Part III

ENDOPARASITIC PLATYHELMINTHS

PLATYHELMINTHES

15

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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

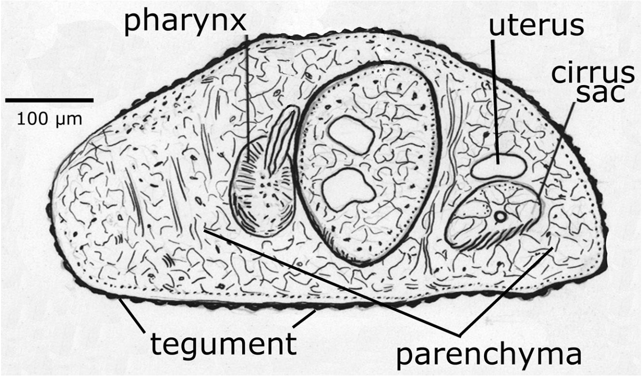

Figure 1. Cross section of a parasitic trematode showing the lack of ciliated epithelium. Source: H. W. Manter. License: CC BY.

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.

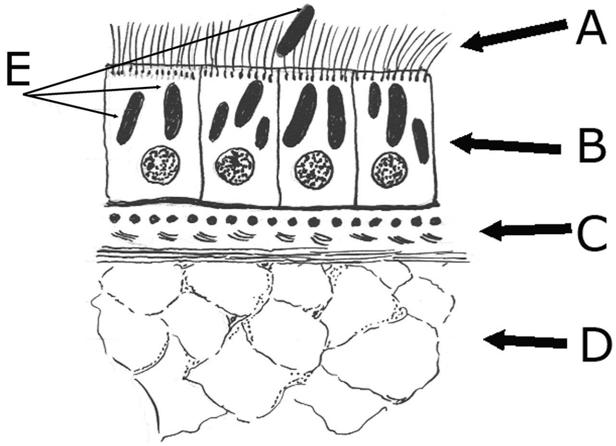

Figure 2. Example section through the body of a turbellarian flatworm. Ciliated tegument (A), epithelial or tegumentary cells (B), circular, longitudinal, and diagonal muscles (C), parenchyma (D), and rhabdites (E). Source: H. W. Manter. License: CC BY.

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

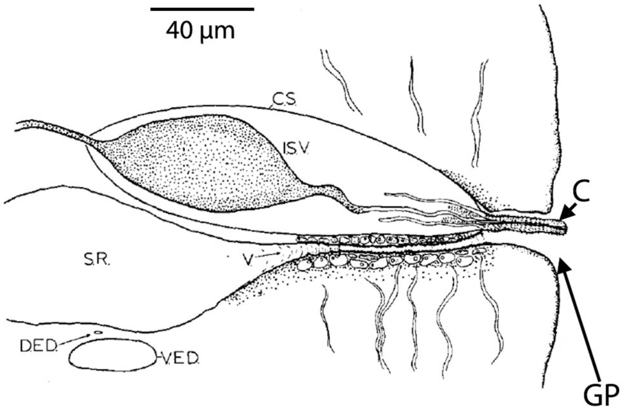

Figure 3. Transverse section through a mature segment of a cestode (tapeworm) showing the dorsal excretory duct (DED) just above the ventral excretory duct (VED). This view is from the posterior end of the worm looking anteriad (meaning, toward the anterior). The seminal receptacle (SR) in this species 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).

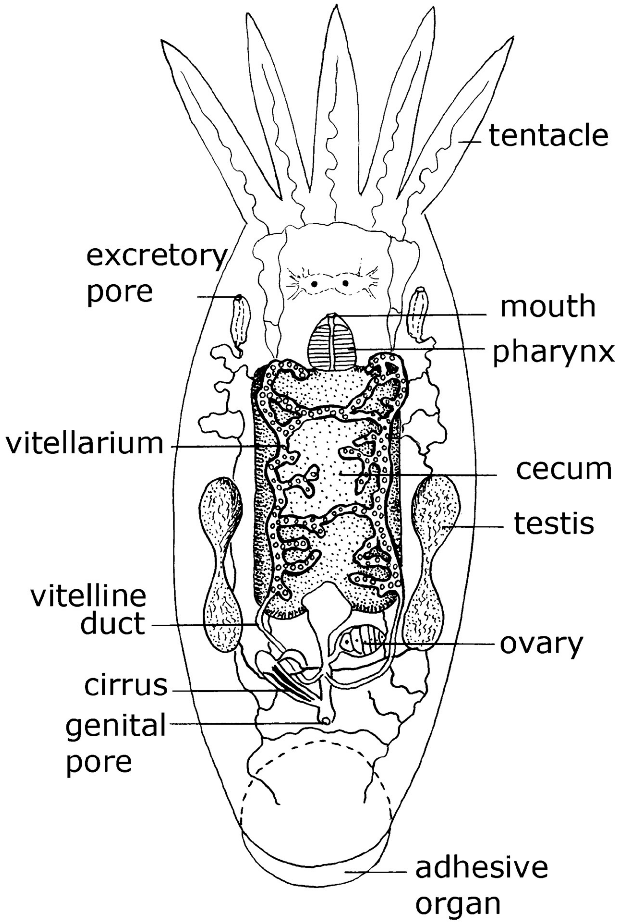

Figure 4. Dorsal view of a marine dwelling flatworm *Temnosewellia semperi* (Weber, 1890) (phylum Platyhelminthes: order Rhabdocoela: family Temnocephalidae) that normally occurs on the external carapace of freshwater crabs. Source: Adapted from Bresslau and Reisinger, 1933. License: CC BY.

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

