqua* include mammals, reptiles, and birds (Mans et al., 2014). Larvae have been found parasitizing different species of rodents (Horak et al., 2012), and adults have been found in nests of birds (Keirans et al., 1976). Results of DNA analysis of the gut meals of females indicated that the ticks had fed on lizards of different species. Nymphs and adult females, therefore, have been shown to successfully feed on lizards in an experimental setting (Mans et al., 2011; 2014). Nymphs and adult females have been found in a variety of microhabitats in different regions of Africa (Mans et al., 2014). Although larvae may generally feed on rodents, the nymphal and adult stages seem to prefer reptiles, it is still premature to conclude that natural hosts of immature individuals or adults may feed exclusively on either mammals or reptiles. All these data may suggest a wider geographic distribution as well as host preference for *N. namaqua.*

Host Range

Ticks have variable degrees of specificity for their hosts, with some species parasitizing very different groups of animals. Some species of ticks only feed on a narrow range of host groups or on a specific host species (host-specific = narrow host range), whereas others are less selective (generalists), using a wide range (broad host range) of vertebrates as hosts (Sonenshine, 2013). Mammals serve as hosts for more tick species than birds, reptiles, and amphibians. Among mammals, rodents are one of the most common host groups, particularly for immature stages of hard ticks.

In general, immature stages of species that have a 2- or 3-host life cycle feed on small animals (for example, rodents), whereas adults prefer medium- and large-sized animals. In ticks that use more than 1 host, as happens with most species of the genus *Amblyomma*, immature stages are less specific than the adults, and may parasitize a greater diversity of hosts (Sonenshine, 2013). Host specificity is influenced by several factors, including host defense mechanisms against tick infestations, such as physical barriers in the body, self-cleaning behavior, and immunological responses.

Both passive and active questing methods are used by ticks to find their hosts. Passive species, such as most nidicolous ticks, remain in their habitat (for example, grassy fields, brushy areas, animal burrows, nests) and depend upon contact with vertebrate animals that invade their space incidentally. Most non-nidicolous ticks are hunters; they use ambush behavior (called questing), referring to ticks living in grass or brush-covered habitats typically climbing to the tips of stems or branches of vegetation where they wait for passing hosts to brush against them (Sonenshine, 2013). The success of a

tick in finding a host depends on several factors, including the height of the vegetation on which ticks of different stages are waiting for a host, as well as the response of ticks to specific stimuli, such as body odor, body heat, and carbon dioxide (CO_2), which are emitted from the host. Also, the type of environment has a direct influence on the qualitative and quantitative availability of hosts for the ticks.

The seasonal variation in the biological cycles and development of a species of tick is determined by the host and by abiotic factors, such as temperature, photoperiod, and relative humidity. Temperature plays an important role in determining the duration of each off-host development phase such as: For example, oviposition, egg incubation, larvae hatching, and ecdysis (molting from one stage to another). The photoperiod has a direct influence in the induction of diapause, mainly in nonequatorial regions, modulating the cycles in seasonal rhythms that assure the ticks the synchronization of their activities with the appropriate climatic conditions (Oliver, 1989; Sonenshine, 2013).

Two types of diapause are known: Behavioral (suppression of host-seeking activity or delay of engorgement) and morphogenetic or developmental (delay during embryogenesis) in the ecdysis of immature stages or in the oviposition (egg laying) of females (Sonenshine, 2013). This is an important strategy in the biology of both nidicolous and non-nidicolous ticks, such as *Amblyomma sculptum*, that use both larval and behavioral diapause (Labruna et al., 2002; 2003).

Taxonomic History

Millions of years ago, during the Paleozoic Era, ticks diverged from other Acari Leach, 1817, probably as parasites of the ancestors of modern vertebrates such as reptiles and amphibians (Dantas-Torres, 2018). Therefore, ticks disappeared when their conquering continental hosts went extinct. Fossil evidence indicates that modern tick lineages originated and diverged during the Mesozoic Era (Mans et al., 2016).

There are 2 fossil species in the family Argasidae Koch, 1844 (soft ticks), both of which are in the genus *Ornithodoros* Koch, 1844, namely, *O. antiquus* Poinar, 1995 and *O. jerseyi* (Klompen and Grimaldi, 2001). The third argasid fossil with an adequate morphological description corresponds to a male of *Ornithodoros* sp. found in Dominican amber from about 25 Ma (= million years ago) (Estrada-Peña and De La Fuente, 2018). These authors suggested that many of the lineage splits were produced when the landmasses were still forming the supercontinent Pangea, or Laurasia and Gondwanaland. Fossil species in the family Ixodidae Koch, 1844 (hard ticks) include *Amblyomma birmitum* Chitimia-Dobler, Araujo, Ruthensteiner, Pfeffer and Araujo, 2017, *Cornupalpatum burmanicum* Poinar and Brown, 2003, *Compluriscutula vetulum* Poinar and Buckley, 2008, *Ixodes succineus* Weidner, 1964, and *Haemaphysalis cretacea* Chitimia-Dobler, Pfeffer and Dunlop, 2018. The only fossil of the genus *Haemaphysalis* Koch, 1844 may actually belong to another genus (Guglielmone et al., 2016; see also Dantas-Torres, 2018). Recently, a fossil species, namely *Deinocroton draculi* Peñalver, Arillo, Anderson and De la Fuente, 2017, was described in a recently proposed fossil family Deinocrotonidae Peñalver, Arillo, Anderson and Pérez-de la Fuente, 2017. This species resembles *Nuttalliella namaqua* Bedford, 1931, which represents a basal lineage within the order Ixodida.

Current Taxonomic Position of Tick Genera

The Ixodida is currently represented by 956 species (948 extant and 8 fossil species) (see the supplementary material for more about this), which we now consider to be distributed into 4 families: **Argasidae** (215 species), **Ixodidae** (733 species), **Nuttalliellidae** Bedford, 1931 (monospecific), and **Deinocrotonidae** (monospecific) (Dantas-Torres, 2018; Du et al., 2018; Kwak, 2018; Barker, 2019; Tomlinson and Apanaskevich, 2019).

Excluding the monospecific families, the genus-level classification of ticks has been a long issue of debate and changes are constantly proposed, particularly in the family Argasidae.

Following are detailed descriptions of some groups of ticks.

Family Argasidae: The Soft Ticks

The family Argasidae, or the soft ticks, includes 215 extant and 2 fossil species (Dantas-Torres, 2018), many of which have not been yet adequately described. Estrada-Peña and colleagues (2010) suggest that there is still a long way to go to achieve an accurate view of the main evolutionary lines of the family. The soft ticks are so-named because they have no hard plate on their back (called the scutum in hard ticks; see below). They also commented that there is no consensus about the relevant morphological features for the determination of argasid species nor there is consensus on the appropriate genus for many species. According to Venzal and colleagues (2008), only larval morphological features have been adequately defined for a specific determination, mainly in the absence of DNA sequence data.

In this chapter, the genus-level classification adopted by the last lists of ticks of the world is used (Guglielmone et al., 2010; Dantas-Torres, 2018). Also included are 2 recent genera proposed by Barker and Burger (2018) and new species described in 2019 (Barker, 2019; Martins et al., 2019; Tomlinson and Apanaskevich, 2019; Sun et al., 2019). However,

the genus-level classification of argasids is still controversial, with some subgenera perhaps deserving to be elevated to the rank of genera (Burger et al., 2014; Mans et al., 2019). Based on 4 classification schemes for the argasid genera (the Soviet, American, French, and Cladistic schools), the subfamily Argasinae (Trouessart, 1892, pro parte) Pospelova-Shtrom, 1946 (ectoparasites of chickens and wild birds) is well supported by molecular data (Burger et al., 2014). However, this is not true for the subfamily Ornithodorinae Pospelova-Shtrom, 1946. After sequencing the mitochondrial genomes of 12 species, Burger and colleagues (2014) concluded that there is a clade of Neotropical species within the Ornithodorinae that includes the genera *Antricola* Cooley and Kohls, 1942 and *Nothoaspis* Keirans and Clifford, 1975, and the subgenera *Alectorobius* Pocock, 1907, *Parantricola* Cerny, 1966, and *Subparmatus* Clifford, Kohls and Sonenshine, 1964. On the other hand, the genera and subgenera of the Neotropical Ornithodorinae clade were placed in the genus *Carios* Latreille, 1796, as previously proposed by Klompen and Oliver (1993). Probably, the generic classification of argasids adopted here (Guglielmone et al., 2010) will change in the future, considering that new genomic data are becoming available, shedding new light onto this issue. For instance, Mans and colleagues (2019) generated a total of 83 whole mitochondrial genomes, 18S rRNA and 28S rRNA genes and proposed a revised genus-level classification for the family Argasidae. The new classification corresponds broadly with the morphological cladistic analysis of Klompen and Oliver (1993), however, with the erection of different subgenera to the genus level.

The genus *Antricola* is represented by 17 species distributed in the Neotropical region, most of them being restricted to Cuba. Besides Cuba, some species have been described from Mexico, Puerto Rico, Brazil, and Venezuela (Jones et al., 1972; De La Cruz, 1973; 1976; 1978; De La Cruz and Estrada-Peña, 1995; Camicas et al., 1998; Estrada-Peña et al., 2004; Guglielmone et al., 2010). The main contributions to the taxonomy of this genus were produced by De La Cruz, during the 1970s (De La Cruz, 1973; 1976; 1978). The number of species described from 1910 to 2004 is shown in Figure 2A. A key for the currently known species of *Antricola* is available in Estrada-Peña and colleagues (2004).

The genus *Argas* Latreille, 1795 is currently represented by 62 species, distributed in the Afrotropical, Australasian, Neotropical, and Oriental regions (Camicas et al., 1998). The number of species described from 1795 to 2012 is shown in Figure 2B. Most of the species have been described in the last century, and half of them from 1960 to 1980 (35 species). The main contributions were those of Kohls and Hoogstraal (1961), Kohls and colleagues (1970), and Keirans and colleagues (1979). According to Muñoz-Leal (2018), the morphology of nymphs and adults of *Argas* are less informative taxonomically, but some integumental dorsal features may be useful for a specific identification.

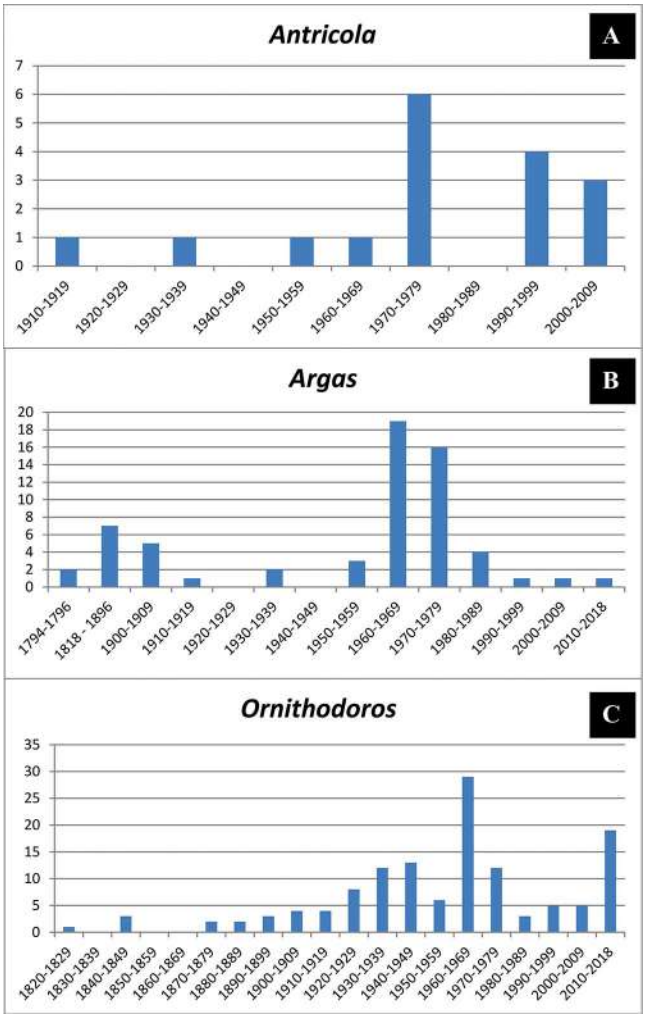

Figure 2. Argasidae genera. A) Number of *Antricola* species chronologically described from 1910 to 2004; B) number of *Argas* species chronologically described from 1795 to 2012; C) number of *Ornithodoros* species chronologically described from 1820 to 2019. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres. License: CC BY.

The genus *Nothoaspis* is composed of 3 species (*N. reddelli* Keirans and Clifford, 1975 in Mexico; *N. amazoniensis* Nava, Venzal and Labruna, 2010 in Brazil; and *N. setosus* (Kohls, Clifford & Jones, 1969) n. comb. (Muñoz-Leal et al., 2019). The last species was previously assigned to the genus *Ornithodoros*, but the morphological and molecular analysis of *O. setosus* larvae recently collected of the local type of this species showed that it belongs to the genus *Nothoaspis.* This genus is restricted to the Neotropical region (Keirans and

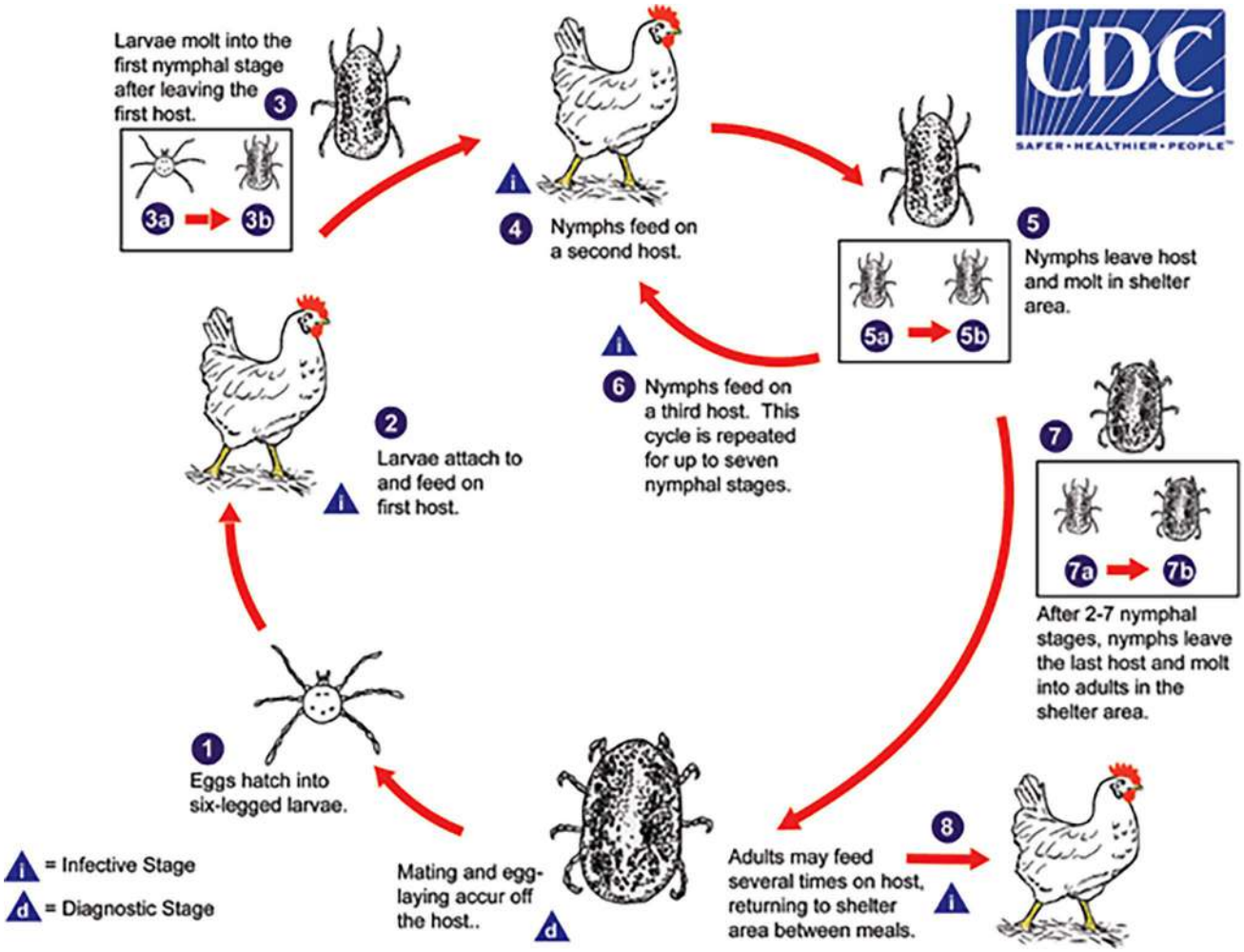

Figure 3. Multihost life cycle for argasid (soft) ticks. Unlike the Ixodidae, members of the family Argasidae have 2 or more nymphal stages, each of which requires a blood meal. This pattern is referred to as the multihost life cycle. Two species of public health concern in the United States, *Ornithodoros hermsi* and *O. turicata*, are vectors of tick-borne relapsing fever (TBRF) spirochetes. In Africa and Asia, *O. moubata* is a vector of TBRF spirochetes. Members of the genus *Carios* are vectors of TBRF spirochetes in Central America and South America. — Mating usually occurs, and egg-laying always occurs, off the host in a sheltered area (usually an animal nest). Eggs hatch into 6-legged larvae (1) in the parents' sheltered area. They quest for a host in the vicinity of the sheltered area. Once a suitable host is found, they feed for anywhere from 1 hour to several days, depending on the species (2). After feeding, the larvae leave the host and molt into the first nymphal instars in the sheltered area (3a–3b). The nymphs quest for, and feed on, the second host (4) rapidly (usually about an hour). The second host is usually the same species, and often the same individual, as the first host. The first nymphal instars leave the host and molt into the next nymphal instars in the sheltered area (5a–5b). This cycle can continue to accommodate up to 7 nymphal instars (6), depending on the species. After the last nymphal instar has fed, it leaves the host and molts into an adult (7a–7b) in the sheltered area. Adults may continue to feed on the host (8), feeding rapidly and detaching after each blood meal. Females of some species lay egg batches after each meal. Humans are usually only incidental hosts for argasid ticks and may be fed upon by any of the stages. Source: United States Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, 2017. Public domain.

Clifford, 1975; Nava et al., 2010; Muñoz-Leal et al., 2019). Note that *N. setosus* is referred to as *O. setosus* in the supplementary list for this chapter.

The genus *Ornithodoros* is the most speciose in the family Argasidae and comprises 131 extant and 2 fossil species (note that the fossil species are not included in the supplementary list for this chapter). They are distributed in the Afrotropical, Australasian, Oriental, Nearctic, Neotropical, and Palearctic regions (Camicas et al., 1998). The number of species described from 1820 to 2019 is shown in Figure 2C. As for *Argas*, most of them were described from 1960–1969 (29 species), and the main contributions are those of Clifford and colleagues (1964), Kohls and Clifford (1964), Kohls and colleagues (1965; 1969a), followed by those described from 2010–2019 (20 species) (Dantas-Torres et al., 2012a; Trape et al., 2013; Venzal et al., 2012; 2013; 2015; Barros-Battesti et al., 2015; Labruna et al., 2016; Muñoz-Leal et al, 2016; 2017; Bakkes et al., 2018; Sun et al., 2019).

The nymphs and adults of *Ornithodoros* are very similar and no reliable keys are currently available for their identification. On the other hand, larvae can be reliably separated by chaetotaxy of dorsum and venter, morphology of hypostome and, if present, dorsal plate.

The genus *Otobius* (Banks, 1912) includes 2 species, namely *O. megnini* (Dugès, 1883) and *O. lagophilus* Cooley and Kohls, 1940. The species *O. megnini* is thought to have had its original center of distribution in the arid lands of southwestern North America (Keirans and Pound, 2003). It was probably introduced into Central America and South America on both cattle and horses, and it was imported into South Africa in the ears of horses from South America or, perhaps, Mexico (Keirans and Pound, 2003). Currently, it is distributed worldwide, occurring in Afrotropical, Nearctic, Neotropical and Oriental regions (Camicas et al., 1998; Flores and Solís, 2018; Hosseini-Chegeni et al., 2018). The species *O. lagophilus* is restricted to the Nearctic region, parasitizing wild rabbits, occurring in Canada and the United States (Herrin and Beck, 1965).

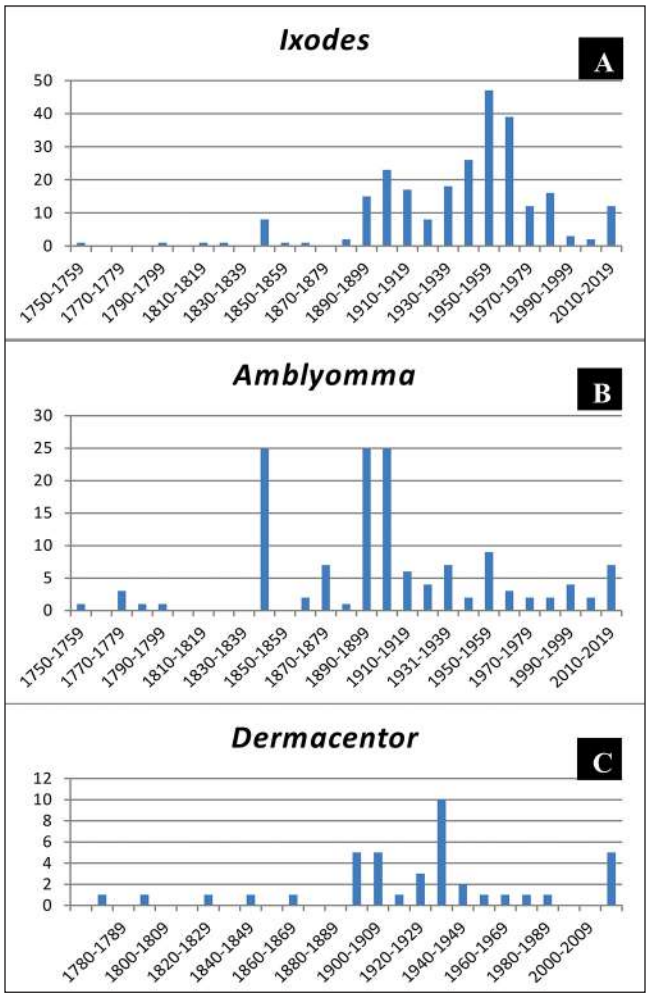

Figure 4. Ixodidae genera. A) Number of *Ixodes* species chronologically described from 1758 to 2019; B) number of *Amblyomma* species chronologically described from 1758 to 2019; C) number of *Dermacentor* species chronologically described from 1776 to 2016. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres. License: CC BY.

Family Ixodidae: The Hard Ticks

The family Ixodidae currently comprises 733 extant and 5 fossil species. This family is divided into 2 lineages (that is, Prostriata and Metastriata) found in all zoogeographic regions of the world (Guglielmone et al., 2014). The ixodids are called hard ticks because they have a big plate on their back called the scutum. The Prostriata (anal groove curves anterior to anus) contains only 1 genus, whereas the Metastriata (when present, anal groove curves posterior to anus) contains 14 genera (Burger et al., 2012; Barker and Burger, 2018). In contrast to what occurs with the family Argasidae, the genus-level classification of Ixodidae is more stable and consensual (Barker and Murrell, 2002; Guglielmone et al., 2014; Dantas-Torres, 2018). Nonetheless, some systematic issues (for example, paraphyly of the genus *Amblyomma* Koch, 1844) are still under debate (Barker and Burger, 2018), but the 2 genera proposed by these authors are here included with the new combinations.

The genus *Ixodes* Latreille, 1795 comprises 255 extant species and 1 fossil species. They are distributed in the Afrotropical, Australasian, Nearctic, Neotropical, Oriental, and Palearctic regions, and combinations of these regions including remote islands, and the polar area (circumpolar)

(Guglielmone et al., 2014; Estrada-Peña et al., 2014; Hornok et al., 2014, 2016; Ash et al., 2017; Apanaskevich and Bermúdez, 2017; Guo et al., 2017; Heath and Palma, 2017; Kwak et al., 2018; Barker, 2019). This is the largest tick genus, and most of the species originated on Gondwanaland (the southern continental landmass that began to break up in the early Jurassic around 184 million years BCE) (Guglielmone et al., 2014). The number of species described from 1758 to 2019 is shown in Figure 4A. The highest number of species were described during the 1950s and 1960s, with 47 and 39 species, respectively, mainly due to the contributions of Arthur (1956; 1960a), Kohls (1953; 1956a; 1956b; 1957; 1969), Kohls and Clifford (1962; 1966; 1967), and Kohls and colleagues (1969b).

The genus *Archaeocroton* Barker and Burger, 2018 was proposed for *Amblyomma sphenodonti* (Dumbleton, 1943), the tuatara tick of New Zealand. This new combination was mainly because this species in *Amblyomma* leaves this genus polyphyletic, and indeed, taxonomically unstable (Barker and Burger, 2018). The species was named *Archaeocroton sphenodonti* (Dumbleton, 1943).

The genus *Amblyomma* is 1 of the largest genera and comprises 137 extant and 1 fossil species, distributed in the Afrotropical, Australasian, Nearctic, Neotropical, and Oriental regions. Some species are found in more than 1 region, presenting Afrotropical-Neotropical, Afrotropical-Oriental, Afrotropical-Palearctic, Australasian-Oriental, Nearctic-Neotropical, Oriental-Palearctic, Afrotropical-Australasian-Oriental, or Australasian-Oriental-Palearctic distributions (Guglielmone et al., 2014; Nava et al., 2014a; 2014b; Krawczak et al., 2015; Apaneskevich and Apaneskevich, 2018). Chitimia-Dobler et al. (2017) commented that the genus *Amblyomma* was split in Gondwanaland, with a concurrent spread into what are now known as Africa, Australia, Asia, and South America. No species occurs exclusively in the Palearctic region (Guglielmone et al., 2014). The Neotropical region is home to the largest number of species within this genus, followed by the Afrotropical region. The number of species described from 1758–2019 is shown in Figure 4B. The highest number of species was described from 1840–1849 and 1890–1899, with 25 taxa each of those decades. The greatest contributions were those of Koch (1844) and Neumann (1899), who described 20 and 21 species of *Amblyomma*, respectively, during those years. In the first 10 years of the last century, Neumann (1901; 1904; 1905; 1906; 1907; 1911) described 16 species belonging to this genus.

The genus *Anomalohimalaya* Hoogstraal, Kaiser and Mitchel, 1970 is represented by 3 species, namely *A. lamai*

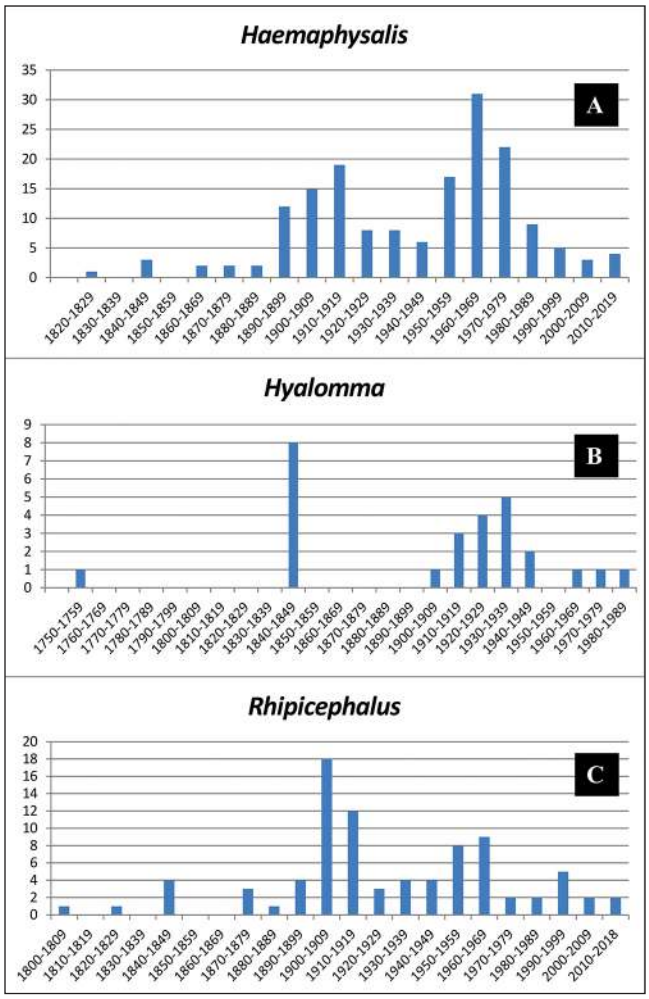

Figure 5. Ixodidae genera. A) Number of *Haemaphysalis* species chronologically described from 1826 to 2019; B) number of *Hyalomma* species chronologically described from 1758 to 1982; C) number of *Rhipicephalus* species chronologically described from 1806 to 2013. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres. License: CC BY.

Hoogstraal, Kaiser and Mitchell, 1970 from Nepal, *A.lotozkyi* Filippova and Panova, 1978 from Tajikistan, and *A. cricetuli* Teng and Huang, 1981 from China. They are exclusive to the Palearctic region and found in lands that once constituted Laurasia (Hoogstraal et al., 1970; Filippova and Panova, 1978; Filippova and Bardzimashvily, 1992; Guglielmone et al., 2014).

The genus *Bothriocroton* Keirans, King and Sharrad, 1994 includes 7 species: *B. undatum* (Fabricius, 1775), *B. hydrosauri* (Denny, 1843), *B. concolor* (Neumann, 1899), *B. oudemansi* (Neumann, 1910), *B. auruginans* (Schulze, 1936), *B. tachyglossi* (Roberts, 1953), and *B. glebopalma* (Keirans, King and Sharrad, 1994). They are found exclusively in the

Australasian region (Klompen et al., 2002; Beati et al., 2008; Burger et al., 2012; Barker and Walker, 2014).

The monospecific genus *Cosmiomma* Schulze, 1919 is found in the Afrotropical region and it is represented only by *C. hippopotamensis* (Denny, 1843) (Arthur, 1960b).

The genus *Dermacentor* Koch, 1844 is represented by 40 species, which are of Afrotropical, Nearctic, Neotropical, Oriental, Palearctic, Australasian-Oriental, Nearctic-Neotropical, and Nearctic-Palearctic distribution (Guglielmone et al., 2014; Rubel et al., 2016; Vongphayloth et al., 2018). According to Nava and colleagues (2017), the species are more prevalent in lands of Laurasian origin than in Gondwanan lands. The number of species described from 1776–2016 is shown in Figure 4C. Most species were described during the 1930s, with 10 taxa described during that decade. The main contributions to this genus were those of Schulze (1933; 1935; 1937; 1939). In the last 5 years, 1 species was described in Central America (Apanaskevich and Bermúdez, 2013a) and 4 taxa were described to in the Oriental region (Apanaskevich and Apanaskevich, 2015a; 2015b; 2015c; 2016).

The genus *Haemaphysalis* comprises 169 extant species and 1 fossil species, distributed in the Afrotropical, Australasian, Nearctic, Neotropical, Oriental, and Palearctic regions, and combinations of these regions: Afrotropical-Neotropical, Afrotropical-Palearctic, Australasian-Oriental, Nearctic-Neotropical, Oriental-Palearctic, Afrotropical-Oriental-Palearctic, and Australasian-Oriental-Palearctic (Guglielmone et al., 2014). This genus is poorly represented in the Nearctic and Neotropical regions, with the majority of species occurring exclusively in the Oriental region. The inclusion of the fossil species *H. cretacea* in this genus (Chitimia-Dobler et al., 2018) has been questioned (Dantas-Torres, 2018). The number of species described from 1826–2019 is shown in Figure 5A. The greatest numbers of species were described during the 1960s with 31 taxa, followed by the 1970s with 22 species. The greatest contributions were those of Hoogstraal and colleagues (1965; 1969), Hoogstraal and Trapido (1966), and Hoogstraal and Kim (1985). In the last decade, 6 species were described (Tomlinson and Apanaskevich, 2019).

The genus *Hyalomma* Koch, 1844 is represented by 27 species, distributed in the Afrotropical, Oriental, Palearctic, Afrotropical-Palearctic, Oriental-Palearctic, and Afrotropical-Oriental-Palearctic regions (Guglielmone et al., 2014). The greatest number of species is found in the Afrotropical, followed by the Palearctic regions. According to these authors, the genus is absent in the Australasian, Nearctic, and Neotropical regions. The highest numbers of species were described during the 1840s with 8 taxa described, and the main contributions were those of Koch (1844) who described 7 species during that decade. The number of species described from 1758–1982 is shown in Figure 5B.

The genus *Margaropus* Karsch, 1879 is represented by 3 species, namely *M. reidi* Hoogstraal, 1956, *M. wileyi* Walker and Laurence, 1973, and *M. winthemi* Karsch, 1879, which occur only in the Afrotropical region (Arthur, 1960b; Walker and Laurence, 1973; Guglielmone et al., 2014).

The genus *Nosomma* Schulze, 1919 is represented only by 2 species, namely *N. monstrosum* (Nuttall and Warburton, 1908) and *N. keralensis* Prakasan and Ramani, 2007. Both species are exclusively from the Oriental region (Guglielmone et al., 2014).

The genus *Rhipicentor* Nuttall and Warburton, 1908 is represented by 2 species, namely *R. bicornis* Nuttall and Warburton, 1908 and *R. nuttalli* Cooper and Robinson, 1908, both of which are exclusively found in the Afrotropical region (Guglielmone et al., 2014).

The genus *Rhipicephalus* Koch, 1844 includes 85 species. According to Guglielmone and colleagues (2014), 63 species are exclusively found in the Afrotropical region, 7 species occur exclusively in the Palearctic region, and 3 are found only in the Oriental region. The remaining species are distributed in the Australasian, Nearctic, and Neotropical regions, but they are not exclusively from these regions. The number of species described from 1806–2013 is shown in Figure 5C. The highest numbers of species described were from 1900–1910 with 18 taxa, followed by the 1910s with 12 species. The greatest contributions were those of Neumann (1899; 1901; 1904; 1905; 1906; 1907; 1911; 1913). The 2 latest species described in this genus were in 2013 (Apanaskevich et al., 2013b; Horak et al., 2013).

The genus *Robertsicus* Barker and Burger, 2018 was proposed for *Amblyomma elaphensis* (Price, 1959), from the Chihuahuan Desert of Mexico and the southeastern United States. This new combination was meant to solve for the polyphyly of the genus *Amblyomma* that is in the same situation (Barker and Burger, 2018). These authors named the species *Robertsicus elaphensis* (Price, 1959).

Family Nuttalliellidae

Nuttalliellidae is a monospecific family, which presently is restricted to the Afrotropical region (Bedford, 1931; Keirans et al., 1976; Camicas et al., 1998; Mans et al., 2011; 2016). Based on analysis of mitochondrial genome and nuclear ribosomal RNA (18S and 28S) sequence data, Mans and colleagues (2011; 2019) suggested that the Nuttalliellidae is basal to other tick families, representing the closest extant lineage to the last common ancestral tick lineage.

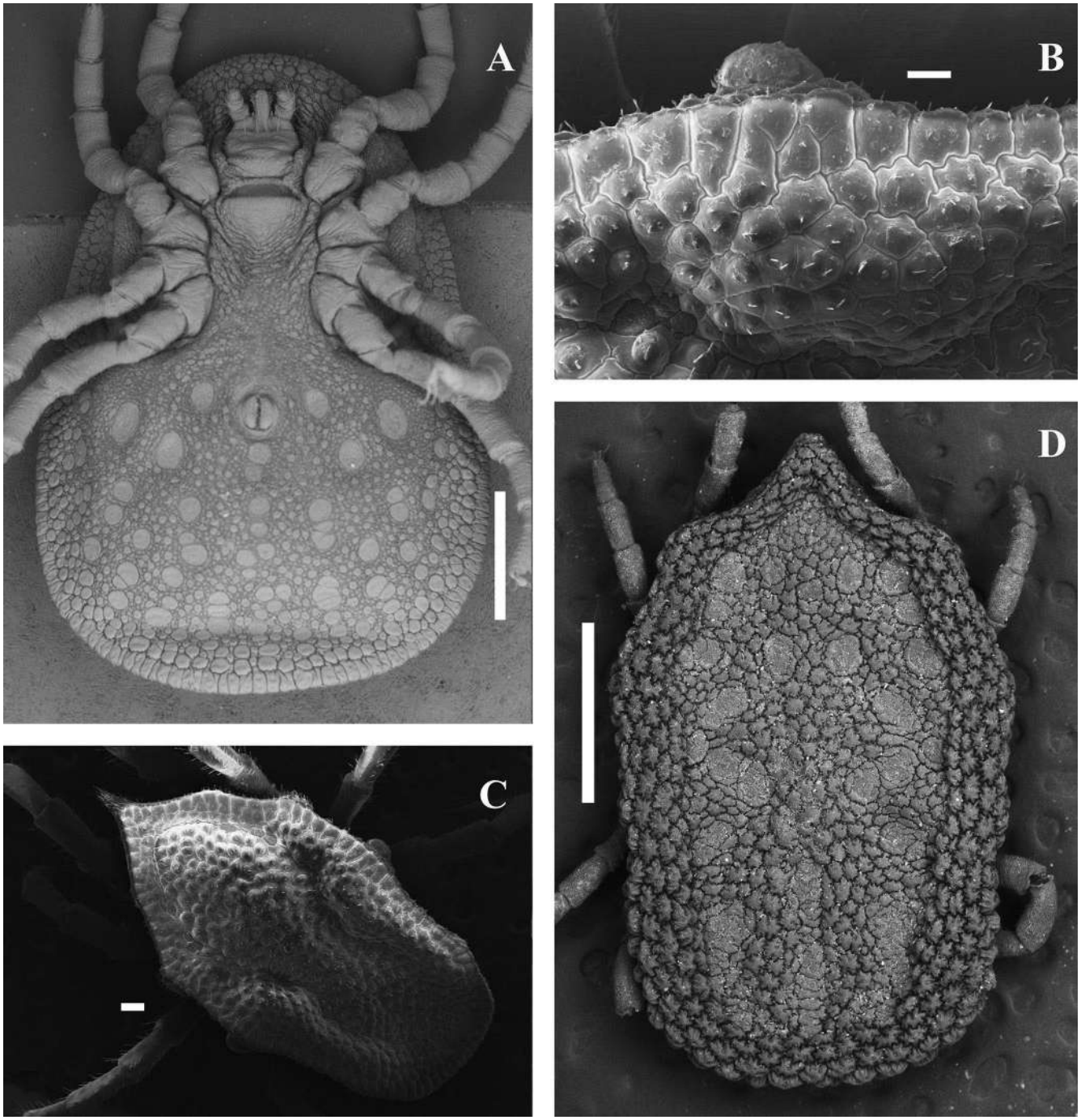

Figure 6. Adults of Argasidae genera. A) *Argas miniatus* female, ventral view; B, C) *Antricola guglielmonei*, spiracular plate and dorsal view; D) *Ornithodoros* sp., dorsal view. Scale bars: A, D = 1,000 µm; B = 100 µm; C = 200 µm. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres.

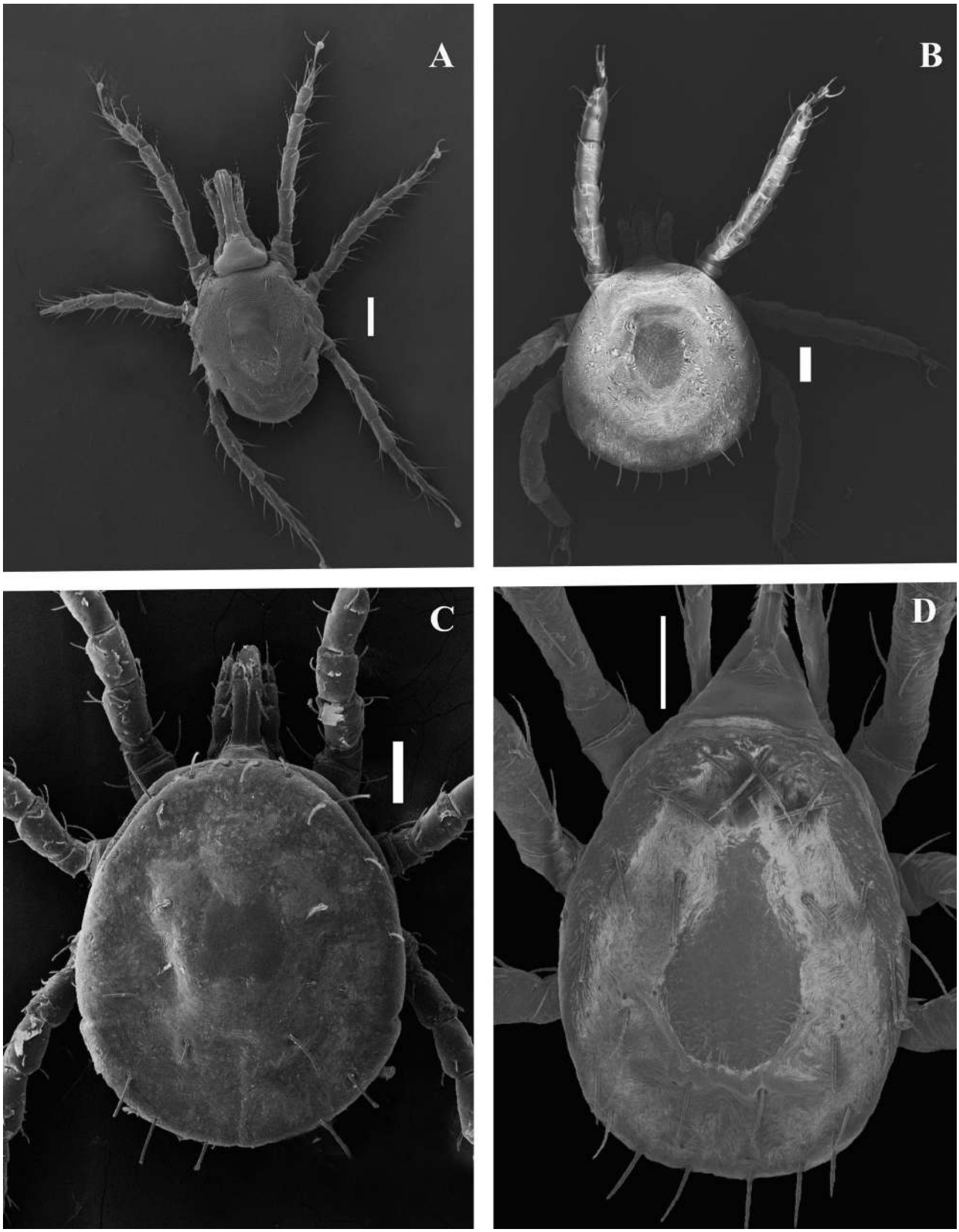

Figure 7. Larvae of Argasidae genera. A) *Otobius megnini*, dorsal view; B) *Argas miniatus*, dorsal view; C) *Ornithodoros brasiliensis*, dorsal view; D) *Or. fonsecai*, dorsal view. Scale bars: A–C = 100 µm; D = 120 µm. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres. License: CC BY-NC-SA 4.0.

According to these authors, nuttalliellids almost became extinct during the great end-Permian mass extinction event, leaving *Nuttalliellia namaqua* as the closest living relative to the ancestral tick lineage.

Family Deinocrotonidae (Fossil)

Deinocrotonidae is a fossil tick family recently described based on fossil material retrieved in 99-million-year-old Cretaceous amber from Myanmar (Peñalver et al., 2017). *Deinocroton draculi* was found in association with *Cornupalpatum burmanicum*, suggesting that both deinocrotonids and ixodids fed on blood from feathered dinosaurs (Peñalver et al., 2017). Morphologically, deinocrotonids resemble nuttalliellids, but no DNA sequences from the former are available to assess their phylogenetic relationship.

Descriptions of Selected Tick Genera

In this section, morphological descriptions are presented for the identification of several tick genera as used in scientific papers. The fossil family Deinocrotonidae and the fossil genera *Deinocroton*, *Cornupalpatum*, and *Compluriscutula* are not included.

ARGASID TICK GENERA

Genus *Argas*

The following morphological descriptions are based on Cooley and Kohls (1944) and Kohls and colleagues (1970).

Larva: Dorsal surface with around 25–30 pairs of setae, dorsal plate oval and elongated; ventral surface with less than 7 pairs of setae and 1 pair on valves; posteromedial seta present or absent; 2 pairs of short post-hypostomal setae; hypostome rounded at apex, dentition 2/2 at basis to 3/3 at apex. *Nymph*: Outline oval, discs present, distributed more or less symmetrically dorsally; idiosoma mamillated, flattened dorsoventrally, with suture and lateral margin demarcating the dorsal and ventral surfaces; Haller's organ with transversely slit-like aperture, placed slightly laterally. *Adults*: Idiosoma flattened, dorsal and ventral surface equal, margin distinct flattened, made up a radial striae or quadrangular plates; sutural line present; flattened margin not obliterated even when tick is fully fed; capitulum ventral; integument leathery, minutely wrinkled in folds, of many shapes often intermingled with small, rounded, buttons each with a pit on top and often bearing a hair in the pit; discs present on both dorsal and ventral surfaces and placed in more or less radial lines; eyes absent.

Genus *Antricola*

The following morphological descriptions are based on Cooley and Kohls (1944), Estrada-Peña and colleagues (2004), and Barros-Battesti and colleagues (2013).

Larva: Dorsal surface with 14 pairs of setae, typically 14 (11 dorsolateral, 3 central dorsal); dorsal plate, large and elongated with lateral margins parallel, narrowing anteriorly; eyes absent; ventral surface with 11 pairs of setae (3 sternal setae, 3 post-coxal setae, 4 circumanal + 1 on valves), and 1 posteromedial seta; 2 pairs of long post hypostomal setae, hypostome pointed, dentition 3/3 in anterior three-fourths, then 2/2 posteriorly to basis; palps with 18 setae, number of setae on palpal article 1–4, respectively 0, 4, 5 and 9; pulvilli large, claws absent (except in *A. marginatus*); dorsal hump absent; Haller's organ with a rounded capsule, open only in a small central portion. *Nymph*: Body outline suboval, pointed anteriorly, covered by tubercles, most of them bearing short setae, some single, others in groups; hypostome short, broad and rounded apically, with small denticles on anterior and lateral margins; cheeks absent; spiracular plates oval, relatively large, expanded and dorsally visible in some specimens, with numerous minute pores. *Adults*: Dorsal surface flattened and marginated; cuticle semi-translucent and smooth, shining, and with tubercles and tufts of setae; dorsomarginal grooves well defined; transverse post-anal groove present. Basis capituli slightly longer than wide, rounded laterally, hypostome small, slightly longer than wide, scoop-like, without denticles.

Genus *Ornithodoros*

The following morphological descriptions are based on Venzal and colleagues (2006) and Barros-Battesti and colleagues (2013).

Larva: Dorsal surface of idiosoma usually with 13–14 pairs of setae (with some exceptions); dorsal plate absent in few species, but present in the majority, varying in shape, from triangular to pyriform (bat-associated group) to elongated sub-rectangular with anterior extremity narrowed; venter with 7–8 pairs + 1 pair on anal valves, and 1 posteromedial seta (which may be absent). Basis capituli with lateral angles slightly rounded, lateral auriculae present or absent, hypostome with apex rounded or pointed, dental formula: 5/5 to 2/2 at apex, 4/4 to 2/2 in medial portion and 2/2 at basis; Haller's organ with capsule aperture transversely slit-like, large, occupying all of the dorsum with many small setae, or small occupying part of the dorsum. *Nymph*: Body outline oval, slightly pointed anteriorly, idiosoma covered by tile-like mammillae; presence of 4 pairs of bulging lateral structures resembling large mammillae on supracoxal

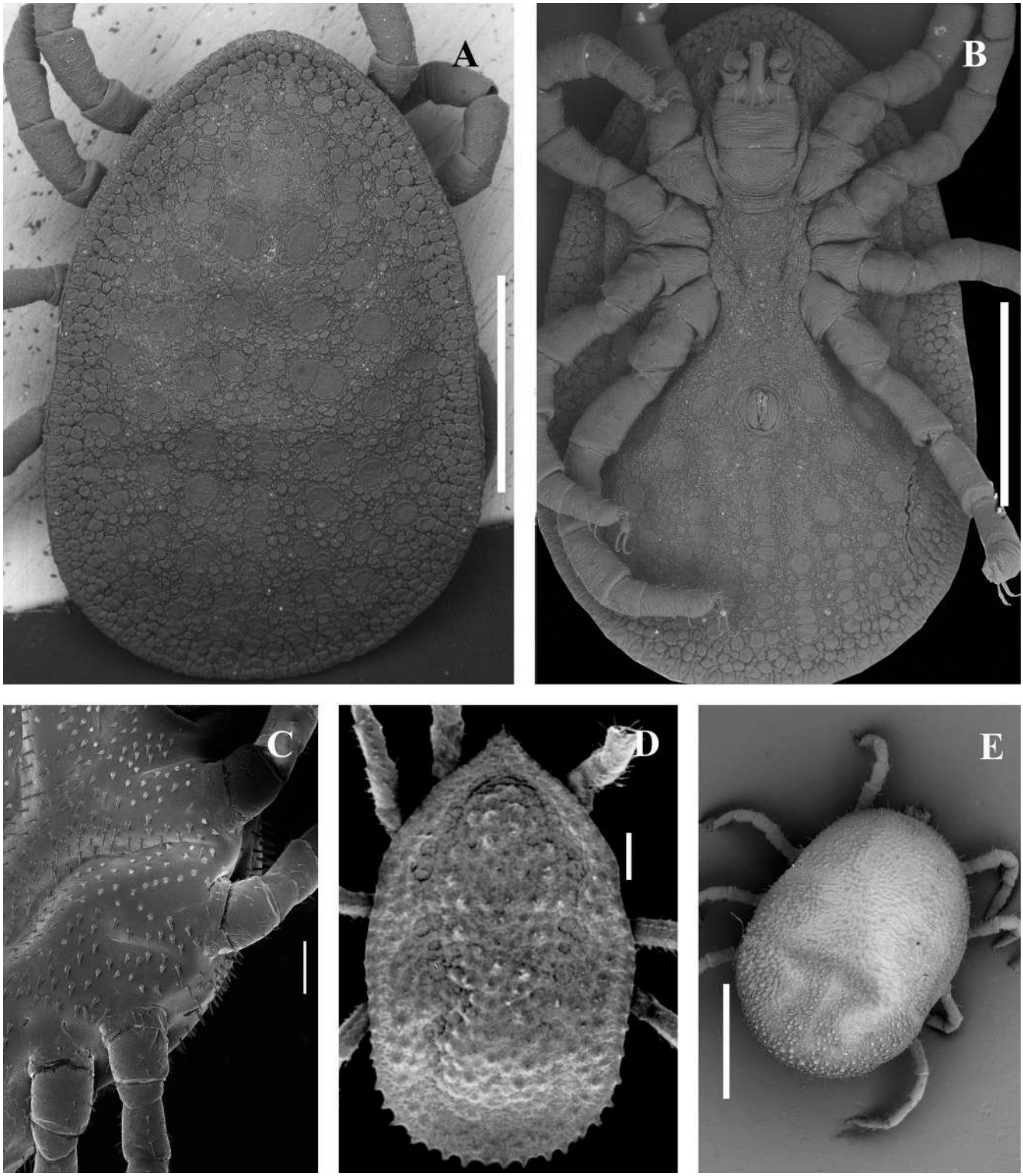

Figure 8. Nymphs of Argasidae genera. A, B) *Argas miniatus*, dorsal and ventral view, C) *Otobius megnini*, ventral view; D) *Antricola guglielmonei*, dorsal view; E) *Ornithodoros brasiliensis* dorsal view. Scale bars: A, B, E = 1,000 µm; C = 300 µm; D = 200 µm. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres. License: CC BY-NC-SA 4.0.

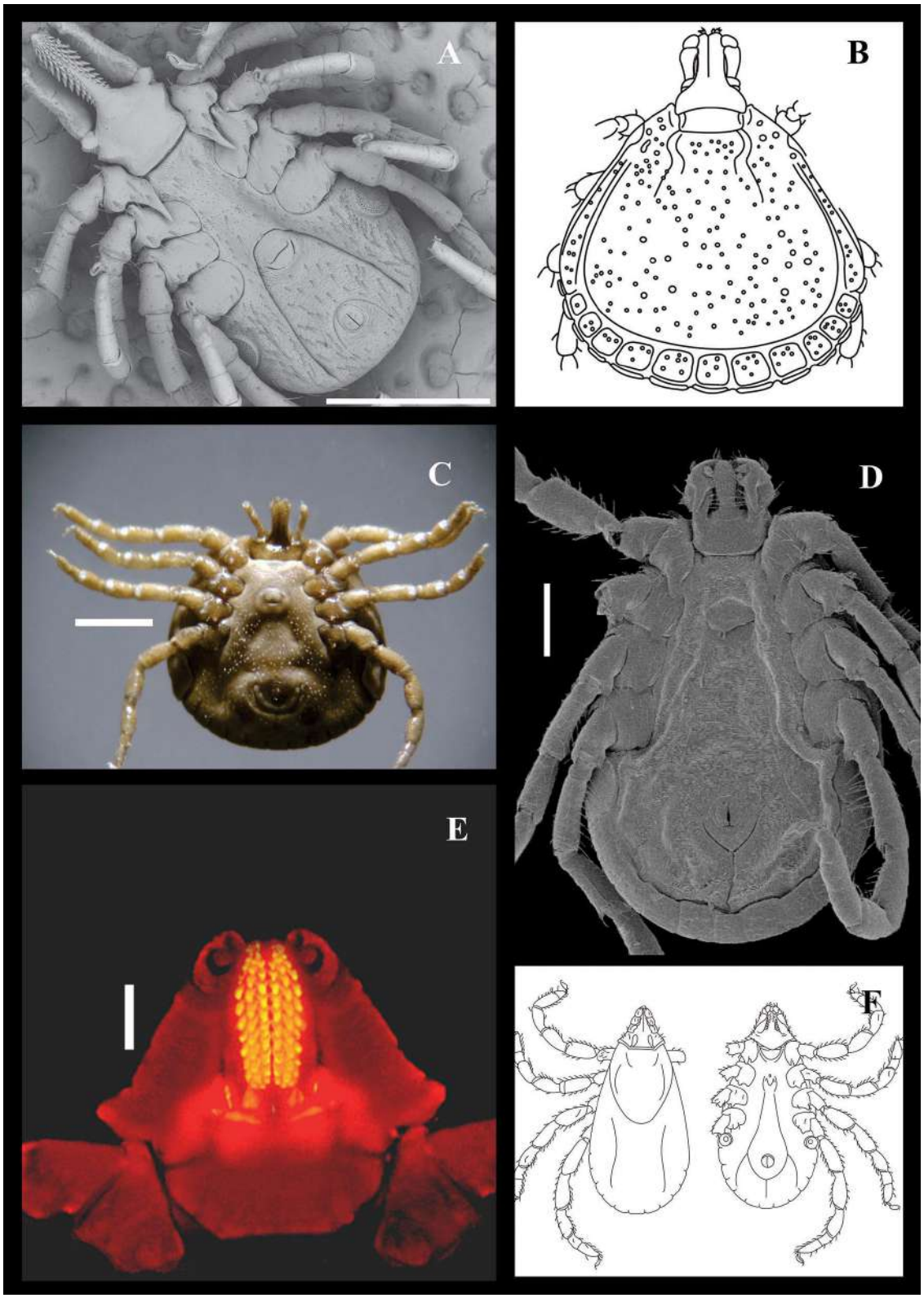

Figure 9. Adults of Ixodidae genera. A) *Ixodes aragaoi* female, ventral view; B) *Bothriocroton* male, dorsal view; C) *Amblyomma (Aponomma) quadricavum* female, ventral view; D) *Haemaphysalis juxtakochi* male, ventral view; E) *H. leporipalustris,* gnatosoma ventral view; F) *Anomalohimalaya* female, dorsal and ventral view. Scale bars: A = 500 µm; C = 1,000 µm; D = 300 µm; E = 100 µm. Sources: A, C–E) D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres; B) adapted from Baker and Walker, 2004; F) adapted from Hoogstraal et al., 1970.

folds between legs I–IV (soil-living group) or absent (bat-associated group), hypostome rounded on apex; humps present (only in the soil-living group) or absent (bat-associated group), Haller's organ similar to the larvae. *Adults*: Idiosoma suboval, with rounded margins, without marginal lateral sutures; well-developed hypostome with well-defined rows of denticles; hood present; sometimes cheeks present; eyes, when present are arranged anterolaterally to the supracoxaal folds; integument leathery, with tiny mammillated elevations, interspersed by discs on both the dorsal and ventral surfaces.

Genus *Otobius*

The following morphological descriptions are based on Cooley and Kohls (1940; 1944), Guglielmone and colleagues (2006), and Barros-Battesti and colleagues. (2013).

Larva: Integument striated, dorsal surface with 7–10 pairs of setae, dorsal plate large, elongate tapering slightly posteriorly; 2 pairs of eyes; ventral surface with 5 pairs of setae + 1 pair on valves; pulvilli present on all tarsi, not enlarged, claws present, Haller's organ with capsule aperture large and rounded, with posterior projections; hypostome long without corona, dental formula 2/2. *Nymph*: Camerostome and hood absent; hypostomal dentition 4/4; idiosoma panduriform, integument striated and spinous; spiracular plate cone-shaped; Haller's organ with capsule aperture transversely slit-like, elevated and large, bordered with prolonged pointed projections and with small setae internally. *Adult*: Integument granulated and with no change of pattern at the sides; small discs present; hood and eyes absent; hypostome vestigial, not functional to the hematophagy. The morphology is very similar between the 2 species, but the distance between the dorsal small discs in *Otobius megnini* is larger than in *O. lagophilus.*

Genus *Nothoaspis*

The following morphological descriptions are based on Nava and colleagues (2010) and Barros-Battesti and colleagues (2013) and Muñoz-Leal and colleagues (2019).

Larva: Dorsal plate with isosceles triangle shape occupying entire length of the dorsum of unfed specimens with a curvy-notched posterior margin; lateral margins of basis capitulum provided with a small bulge dorsal; surface with 12–13 pairs of setae; hypostome with apex pointed, dental formula 2/2 with 20 denticles in each row, corona absent. *Nymphs*: Idiosoma twice as longer as wide, anteriorly more abruptly narrowing than posteriorly; false shield covered by cells (irregular in shape and size) occupying the anterocentral area of dorsum, most of them at least with 1 seta; setae short, except for posterior margin of idiosoma, where setae are larger. Ventral surface with integument also covered by cells (irregular in shape and size), except for a narrow area located between coxae I and III; spiracular plate small; basis capituli subrectangular in outline, with 1 pair of post-hypostomal setae and at least 7 pairs of sublateral setae, bordered posteriorly by integumental fold; postpalpal setae absent; hood large, broadly rounded, not entirely covering capitulum, cheliceral blades, palpal articles II–IV visible dorsally; ventrally, article I forms elongate flaps protecting the pointed hypostome, dental formula 4/4 apically, 5/5 at base. *Adults*: Presence of false shield or nothoaspis (pseudoscutum), an anteriorly projecting hood covering the capitulum, a medial extension of palpal article I (flaps), genital plate extending from coxa I to IV, absence of 2 setae on the internal margin of the flaps, a small hypostome without denticles, presence of a central pore in the base of hypostome, and a reticulate surface pattern on the posterior half of the nothoaspis in males.

IXODID TICK GENERA

Genus *Ixodes*

The following morphological descriptions are based on Coley and Kohls (1945), Clifford and Anastos (1960), Clifford and colleagues. (1973), Nava and colleagues (2017), Apaneskevich and Lemon (2018), and Kwuat and colleagues (2018).

Larva: Anal groove anterior to anus; sensilla sagittiformia absent; with 2 pairs of post-hypostomal setae; eyes and festoons absent; 6 legs. *Nymph*: Anal groove anterior to anus; eyes and festoons absent; genital pore absent; spiracular plates circular ; 8 legs; nymphs are smaller than adults. *Adults*: An anal groove is present anterior to the anus, forming an arch; eyes and festoons are absent; an inornate scutum is present; spiracular plates are semicircular or oval; and there is a spur on the coxae. Sexual dimorphism is pronounced. The male venter is largely covered by 7 sclerotized plates. Males have ventral plates. Females have porose areas. The denticles of the female hypostome are well developed, while those of the male are usually few and small, often appearing only as mild crenulations.

Genus *Dermacentor*

The following morphological descriptions are based on Arthur (1960b), Yunker and colleagues (1986), Apanaskevich and Bermúdez (2013a), and Barker and Walker (2014).

Larva: Sensilla sagittiformia present; eyes present; a pair of posthypostomal setae; anal groove absent; 3 marginal dorsal setae anterior to the sensilla sagittiformia on dor-

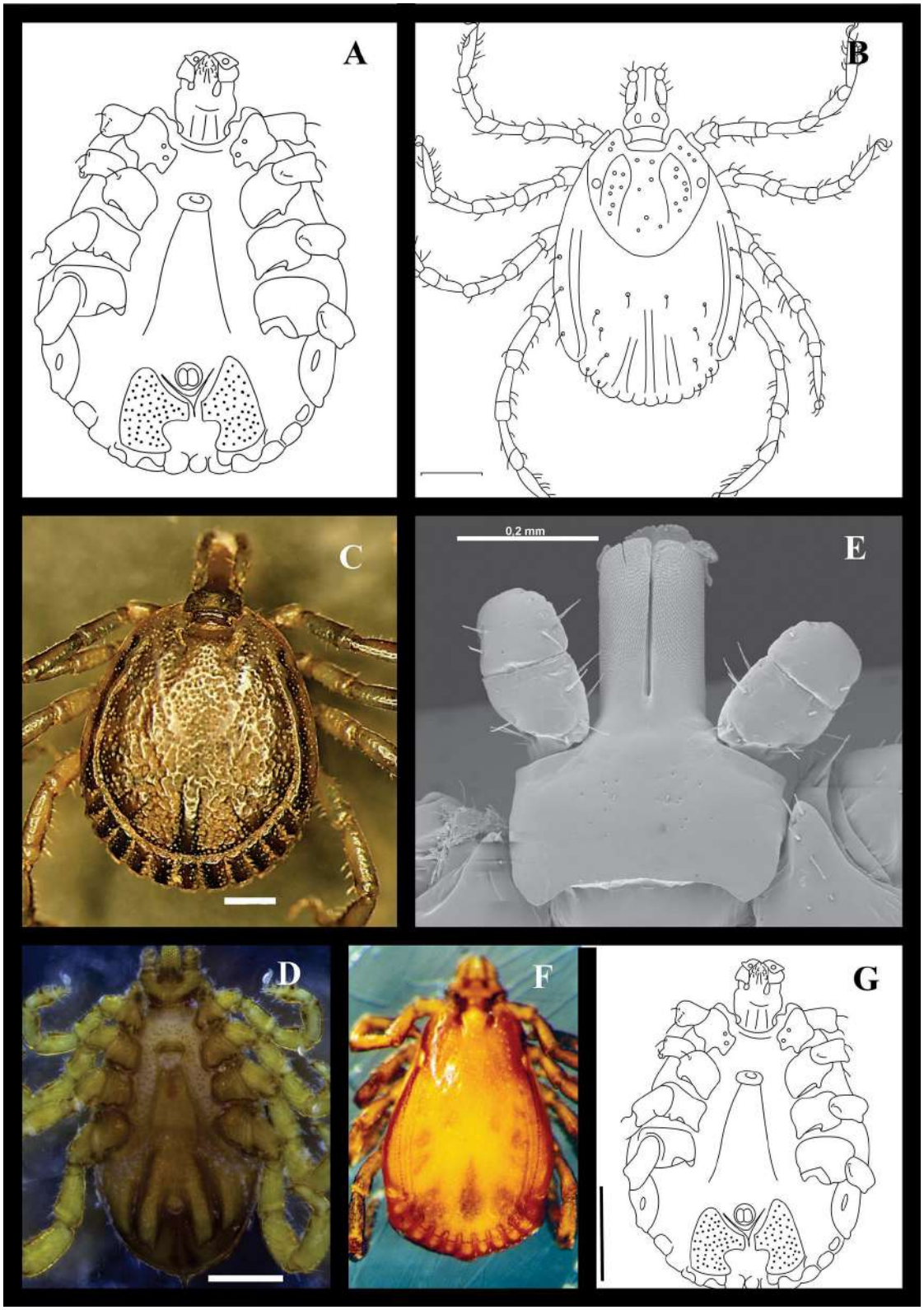

Figure 10. Adults of Ixodidae genera. A) *Nosomma* male, ventral view; B) *Hyalomma* female, dorsal view; C) *Amblyomma sculptum* male, dorsal view; D) *Rhipicephalus (Boophilus) microplus* male, ventral view; E) *Dermacentor* male, gnathosoma dorsal view; F) *Rhipicephalus sanguineus* s. l. male, dorsal view; G) *Rhipicentor* male, ventral view. Scale bars: C = 250 µm; D = 500 µm; E = 250 µm. Sources: A) Adapted from Prakasan and Ramani, 2007; B) adapted from Walker et al., 2003; C–F) D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres; G)adapted from Nuttall and Warburton, 1908.

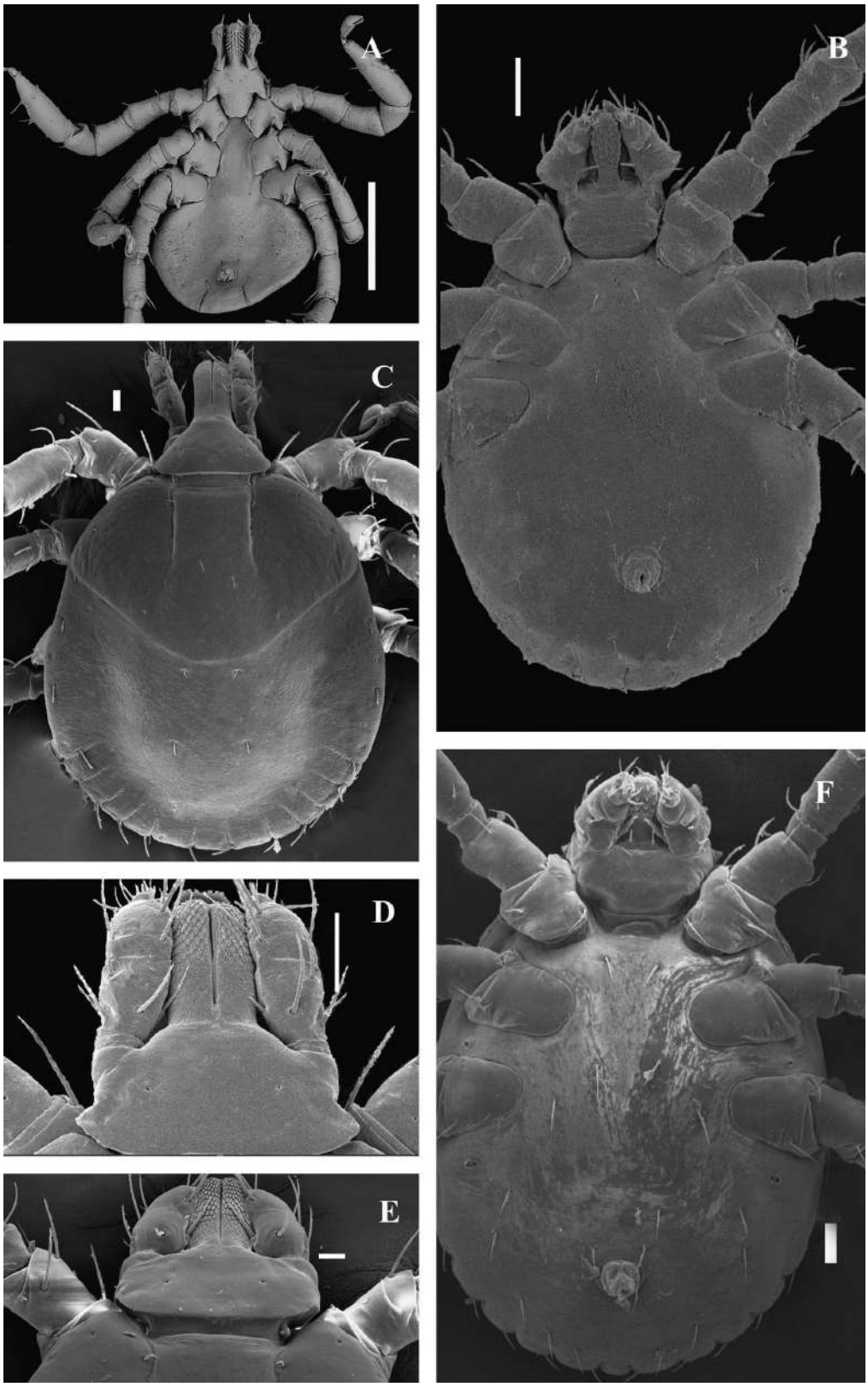

Figure 11. Larvae of Ixodidae genera. A) *Ixodes auritulus* group, ventral view; B) *Amblyomma romitii*, dorsal view; C) *Dermacentor nitens*, gnathosoma dorsal view; D) *Haemaphysalis juxtakochi*, ventral view; E) *Rhipicephalus microplus*, gnatosoma dorsal view; F) *Rhipicephalus sanguineus*, ventral view. Scale bars: A = 250 µm; B, F = 30 µm; C = 40 µm; D = 60 µm; E = 20 µm. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres. License: CC BY-NC-SA 4.0.

sal surface; idiosoma with 9 festoons. *Nymphs*: Eyes present; anal groove absent; spiracular plate circular to suboval with few and large goblet cells. *Adults:* Scutum in females usually ornate (inornate in *Dermacentor nitens* Neumann, 1897); anal groove contouring the anus behind; spiracular plates subcircular to comma-shaped (subcircular in *D. nitens* with large goblet cells); basis capituli more broad than long, rectangular dorsally; eyes on the scutum usually present and distinct. *Males*: Scutum usually ornate (inornate in *D. nitens*); anal groove posterior to the anus; coxae I–IV increase progressively in size; dorsal and posterior margins with festoons.

Genus *Amblyomma*

The following morphological descriptions are based on Klompen and colleagues (1996), Barbieri and colleagues (2007), and Nava and colleagues (2017).

Larva: Anal groove absent, sometimes indistinct; sensilla sagittiformia present on idiosomal dorsal segment VIII and sometimes on segment V; hypostome with denticles arranged in rows; with 1 pair of posthypostomal setae; eyes and festoons present. *Nymph*: Anal groove posterior to the anus; eyes and festoons present; spiracular plates comma-shaped. *Adults*: Scutum ornate with rare exceptions; anal groove posterior to the anus; eyes and festoons present; spiracular plates in comma shape; spurs on coxae usually present; ventral plates absent in most males; porose areas present in females.

Genus *Hyalomma*

The following descriptions are based on Apanaskevich and colleagues (2008).

Larva: Eyes present; portion of scutum posterior to eyes 1/5 to 1/4 of scutal length; apex of spur on coxae I directed posteriorly or medially; narrower palps and hypostome; shorter legs. *Nymph*: posterior margin of scutum broadly rounded with moderate posterolateral depressions on either side of its extremity; spiracular plates with relatively large, wide, blunt dorsal prolongation. *Adults:* Deep cervical grooves; in females scutum with sparse large punctations, small punctations usually very sparse or absent; narrow V-shaped genital operculum; preatrial fold of genital operculum flat or very slightly convex; posteromedial spur of coxa I broad and with blunt apex; males with broadly oval shape of conscutum; deep and long cervical grooves; short marginal grooves; large punctations sparse; smaller punctations normally sparse or absent; adanal plates distinctly curved medially; subanal plates moderate in size; dorsal prolongation of spiracular plates long.

Genus *Rhipicentor*

The following morphological descriptions are based on Cooper and Robinson (1908) and Clifford and Anastos (1960).

Larva: Palps short with 3 articles (article 1 absent); 3 marginal dorsal setae anterior to the sensilla sagittiformia; eyes present; idiosomal with 9 festoons. *Adults:* females with scutum as long as broad, with few punctations, posterior border sinuous; eyes present slightly anterior to the lateral angles; cervical grooves parallel; spiracular plates short, comma-shaped; basis capituli protuberant laterally, small cornua present. Male of *Rhipicentor nuttalli* has idiosoma oval, narrow in front, slightly concave just behind level of eyes; scutum covers entire dorsum, glabrous, polished, punctations not numerous, coarse and showing a tendency to arrange linearly; numerous fine punctations at posterior end of body immediately anterior to festoons; cervical grooves short and deep, crescentic with convexity outwards; marginal grooves well-defined, commencing a little distance behind the eyes and terminating at the external festoon on either side; posteromedian and accessory grooves shallow and ill-defined; eyes large and pale. Venter yellowish-brown with few scattered pale hairs; genital grooves parallel anteriorly, divergent behind coxae and extending to festoons; spiracles comma-shaped. Capitulum short (length 1.1 mm), basis capituli large, with pronounced lateral angles, cornua strong, short and blunt; palps short and broad; articles 2 and 3 rounded laterally; slight ventral retrograde tooth on article 3; hypostome slightly spatulate, dentition 3/3. Legs strong, coxa I with 2 strong spurs placed close together, the internal pointed, the external blunt; coxae II and III with very stumpy blunt spurs; coxa IV very large, with 2 long almost equal spurs, widely separated and slightly divergent.

Genus *Rhipicephalus*

The following morphological descriptions are based on Neumann (1900; 1901; 1904; 1905; 1907; 1911), Apanaskevich and colleagues (2013b), Horak and colleagues (2013), and Nava and colleagues (2018).

Larva: Eyes present, with 4 pairs of marginal dorsal setae anterior to the sensilla sagittiformia; idiosomal with 9 festoons; lateral sides of basis capituli acute or slightly angular. *Nymph*: Eyes present; basis capituli hexagonal, hypostomal dentition 3/3, small auriculae present in *Rhipicephalus sanguineus* (Latreille, 1806) and related species that forms the *R. sanguineus* group; anal groove distinct or indistinct. *Female*: Scutum inornate; eyes present; basis capituli dorsally hexagonal; palps short and round apically, dental formula 3/3 to 4/4;

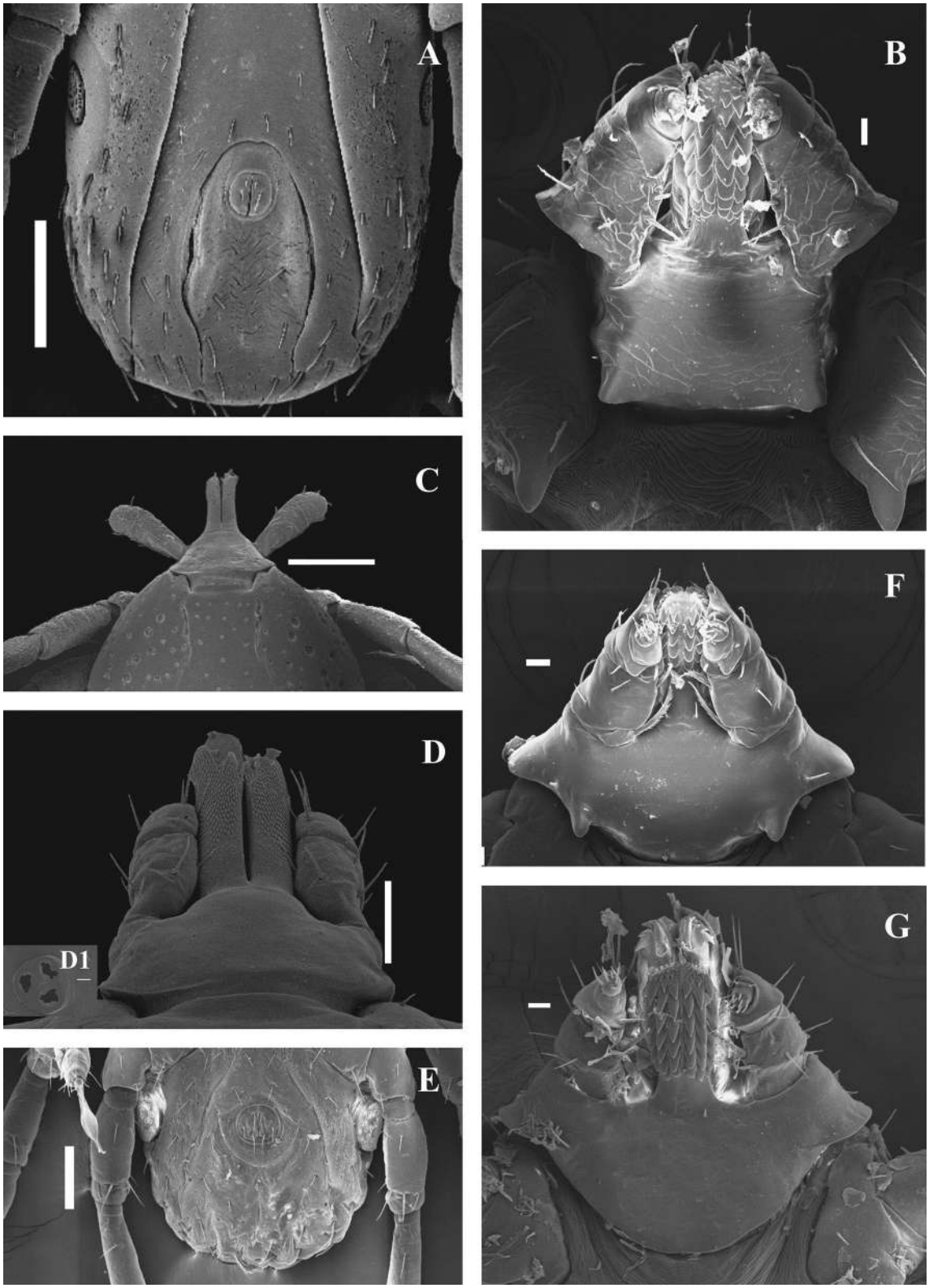

Figure 12. Nymphs of Ixodidae genera. A) *Ixodes luciae* anal groove, ventral view; B) *Haemaphysalis juxtakochi* gnathosoma, dorsal view; C) *Amblyomma longirostre*, dorsal view; D) *Dermacentor nitens* gnathosoma, dorsal view; d1) *Dermacentor nitens* spiracular plate; E) *Rhipicephalus sanguineus* s. l. anal groove, ventral view; F) *Rhipicephalus sanguineus* s. l. gnathosoma, ventral view; G) *Rhipicephalus microplus* gnathosoma, ventral view. Scale bars: A, E = 100 µm; B, G = 20 µm; C = 300 µm; D = 80 µm; d1 = 40 µm. Source: D. Moraes Barros-Battesti, V. Castilho Onofrio, and F. Dantas-Torres. License: CC BY-NC-SA 4.0.

anal groove distinct or indistinct; spiracular plates rounded to elongated. *Male*: Eyes present; basis capituli hexagonal dorsally; small cornua present or absent; palps short, palpal articles II and III with short, retrograde, internal process; dental formula 3/3 to 4/4; anal groove distinct or indistinct; spiracular plates round to elongated; adanal plates present, some species with caudal appendage.

Genus *Archaeocroton*

The description presented below was that proposed by Dumbleton (1943) and by Kaufman (1972) for *Archaeocroton sphenodonti.*

Larva: Large elongate cervical grooves. *Nymph*: not available. *Adults*: Idiosoma suboval in both, scutum light brown, inornate, with small and very numerous punctations evenly distributed; eyes absent. Male with cervical and marginal grooves, cervical grooves short, slightly divergent; lateral grooves distinct, incomplete, extending half the distance between the first festoons and scapulae; basis capitulum subtriangular, cornuae distinct, blunt; palps elongate and somewhat thickened; hypostome spatulate, dentition 3/3 distally and 2/2 proximally, with large corona; 2 files with about 5–6 stout denticles and partial innerfile with 5 very fine denticles; all coxae with a single subtriangular spur; tarsi very elongate. Female with scutum subcordiform, broader than long, cervical groove present; cervical pits deep, slightly concave externally; cervical grooves short and slightly divergent; cornua extremely broad, very blunt; palps elongate, thickened, article (segment) 2 about twice as long as article (segment) 3; a pair of very large conical spurs on either side of the midline of the ventral basis capituli; hypostome spatulate, dentition 3/3 distally and 2/2 proximally, with large corona; 2 files with circa 5–6 stout denticles and partial innerfile with 5 very fine denticles; genital aperture opposite level of coxae II; genital groove divergent; spiracular plate subcircular, as wide as long; all coxae with a subtriangular spur that is as long as broad, and tarsi very slightly humped, noticeably elongate and without spurs.

Genus *Anomalohimalaya*

The following morphological descriptions are based on Hoogstraal and colleagues (1970) and Filippova and Bardzimashvily (1992).

Larva: Basis capituli dorsally 3.5 times as broad as long, the ventrally posteroexternal junctures are at an angle; dorsally with 2 small sensilla hastiformia, ventrally 1 pair post-hypostomal setae, palps 2 times as long as broad; scutum 1.7 times as broad as long; anterior emargination broad, shallow; scapulae slightly rounded; external margin gradually diverging to convex posterior margin; eyes are large, slightly convex, pale area in each posteroexternal juncture; festoons present. *Nymph*: The nymph of *Anomalohimalaya cricetuli* has a smooth nitidous scutum with closely moved lateral and cervical grooves, forming a narrow deep short furrow, whereas in 2 other species the scutum is dull, the furrow between the lateral and cervical grooves is short and nearly reaches posterolateral margins of the scutum. Shape and location of the lateral projections of the basis capituli corresponds to those of female. Anal valves equally get narrow forward and backward, whereas in *A. lotozkyi* they are narrower anteriorly; eyes absent in *A. lamai*. *Adults*: Basis capituli dorsally broadly quadrangular, externally converging to narrower, straight posterior margin; anteroventrally flanged; palps clavate, 2 times as long as broad, article I extended ventrointernally; eyes absent; scutum broadly pyriform, rugose, lacking lateral grooves, cervical grooves indistinct, punctations especially large and numerous. Basis capituli hexagonal in females; the dorsal scutum in *A. cricetuli*, is pointed posteriorly and the genital opening is U-shaped, whereas in 2 other species the scutum is rounded posteriorly and the genital opening is V-shaped. *Anomalohimalaya cricetuli* differs from *A. lotozkyi* in that it has less pointed and shorter lateral projections of the gnathosoma basis, apexes of which are moved forward from the posterior margin, and by concave laterally porous area. It differs from *A. lama* by the absence of the dorsal process on the spiracular plate, and a short tooth on coxae IV.

Genus *Bothriocroton*

The following morphological descriptions are based on Klompen and colleagues (2002), Barker and Walker (2014), and Beati and colleagues (2008).

Larva: Hypostomal dentition in the adults 2/2 or 3/3, internal row much smaller than other rows; 3 large wax glands lateral near setae s6 (= marginal dorsal setae Md3), and anterior to the first festoons; large wax glands on festoon 5 absent; eyes absent; idiosomal setation pattern generally as in other Metastriata; scutum more broad than long; leg and palpal chaetotaxy as in *Amblyomma* sensu lato *Nymph*: Scutum similar to the females, with conspicuous posterolateral indentations formed by confluence of larger punctations; eyes absent; anal groove posterior to the anus; spiracular plates extruding from lateral body margins in *Bothriocroton oudemansi*. *Adult*s: Basis capituli subpentagonal in shape; eyes absent, but large punctations could be mistaken for an eye; hypostomal dentition 3/3 to 4/4; scutum of the males with partial or complete lateral grooves, with white ornamentation in some species; trochanters with a single subterminal ventral spur (absent in *B. glebopalma*); coxae I with anterior projection visible in some species; anal groove posterior to

the anus; large spiracular plates anterior to first festoon, extruding from lateral body margin in male and female of *B. oudemansi.*

Genus *Cosmiomma*

The following morphological descriptions are based on adults (Arthur, 1960b); immature stages are unknown.

Adults: Basis capituli subtriangular dorsally; palpal article 2 appreciably narrower than article 3, and about twice as long; enamel pigmentation on the palps, basis capituli, scutum, and legs; eyes well developed; 11 festoons in the female more or less clearly defined, with large spiracular plate abutting against the margins of the anterior festoons; female with anal groove encircling the anus and produced behind in a median groove; male with 1 pair of adanal plates, accessory and subanal plates absent. Coxa I with long external spur and prominent internal spur, divergent.

Genus *Haemaphysalis*

The following descriptions are based on Apanaskevich and colleagues (2007).

Larva: Eyes absent; scutum inornate; 2 marginal dorsal setae anterior to the sensilla sagittiformia on each side; article II of palps laterally produced beyond the basis capituli; idiosoma with 11 festoons; anal groove posterior to the anus. *Nymphs*: Eyes absent; basis capituli rectangular dorsally; scutum inornate; article II of palps laterally produced; article III of palps with retrograde ventral spur; spiracular plate suboval; anal groove posterior to the anus. *Adults:* Article II of palps laterally produced; article III of palps with retrograde ventral spur; eyes absent; scutum inornate; article II of palps laterally produced; article III of palps with retrograde ventral spur; in females the scutum is 1.3 times as long as broad, cervical grooves narrow arcs extending 2/3 of total scutal length; posterior lip of genital aperture broadly U-shaped; spiracular plates varying in size, irregularly suboval or subcircular, dorsal projection short, broadly triangular; in males the spiracular plate is variable in size, usually slightly broader than long, suboval, dorsal projection triangular, submarginal row of perforations on spiracular plate complete; coxal pore absent. In male, the basis capituli rectangular dorsally; article II of palps laterally produced; article III of palps with retrograde ventral spur; marginal groove absent.

Genus *Margaropus*

The following morphological descriptions are based on Arthur (1960b), Clifford and Anastos (1960), Walker and Laurence (1973), and Walker and colleagues (2003).

Larva: 5 marginal dorsal setae, Md5, located anteriorly to the dorsal sensilla sagittiformia, all other larval morphology resembles those of the genera *Dermacentor* and *Rhipicephalus*. *Nymph*: Idiosoma with long setae; basis capituli 3 times as wide as long, with straight basal margin, rounded junctures, and divergent lateral margins; eyes present; palps 4 times as long as wide; segment I forming a slight pedicle; segments 2 and 3 of approximately equal length and subrectangular; apex more or less bluntly rounded; segment 3 ventrally with a short, wide spur not reaching basal margin of segment; hypostome similar with smaller corona and 3/3 dentition in files of 8 denticles; spiracular plates subcircular, with 6 large goblets in a circle. *Adults*: Festoons absent; in females the scutum is inornate, widest at midlength between the scapulae and the eyes, posterior margin bluntly pointed; eyes present which may be indistinct; porose areas vertically subtriangular; palpal articles 2 and 3 separated by a slight constriction; coxae conical, unarmed but for a small spur posteriorly on coxa I, tarsi elongate, narrow, tapering with a large apical hook-like projection; integument bears conspicuous hairs posteriorly; spiracular plate subcircular, 3 rows of large goblets around the ostium; males with expanded leg articles that are more or less deeply separated from each other; scutum with lateral margins convex, more strongly convergent anteriorly, bluntly rounded behind, about a third as long again as it is wide; spiracular plates similar to the females; a pair of adanal plates present; caudal appendage present in *Margaropus winthemi*, broad and has a hook on the ventral surface; hypostome about twice as long as broad, slightly notched in the mid-line distally, and behind a well-defined corona the dentition is 4/4. This species is distinguished from *Rhipicephalus* (*Boophilus*) by the thick legs that are very conspicuous in males.

Genus *Nosomma*

The following morphological descriptions are based on Arthur (1965), Singh (1968), and Prakasan and Ramani (2007).

Larva: Basis capituli triangular dorsally, posterior margin slightly convex, lateral margins slightly sinuous and meet posterior margin to form sharp lateral points; ventrally basis triangular with posterior margin bow shaped; palps long and slender, reaching to the apex of hypostome, hypostome slender, dentition 2/2; eyes flat; cervical grooves shallow, narrow anteriorly but broadening posteriorly to almost reach the hind margin of scutum; dorsally without marginal grooves but with 9 distinct festoons. *Nymph*: Basis capituli dorsally triangular; posterior margin almost straight; posterolateral angle sharp and pointed. Ventrally posterior margin bow shaped; palps long and slender, reaching to the tip of hypostome, article 2 twice as long as article 3, dentition 2/2; posterior margin of scutum broadly rounded; eyes flat and situated at posterolateral corners of scutum, cervical grooves subparallel anteriorly

and diverging posteriorly reaching posterior margin; dorsal integument with faint marginal grooves and 11 well marked festoons; spiracular plates oval. *Adults: Nosomma* resembles *Dermacentor* in the shape of the capitulum and in having short palps; palpal articles 1, 2, and 3 of *Nosomma* are unlike those of known *Dermacentor* species in the possession of long, broad, strong sabre-like hairs on their infra-internal margin. Basis capituli of female is almost twice as broad as long; cornua prominent, basal breadth exceeding their length, broadly rounded; idiosomal with distinct median and paramedian grooves on the dorsal surface, posterior extremity of median groove continuous with depressions separating parma from adjacent festoons; 11 festoons present. Basis capituli of male is rectangular dorsally; palps conical lacking basolateral salience; median ridge like dorsal palpal spur; hypostome reaching apex of palps; hypostomal denticles formula 4/4; scutum outline elongated oval, brownish, whitish ornamentation present; cervical grooves short and deep; pseudoscutum well marked; eyes prominently colored; 11 festoons present, festoon 2 dorsally separated by well-marked sutures; palpal article III more broad than long with a stronger ventral process, hypostomal dentition 3/3; coxa I with 2 separated spurs; coxae II–IV with small spurs; tarsus IV with 2 ventral spurs, the distal one stronger than the proximal; ventral plates represented by adanal and accessory subadanal plates trilobed, middle lobes come close to each other behind the anal groove; anal groove posterior to the anus; spiracular plates comma-shaped.

Genus *Robertsicus*

Baker and Burger (2018) did not repeat the description for *Amblyomma elaphense* when the new combination for *Robertsicus elaphense* was proposed, but we present the one previously detailed by Keirans and Degenhardt (1985).

Larva: Outline suboval, widest at midlenght with 11 festoons; setae dorsally 13 pairs, all minute except for scutal central 1 (SC1); 2 central dorsal pairs; 8 marginal dorsal pairs, 2 of which are anterior to sensilla sagittiformia; supplementary setae absent; 3 scutal pairs; ventrally 15 pairs, 3 sternal pairs, 2 preanal pairs, 4–5 premarginal pairs, 5 marginal pairs, 1 pair on anal valves; palpal setae 10 on segment 4, 3 dorsally, 1 laterally, 2 ventrally on segment 3; 3 dorsally, 1 laterally, 2 ventrally on segment 2; 0 on segment 1; hypostome bluntly rounded apically with few minute hooklets, dental formula 2/2; scutum inornamented; eyes absent; cervical grooves and punctations absent; legs with small triangular external spur on coxae I–III, internal spurs absent; setae: coxa I with 3, coxae II and III with 2 setae each; Haller's organ with roof bifurcate; anterior pit setae: 1 porose, 2 fines, 1 fine or perhaps setiform. *Nymph*: Small, suboval, about as wide as long; scutum with scale-like markings over the surface, otherwise as in female; capitulum dorsally broadly triangular, corona absent; ventrally with hypostomal dentition 2/2, a small corona of minute denticles apically; legs each with a very small bluntly rounded spur on coxae I–IV; spiracular plate suboval, without dorsal prolongation. *Adults*: Inornate, light brown. Male with scutum smooth, without cervical or marginal grooves, setae and punctations minute, inapparent under binocular microscopy; capitulum dorsally subtriangular, lacking cornua; ventrally with hypostomal dentition 2/2 throughout, apically with a large corona of fine denticles; palps elongate; legs each with a single triangular spur on coxae I–IV; Haller's organ roof slit-like and slightly bifurcate medially, 5 anterior pit setae; spiracular plate suboval with a long narrow dorsal prolongation, globet cells minute. Female with scutum more broad than long, cordiform, smooth, without cervical grooves, setae and punctations minute; capitulum dorsally subtriangular, cornua absent; porose areas subcircular, shallow; ventrally with hypostomal dentition 2/2 (although the hypostome figured has a single supernumerary tooth between file 1 and 2 on the left side of the hypostome as viewed from above); legs with coxae as in male; genital aperture at level of coxae II; spiracular plate suboval with a short dorsal prolongation, goblet cells minute.

Genus *Nuttalliella*

The following morphological descriptions are based on Bedford (1931), Latif and colleagues (2012), and Mans and colleagues (2018).

Larva: Dorsum with a sclerotized scutum; cervical grooves and eyes absent; preanal groove present; anal plate with rows of denticles separated by the median post-anal groove; 5 posthypostomal setae present; apex of hypostome distinctly rounded, forming a ball-like structure, with 11 prominent denticles arranged in 2 rows. *Nymph*: Idiosoma circular; pseudoscutum with elevation between the cervical grooves; eyes absent; surface of alloscutum with dense-elevated and convoluted rosettes and setae in rosette pits; posthypostomal setae present; hypostomal denticles rudimentary; spiracular plates fenestrated and located posterior to coxa IV. *Female*: Idiosoma covered by leathery integument; scutum semi-sclerotized wider than long; preanal groove present; gnathosoma ventroapically, a pair of posthypostomal setae present; hypostomal denticles large and distinct arranged in 2 rows; palps 4-segmented; coxal organ absent; and spiracular plates fenestrated and located posterior to coxa IV. *Male*: Pseudoscutum present, covering most of the dorsum; chelicerae forming a unique rod-like structure similar to a spematodactyl in mites.

Medical and Veterinary Significance of Ticks

Ticks are obligate blood-sucking parasites with an almost worldwide distribution. As the second largest group of vectors of human disease agents (only trailing mosquitoes), ticks are among the most important vectors of pathogens causing disease in humans and other animals (Dantas-Torres et al., 2012). They are the most important ectoparasites of livestock in tropical and subtropical areas, and the diseases and direct damage caused by ticks are responsible for severe economic losses in livestock production (Jongejan and Uilenberg, 2004).

Human-tick interactions are extremely common, resulting in a great impact on human health. Due to saliva secretion during blood feeding, ticks transmit pathogens, such as viruses, bacteria, protozoa, and helminths, readily to hosts. Aside from mere irritation, their bite can also lead to allergy and even severe toxic conditions, such as paralysis and toxicosis in humans and other animals. Infection with multiple tick-transmitted pathogens can occur in an individual host after exposure to coinfected ticks or multiple ticks infected with different pathogens. The coinfection of individual ticks is a relatively frequent phenomenon and the same tick species may be a vector for different pathogens (Milutinovic et al., 2008; Nicholson et al., 2010), which may partially explain variations in clinical presentation, pathogenicity, and host response to therapy.

Pathogens ingested by a single larval tick may be passed through to subsequent developmental stages (that is, nymph and adult) through transstadial transmission from host to host (also called horizontal transmission) and, if a female is infected, may eventually be spread to her offspring through vertical or transovarial transmission. Female ticks are extremely fecund and may lay thousands of eggs, which enables effective dissemination of infectious agents.

Wildlife and ticks are the main reservoirs and vectors of tick-borne pathogens of medical and veterinary importance (Dantas-Torres et al., 2012b). Species of ticks that parasitize domestic animals are the most studied, while those that parasitize wildlife are still poorly understood as to their ability to transmit pathogens. Wild and domestic carnivores are considered the primary source of tick-borne zoonotic agents affecting humans (Otranto et al., 2015).

Ticks and tick-borne diseases have a zoogeographical range restricted by host movement and climatic factors. However, the increased mobility of humans and of domestic animals has resulted in a rapid extension of the zoogeographical ranges for many tick species and tick-transmitted pathogens. As such, the incidence of tick-borne diseases in humans and animals has increased in the 21st century (Estrada-Peña and Jongejan, 1999; Guglielmone et al., 2006). Additional factors associated with the emergence or re-emergence of vector-borne diseases include global warming (and resultant climate change), increased outdoor recreation, global travel, urbanization, encroachment of human development on natural environments, deforestation, and habitat fragmentation, which together promote greater contact between ticks, wildlife, humans, and domestic animals (Beugnet and Chalvet-Monfray, 2013; Dantas-Torres, 2015).

The major zoonoses whose causative agents are transmitted by ticks are rickettsioses, borrelioses, ehrlichiosis, and babesiosis. Rickettsioses are mainly associated with ticks of the genera *Amblyomma, Dermacentor, Ixodes*, and *Rhipicephalus*, borrelioses to *Ixodes* and *Ornithodoros*, and ehrlichiosis and babesiosis mostly to *Rhipicephalus* (Barros-Battesti et al., 2006). These diseases and other zoonotic tick-borne illnesses, such as those of viral origin, characterized by encephalitis and hemorrhagic fevers, are the major cause of host morbidity and mortality (Jongejan and Uilenberg, 2004; Dantas-Torres et al., 2012b). In the following sections, the main tick-borne diseases of humans and other animals are summarized.

Anaplasmosis

Anaplasmosis is a disease caused by gram-negative bacteria of the order Rickettsiales, family Anaplasmataceae, and genus *Anaplasma* (Dumler et al., 2001).

Bovine anaplasmosis occurs in tropical, subtropical, and temperate regions of the world, and is caused by the intraerythrocytic rickettsia *Anaplasma marginale*, a member of the ehrlichial genogroup II (Dumler et al., 2001); *A. marginale* is transmitted biologically by ixodid ticks, by hematophagous insects, and mechanically by needles contaminated with blood of infected animals.

After inoculation of a suitable bovine host, and after an incubation period of 20 to 40 days, there is an increase in rickettsemia, resulting in anemia, weight loss, abortion, and death (Richey, 1981). The disease can have serious consequences, especially when susceptible animals are introduced into endemic areas. In this case, mortality may exceed 50%, causing serious problems to genetic breeding programs, based on the importation of animals from disease-free areas (Machado, 1995).

Combining tick control and vaccination results in the most effective measure against this disease (Palmer, 1989). Immunized cattle may develop persistent field infections, acting as reservoirs of *Anaplasma marginale* helping to maintain the pathogen circulation in endemic areas.

The most important species in dogs is *Anaplasma platys*, which mainly infects platelets and causes infectious canine cyclic thrombocytopenia (Ferreira et al., 2007). It is generally found in coinfections with *Babesia* and *Ehrlichia*, and

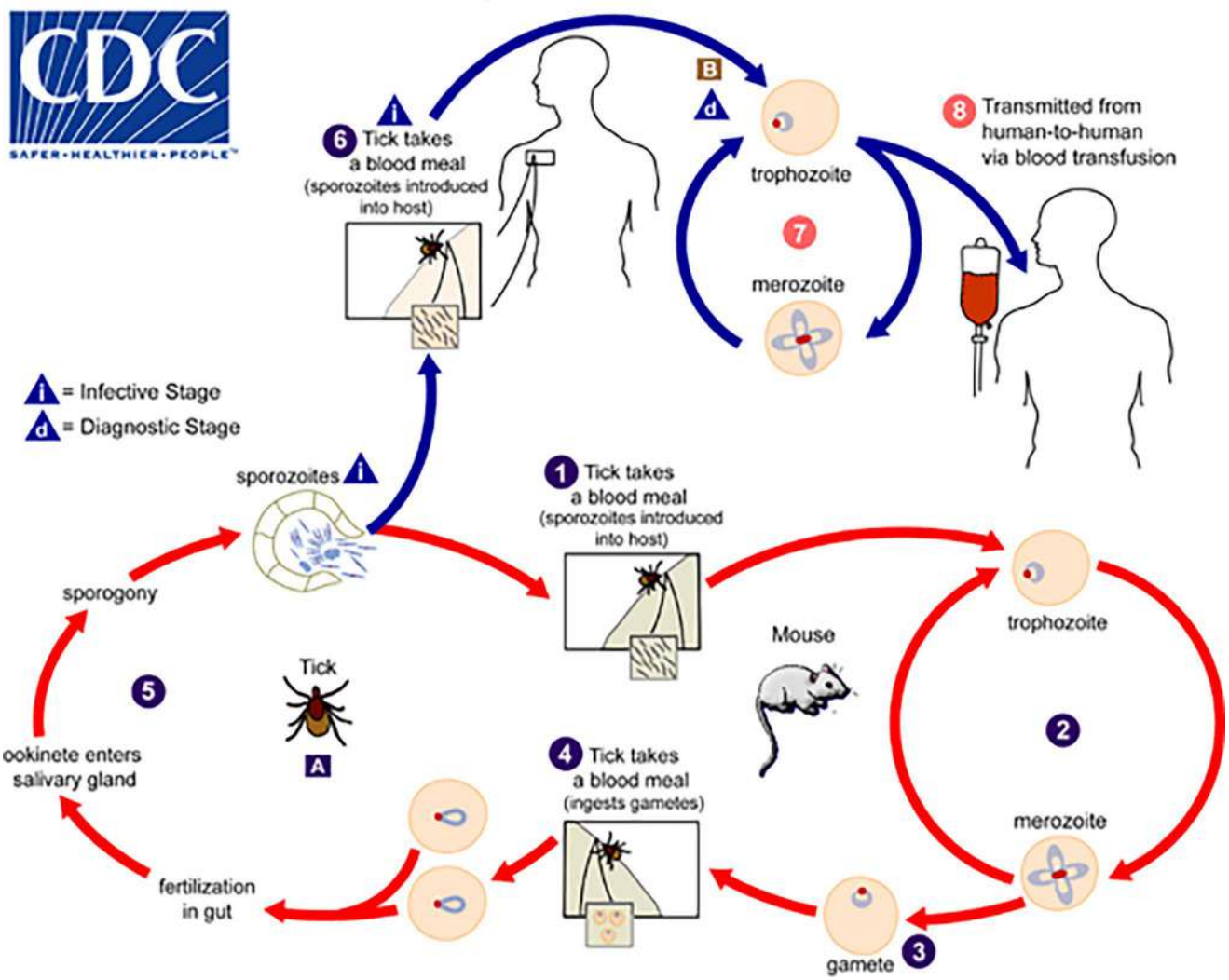

Figure 13. Babesiosis life cycle. Babesiosis is caused by apicomplexan parasites of the genus, *Babesia*. While more than 100 species have been reported, only a few have been identified as causing human infections, including *B. microti*, *B. divergens*, *B. duncani*, and a currently unnamed strain designated MO-1. — The *Babesia microti* life cycle involves 2 hosts, which includes a rodent, primarily the white-footed mouse, *Peromyscus leucopus*, and a tick in the genus *Ixodes*. During a blood meal, a *Babesia*-infected tick introduces sporozoites into the mouse host (1). Sporozoites enter erythrocytes and undergo asexual reproduction (budding) (2). In the blood, some parasites differentiate into male and female gametes although these cannot be distinguished at the light microscope level (3). The definitive host is the tick. Once ingested by an appropriate tick (4), gametes unite and undergo a sporogonic cycle resulting in sporozoites (5). Transovarial transmission (also known as vertical, or hereditary, transmission) has been documented for "large" *Babesia* spp. but not for the "small" babesiae, such as *B. microti* (A). — Humans enter the cycle when bitten by infected ticks. During a blood meal, a *Babesia*-infected tick introduces sporozoites into the human host (6). Sporozoites enter erythrocytes (B) and undergo asexual replication (budding) (7). Multiplication of the blood stage parasites is responsible for the clinical manifestations of the disease. Humans are, for all practical purposes, dead-end hosts and there is probably little, if any, subsequent transmission that occurs from ticks feeding on infected persons. However, human to human transmission is well recognized to occur through blood transfusions (8).

Rhipicephalus sanguineus sensu lato is suspected to be a vector, though its role remains unproven (Dantas-Torres, 2008; Ribeiro et al., 2017). This pathogen is widespread on several continents but has a predilection for tropical and subtropical regions (Ferreira et al., 2007).

Anaplasma phagocytophilum is incriminated as the causal agent of the human granulocytic anaplasmosis (HGA) in the Northern Hemisphere, tick-borne fever in cattle and sheep in Europe, and equine and canine granulocytic anaplasmosis in the United States (Woldehiwet, 2010; André, 2018).

Babesiosis

Babesiosis is caused by tick-transmitted intraerythrocytic protozoa of the order Piroplasmida, family Babesiidae, and genus *Babesia* (see Figure 13). *Babesia* protozoa are one of the most common blood parasites in the world and they have a wide host range, including mammals and bird species (Schnittger et al., 2012). Hard ticks are the known vectors of these protozoa, such as *Rhipicephalus sanguineus* sensu lato that transmits *B. vogeli* (Gray et al., 2010; René et al., 2012; Silva et al., 2012).

The disease can occur in subclinical, acute, hyperacute, or chronic forms, ranging from mild clinical signs to fatal disease. Severity of illness depends on many factors, such as *Babesia* species and immunocompetence of the patient (Schetters et al., 1997; Gray et al., 2010; Yabsley and Shock, 2013).

Bovine babesiosis (BB) is a tick-borne disease of cattle caused by protozoan parasites of the genus *Babesia* (phylum Apicomplexa, order Piroplasmida). The principal species of *Babesia* that cause BB are: *Babesia bovis, B. bigemina*, and *B. divergens.* Other species that can infect cattle include *B. major, B. ovata, B. occultans,* and *B. jakimovi. Rhipicephalus* tick species are most commonly involved in the transmission of this disease, generally in tropical and subtropical countries (WOAH, 2021).

Canine babesiosis may be caused by several species of *Babesia*, which are usually classified as small and large *Babesia*. Small *Babesia* species include *B. gibsoni*, *B. conradae*, and *B. microti*-like (also referred to *Theileria annae* and *B. vulpes*, but these are nomina nuda, meaning that the scientific names are used but without the necessary accompanying scientific description), whereas large *Babesia* species include *B. canis*, *B. vogeli*, *B. rossi*, and an unclassified species ("*Babesia* sp. Coco") found in dogs in North Carolina, United States (Citard et al., 1995; Schetters et al., 1997; Köster et al., 2015; Solano-Gallego et al., 2016). *Babesia gibsoni* is transmitted by *Haemaphysalis longicornis* Neumann, 1901 in Asia and possibly by blood exchange during dog fights. *Babesia canis* is transmitted by *Dermacentor reticulatus* in Europe, *B. vogeli* is transmitted by *R. sanguineus* sensu lato in tropical and subtropical regions, and *B. rossi* is transmitted by *H. elliptica* (Koch, 1844) in southern Africa (Uilenberg et al., 1989; Sasaki et al., 2007; Köster et al., 2015). The vectors of *B. conradae* and *B. microti*-like remain unknown.

Ehrlichiosis

Ehrlichiosis is a disease caused by several species of obligate intracellular gram-negative bacteria of the genus *Ehrlichia* that infect humans and other animals in different parts of the world (Dumler et al., 2001).

Human ehrlichiosis is caused by *Ehrlichia chaffeensis* (human monocytic ehrlichiosis), *E. ewingii* (human granulocytic ehrlichiosis), or *E. muris eauclairensis* (undetermined ehrlichiosis) (Dantas-Torres et al., 2012b; Pritt et al., 2017). *Ehrlichia chaffeensis* is the most common causative agent of human ehrlichiosis in the United States. It is maintained in a cycle that involves the white-tailed deer (*Odocoileus virginianus*) and the lone star tick *Amblyomma americanum* (Linnaeus, 1758), which play a role as primary reservoir and vector, respectively (Skotarczak, 2003; Yabsley, 2010). Even though white-tailed deer seem to be the main host for *E. chaffeensis*, serological and molecular evidence of infection by this agent has been reported in wild carnivores (André, 2018). *Amblyomma americanum* also transmits *E. ewingii*, while the vector of *E. muris eauclairensis* is *Ixodes scapularis* Say, 1821.

Canine monocytic ehrlichiosis (CME) is a life-threatening disease in dogs that requires rapid and accurate diagnosis in order to initiate appropriate therapy (Skotarczak, 2003). *Ehrlichia canis*, the agent of CME, infects monocytes and macrophages of domestic dogs and wild carnivores (Stich et al., 2008). *Rhipicephalus sanguineus* sensu lato and *Dermacentor variabilis* (Say, 1821) are the recognized vectors for *E. canis* (Johnson et al., 1998; Dantas-Torres, 2008). The disease is described around the world, but CME appears to be particularly prevalent in tropical regions where it is principally vectored by *R. sanguineus* sensu lato (Cicuttin et al., 2015). In South America, the occurrence of CME in tropical regions is related to the difference in vector competence. Populations of *R. sanguineus* sensu lato belonging to the tropical lineage are highly competent vectors of *E. canis*, while South American populations of *R. sanguineus* sensu stricto (= temperate lineage) are incompetent vectors of *E. canis*, which partly explains the scarcity or absence of CME in colder regions of South America (Nava et al., 2012; Moraes-Filho et al., 2015).

Lyme disease (or Lyme borreliosis)

Lyme borreliosis (LB) is the most frequent tick-borne disease in the Northern Hemisphere. The disease is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex, which are transmitted by several tick species of the genus *Ixodes* (Gray et al, 2002; Rauter and Hartung, 2005). LB is recognized as the most commonly reported arthropod-borne disease in North America and Europe, accounting for thousands of new cases yearly in both regions (Piesman and Eisen, 2008; Marques, 2010; CDC, 2017).

In most cases, the tick must be attached to its mammalian host for 36 to 48 hours or more before the bacteria can be transmitted. Typical symptoms include fever, headache, fatigue, and a characteristic skin rash called erythema migrans (CDC, 2017). The complications of untreated LB in humans can be severe and disabling (Dennis and Hayes, 2002).

Rickettsioses

Tick-borne rickettsioses are caused by intracellular bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia* and are among the oldest known vector-borne diseases of humans. The importance of the recognized rickettsial pathogens has increased in the past several years. Several species of tick-borne rickettsiae that were considered nonpathogenic for decades are now associated with human infections, such as *R. slovaca, R. aeschlimannii, R. massiliae, R. monacensis*, and *R. parkeri*. New species of *Rickettsia* of undetermined pathogenicity continue to be detected in or isolated from ticks around the world (Labruna et al., 2011; Parola et al., 2013).

Ticks can be reservoirs and vectors for most species of *Rickettsia*. Bacteria remain in tick populations by transovarial and transstadial transmission. However, some rickettsiae may also be deleterious to ticks, such as *R. rickettsii* (Labruna, 2009).

Vertical transmission of rickettsial agents in arthropods helps to maintain the infection in nature, but for some species of rickettsiae, a life cycle including infected arthropods and 1 or more amplifying hosts is required to guarantee survival of the bacteria (Davoust et al., 2010). Humans are only occasional hosts for ticks and, thus, play no role in maintaining these bacteria in nature (Socolovschi et al., 2009).

Rickettsia rickettsii is the most pathogenic *Rickettsia* species, and the disease caused by this agent is generally called Rocky Mountain spotted fever (RMSF), because it was first reported in the Rocky Mountain region of the United States (CDC, 2006). In Brazil, the disease is Brazilian spotted fever and has a high fatality rate (Oliveira et al., 2016). Despite the availability of accurate diagnostic tools and efficacious therapy, RMSF continues to be a life-threatening disease, with high lethality rate in several endemic geographic foci. The disease has been shown to have a complex ecology with participation of different vertebrate animals and tick species (CDC, 2006).

Several tick species have been implicated as vectors of *Rickettsia rickettsii* accordingly to different geographic areas. While *Dermacentor andersoni* Stiles, 1908 and *D. variablilis* are the main vectors in the United States, ticks of the *Amblyomma cajennense* (Fabricius, 1787) species complex, such as *A. cajennense* sensu stricto and *A. sculptum* (Berlese, 1888), have been implicated as the most important vectors in South America, mainly in Brazil (Labruna et al., 2017). Moreover, *R. sanguineus* sensu lato has been implicated as a vector in Mexico and the United States (Dantas-Torres, 2007).

Rickettsia parkeri is another SFG rickettsia recognized as a human pathogen, with several confirmed cases in the United States. The first confirmed human infection with *R. parkeri* was reported in the United States in 2004, more than 60 years after this bacterium was first isolated in that country, from the Gulf Coast tick *Amblyomma maculatum* Koch, 1844 (Paddock et al., 2004). *Rickettsia parkeri* rickettsiosis can be difficult to distinguish from RMSF and other spotted fevers, especially during the early stages. A retrospective study provided serological evidence that a number of cases previously diagnosed as RMSF in the United States were actually caused by *R. parkeri,* suggesting that both rickettsioses have been misidentified in that country. This disease is characteristically less severe than RMSF and almost always associated with an inoculation eschar (an ulcerated, necrotic lesion) at the site of tick attachment (Paddock et al., 2004).

Several other SFG rickettsia (for example, *Rickettsia conorii*) are important human pathogens and may also infect and cause disease in animals, such as dogs, in Europe and elsewhere in the world. Comprehensive information about other rickettsiae infection humans can be found elsewhere (Dantas-Torres et al., 2012a; Parola et al., 2013; Portillo et al., 2015).

Epidemiological Tick Control and Preventative Measures

Human behavior (for example, sitting on logs, gathering wood, leaning against trees, and walking) might increase the risk of exposure to ticks (Lane et al., 2004). For instance, people visiting forested areas might be exposed to hard ticks whereas people entering tick-infested caves and encountering rodent burrows might be exposed to soft ticks.

Strategies to reduce populations of vector ticks through area-wide application of acaricides and control of tick habitats (for example, clearing leaf litter and brush) have been

effective in small-scale trials. Community-based, integrated, tick-management strategies may prove to be an effective public health response to reduce the incidence of tick-borne infections. However, limiting exposure to ticks is currently the most effective method of prevention (Dantas-Torres, 2007; Pinter et al., 2011).

Whenever possible, areas that are likely to be infected with ticks should be avoided, particularly in the seasons in which larvae and nymphs feed and can be found in abundance. Ticks are commonly found in humid and shady environments, especially grassy or litter areas with low-lying vegetation.

From a practical perspective, it is unreasonable to assume that a person can eliminate all activities that may result in tick exposure. Therefore, measures should be aimed at personal protection (Dantas-Torres, 2007; Piesman and Eisen, 2008; CDC, 2006; 2017). The following measures are recommended:

- When walking through forested areas or with shrubby vegetation, avoid places potentially infested by ticks, and if possible, walk in the center of trails.
- Wear long-sleeved shirts and long trousers (not shorts) and tuck pant bottoms into tops of socks or boots. Wear light colored clothing which makes it easier to find crawling ticks.
- Check often for ticks especially after leaving forested areas. Common sites of attachment include the groin, the underarms, the nape of the neck, around the waist, and behind the knee.
- Examine children more often, paying special attention to the head, neck, and ears. Teach them to avoid tall grass and low brush.
- Do not let pets roam freely in these areas, and if they are allowed to go to these spots, check them daily, especially if allowed indoors. Free-roaming pets may carry ticks of all life stages and can be infected with tick-borne diseases. This is rather important for companion animals living in close contact with humans.
- To remove attached ticks, use fine-tipped tweezers or shield your fingers with a tissue, paper towel, or rubber gloves. Avoid removing ticks with bare hands.
- Grasp the tick with the tweezers as close as possible to the surface of the skin, turn it gently, and from time to time pull upward with steady, even pressure.
- Do not squeeze, crush, or puncture the body of the tick because its fluids (saliva, body fluids, gut contents) may contain infectious organisms.
- Save the tick for identification and potentially test for pathogens. This may help your doctor make an accurate diagnosis if you become sick.

Supplemental Materials

Supplemental documents are available online including keys for the identification of tick families and genera, and a list of extant species described chronologically from 1758 to October 2019.

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Arthropoda

Acari (Order): Mites

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Phylum Arthropoda

Order Acari

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Chapter 67

Acari (Order): Mites

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Introduction

Mites (order Acari or order Acarina) are the most diverse and abundant of all arachnids, but because of their small size (usually less than a millimeter in length) they are rarely seen. Red velvet mites are among the giants of the Acari (up to 10 mm) and can often be seen hunting on the ground or on tree trunks. Water mites are rarely more than a few millimeters long, but their bright colors and rapid movement are eye-catching. At the smaller end of the mite size range are species like the human hair follicle mite *Demodex folliculorum* or the honeybee tracheal mite, small enough to raise a family within a human hair follicle or within a bee's respiratory tube, and too small (about 0.1 mm) to see without a microscope.

Mites are also among the oldest of all terrestrial animals, with fossils known from the early Devonian Era, nearly 400 Ma (= million years ago; Norton et al., 1988; Kethley et al., 1989). Three major lineages are currently recognized: superorders Opilioacariformes, Acariformes, and Parasitiformes (Krantz, 1978; Johnston, 1982; Evans, 1992). About 45,000 species of mites have been described; a small fraction (perhaps 5%) of the number of species estimated to be alive today.

Mites are truly ubiquitous. They have successfully colonized nearly every known terrestrial, marine, and freshwater habitat including polar and alpine extremes, tropical lowlands and desert barrens, surface and mineral soils to depths of >10 m, cold and thermal surface springs and subterranean waters with temperatures as high as 50 °C, all types of streams, ponds and lakes, and sea waters of continental shelves and deep-sea trenches to depths of 5,000 m. Some idea of mite abundance and diversity can be gained from analysis of 1 square m of mixed temperate hardwood or boreal coniferous litter, which may harbor upwards from 1 million mites representing 200 species in at least 50 families. Within this complex matrix of decomposing plant matter, mites help to regulate microbial processes directly by feeding on detritus and microbes, and indirectly by predation on other microfauna.

Many mites have complex symbiotic associations with the larger organisms on which they live. Plants, including crops and the canopies of tropical rainforests, are inhabited by myriads of mite species feeding on mosses, ferns, leaves, stems, flowers, fruit, lichens, microbes, other arthropods, and each other. Many mites found on agricultural crops are major economic pests (for example, spider mites) or useful biocontrol agents (for example, phytoseiid mites) of those pests. Mammals and birds are hosts to innumerable species of parasitic mites (for example, scabies and mange mites), as are many reptiles and some amphibians. Insects, especially those that build nests, live in semipermanent habitats like decaying wood, or use more ephemeral habitats like bracket fungi and dung, are hosts to a cornucopia of mite commensals, parasites, and mutualists. None of these mites exceed a cm in length, and the vast majority grow to less than a mm, yet they often have a major impact on their hosts.

Characteristics

The Acari can be defined by the following characteristics:

- Hexapod prelarva (lost in Parasitiformes and many derived Acariformes)
- Hexapod larval stage
- Three octopod nymphal stages (variously abbreviated in derived taxa)
- Gnathosoma delimited by a circumcapitular suture
- Palpcoxal endites fused medially forming a hypostome
- Hypostome with rutella or corniculi (lost in many derived Acariformes)
- Loss of external evidence of opisthosomal segmentation, that is, without tergites or sternites
- Ingestion of particulate food (lost in many derived taxa).

Figures 1–3 include images of morphological characters of mites.

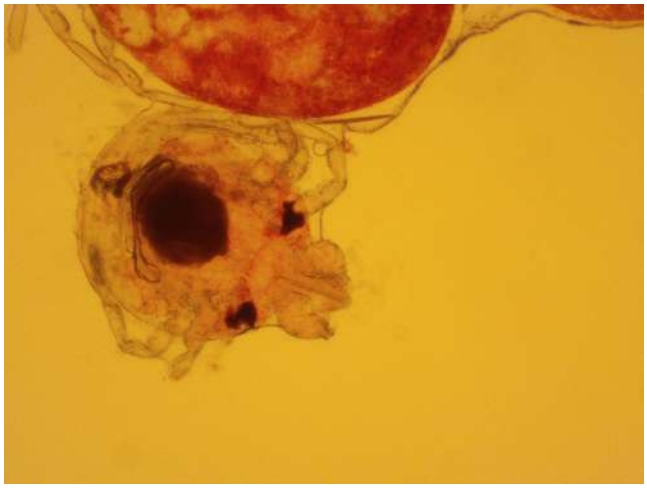

Figure 1. Larval mite of the genus *Hydrachna* that was removed from the wing of a backswimmer (genus *Notonecta*). The lateral dark spots are the eyes. Source: S. L. Gardner, HWML. License: CC BY.

Figure 2. *Ornithonyssys bacoti*, a mite (Acari: Mesostigmata: Macronyssidae) from the skin of a rodent (*Microtus ochrogaster*) collected at Cedar Point Biological Station, near Ogallala, Nebraska, United States, 2015. Source: S. L. Gardner, HWML. License: CC BY.

Scabies

Scabies is an infestation of the skin by the human itch mite (*Sarcoptes scabiei* var. *hominis*). The microscopic scabies mite burrows into the upper layer of the skin where it lives and lays its eggs. The most common symptoms of scabies are intense itching and a pimple-like skin rash. The scabies mite usually is spread by direct, prolonged skin-to-skin contact with a person who has scabies.

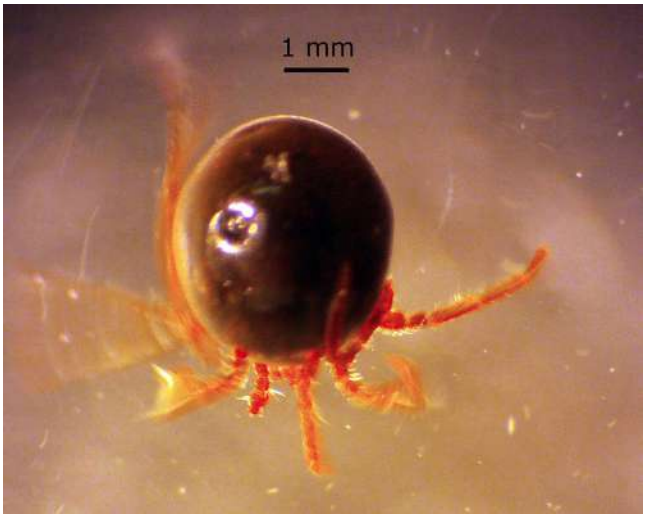

Figure 3. Aquatic mite, adult female collected from a cattle tank in the Sandhills of southwestern Nebraska, United States. The adults are free-living and the larvae are parasitic on backswimmers. Source: S. L. Gardner, HWML. License: CC BY.

Scabies is found worldwide and affects people of all races and social classes. Scabies can spread rapidly under crowded conditions where close body and skin contact is frequent. Institutions such as nursing homes, extended-care facilities, and prisons are often sites of scabies outbreaks. Child-care facilities also are a common site of scabies infestations.

Causal Agent of Scabies

Sarcoptes scabiei var. *hominis* is in the arthropod class Arachnida, order Acari, family Sarcoptidae. The mites burrow into the upper layer of the skin but never below the stratum corneum. The burrows appear as tiny raised serpentine lines that are grayish or skin-colored and can be a cm or more in length. Other races of scabies mites may cause infestations in other mammals, such as domestic cats, dogs, pigs, and horses. It should be noted that races of mites found on other animals may cause a self-limited infestation in humans with temporary itching due to dermatitis; however, they do not multiply on the human host.

Life Cycle of *Sarcoptes scabiei* var. *hominis* (Figure 4)

Sarcoptes scabiei undergoes 4 stages in its life cycle: Egg, larva, nymph, and adult. Females deposit 2–3 eggs per day as they burrow under the skin. Eggs are oval and 0.10 to 0.15 mm in length and hatch in 3 to 4 days. After the eggs hatch, the larvae migrate to the skin surface and burrow into the intact stratum corneum to construct almost invisible, short burrows called molting pouches. The larval stage, which emerges from the eggs, has only 3 pairs of legs and lasts about 3 to 4

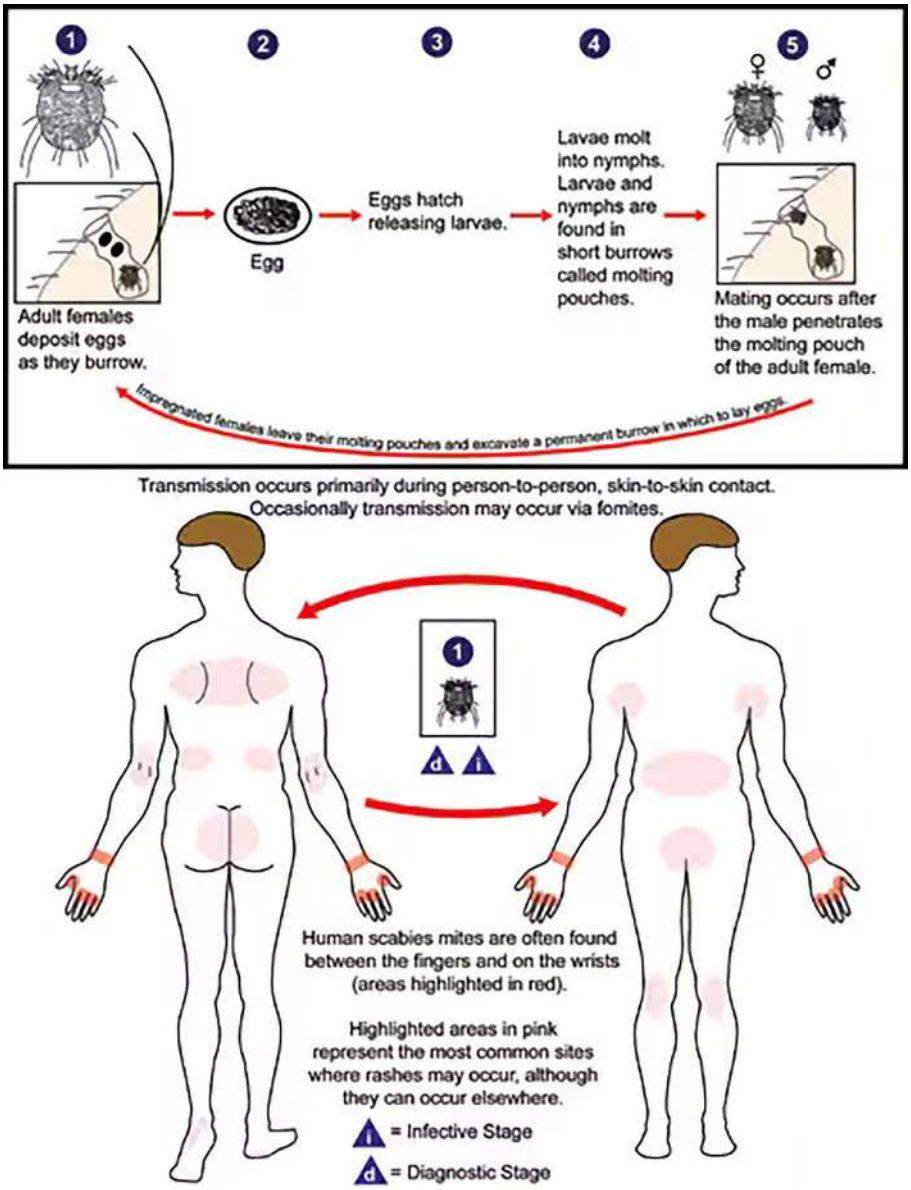

Figure 4. *Sarcoptes scabiei* life cycle. *Sarcoptes scabiei* undergoes 4 stages in its life cycle: Egg, larva, nymph, and adult. Females deposit 2–3 eggs per day as they burrow under the skin (1). Eggs are oval and 0.10 to 0.15 mm in length (2) and hatch in 3–4 days. After the eggs hatch, the larvae migrate to the skin surface and burrow into the intact stratum corneum to construct almost invisible, short burrows called molting pouches. The larval stage, which emerges from the eggs, has only 3 pairs of legs (3) and lasts about 3–4 days. After the larvae molt, the resulting nymphs have 4 pairs of legs (4). This form molts into slightly larger nymphs before molting into adults. Larvae and nymphs may often be found in molting pouches or in hair follicles and look similar to adults, only smaller. Adults are round, sac-like eyeless mites. Females are 0.30 to 0.45 mm-long and 0.25 to 0.35 mm-wide, and males are slightly more than half that size. Mating occurs after the active male penetrates the molting pouch of the adult female (5). Mating takes place only once and leaves the female fertile for the rest of her life. Impregnated females leave their molting pouches and wander on the surface of the skin until they find a suitable site for a permanent burrow. While on the skin's surface, mites hold onto the skin using sucker-like pulvilli attached to the 2 most anterior pairs of legs. When the impregnated female mite finds a suitable location, it begins to make its characteristic serpentine burrow, laying eggs in the process. After the impregnated female burrows into the skin, she remains there and continues to lengthen her burrow and lay eggs for the rest of her life (1–2 months). Under the most favorable of conditions, about 10% of her eggs eventually give rise to adult mites. Males are rarely seen; they make temporary shallow pits in the skin to feed until they locate a female's burrow and mate. Transmission occurs primarily by the transfer of the impregnated females during person-to-person, skin-to-skin contact. Occasionally transmission may occur via fomites (for example, bedding or clothing). Human scabies mites often are found between the fingers and on the wrists. Source: Division of Parasitic Diseases and Malaria, United States Centers for Disease Control and Prevention, 2017. Public domain.

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Mating occurs after the active male penetrates the molting pouch of the adult female. Mating takes place only once and leaves the female fertile for the rest of her life. Impregnated females leave their molting pouches and wander on the surface of the host's skin until they find a suitable site for a permanent burrow. While on the surface of the host's skin, mites hold onto the skin using sucker-like pulvilli attached to the 2 most anterior pairs of legs. When the impregnated female mite finds a suitable location, it begins to make its characteristic serpentine burrow, laying eggs in the process. After the impregnated female burrows into the skin, she remains there and continues to lengthen her burrow and lay eggs for the rest of her life (1–2 months). Under the most favorable of conditions, about 10% of her eggs eventually give rise to adult mites. Males are rarely seen; they make temporary shallow pits in the skin to feed until they locate a female's burrow and mate.

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Some immunocompromised, elderly, disabled, or debilitated persons are at risk for a severe form of scabies called crusted, or Norwegian, scabies. Persons with crusted scabies have thick crusts of skin that contain large numbers of scabies mites and eggs. The mites in crusted scabies are not more virulent than in non-crusted scabies; however, they are much more numerous (up to 2 million per patient). Because they are infested with such large numbers of mites, people with crusted scabies are very contagious to other people. In addition to spreading scabies through brief direct skin-to-skin contact, persons with crusted scabies can transmit scabies indirectly by shedding mites that contaminate items such as their clothing, bedding, and furniture. Persons with crusted scabies should receive quick and aggressive medical treatment for their infestation to prevent outbreaks of scabies.

Phylogenetic Relationships (based on research through 1996)

Traditionally, the mites have been treated as a subclass of the Arachnida, and 3 major lineages have been recognized, though the names used to refer to these groups have varied considerably (Krantz, 1978; Johnston, 1982; Evans, 1992). Here the names are generally followed as used in Parker (1982), and consider 3 superorders (sensu Evans, 1992) of Acari that exist. The superorder Opilioacariformes consists of a single order and family (Opilioacarida, Opilioacaridae) with about 20 known species. The superorder Acariformes contains over 300 families and over 30,000 described species. Two major lineages are recognized, the Sarcoptiformes (Oribatida and Astigmata) and Trombidiformes (Prostigmata). Additionally, 8 families of very early derivative acariform mites are lumped into the Endeostigmata, usually considered a suborder of the Prostigmata, but clearly containing taxa that belong to both major acariform lineages. The superorder Parasitiformes consists of 3 orders: Ixodida, Holothyrida, and Mesostigmata. The Mesostigmata contains in excess of 65 families and 10,000 described species, the other 2 parasitiform orders each comprise 3 families. About 850 species of ticks are known, but only about 30 species of holothyrans have been recognized.

What then is a mite? Aside from being generally tiny chelicerate arthropods with hexapod larvae, a discrete gnathosoma, and a loss of primary segmentation, mites are difficult to characterize. Lindquist (1984) pointed out that many of the characters used to define mites were present in other chelicerate orders, especially in the Ricinulei. He proposed 11 apomorphic characteristics for the Acari (Lindquist, 1984, Table 8, p. 40), but several of these character states are not present in the Parasitiformes and presumably have been secondarily lost. It seems that mites often are most easily recognized by what they are not, other arachnids, rather than by a discrete set of acarine characters.

Among acarologists, arguments about monophyly or diphyly of the Acari have yet to be resolved, although currently the monophyleticists seem to be dominant (see Lindquist, 1984; Evans, 1992). The Parasitiformes and Opilioacariformes are thought to be sister groups, and in turn this taxon (the Anactinotrichida, so named because of the absence in their setae of optically active actinochitin) is considered the sister group of the Acariformes (also called the Actinotrichida). Outside of the acarological community, those interested in chelicerate phylogeny have tended to assume that the Acari were a monophyletic assemblage (for example, Weygoldt and Paulus, 1979; Shultz, 1990; Weygoldt, 1998).

Many acarologists have concluded that mites are closely related to the arachnid order Ricinulei (Lindquist, 1984; van der Hammen, 1989; Evans, 1992). Weygoldt and Paulus (1979) first proposed a sister group relationship between the Ricinulei and the Acari and named this taxon the Acarinomorpha. Schulz (1990) also supported this relationship, but like Weygoldt and Paulus, assumed that the Acari are mono-

phyletic. Van der Hammen (1989) considered the Acari to be diphyletic, and the Acariformes and Parasitiformes to be at most only distantly related. According to van der Hammen, the Ricinulei and Anactinotrichida (Parasitiformes + Opilioacariformes) are sister groups and, within another lineage, the Actinotrichida (Acariformes) and the non-acarine Palpigradi also are sister groups. Lindquist (1984) presented four derived characters linking the Acari and Ricinulei (Lindquist, 1984, Table 9, p. 41) and concluded that, within the Acari proper, the Opilioacariformes and Parasitiformes form a sister group to the Acariformes.

Lindquist's (1984) hypothesis is followed here, which suggests that a monophyletic lineage includes the Ricinulei and the Acari. This hypothesis is based on the characters presented by Lindquist and is in agreement with that of Weygoldt and Paulus (1979) and Schulz (1990), but not with that proposed by Dunlop (1996).

Other Names for the Acari

Other names for the Acari include Acarina, Acaroides, Acaromorpha, Milben, acariens, acaros, Acarida, and mites.

Scope Note for This Textbook Section

This section was adapted by the textbook editors (S. A. Gardner and S. L. Gardner) from Walter and colleagues (1996), an open access contribution to the Tree of Life Web Project made available online under a CC BY-NC 3.0 license, and the public domain United States Centers for Disease Control and Prevention webpages on scabies (CDC, 2020). Since 1996, several other investigations into Acari systematics and genomics have been conducted, so other sources should be consulted to supplement this introduction to the topic.

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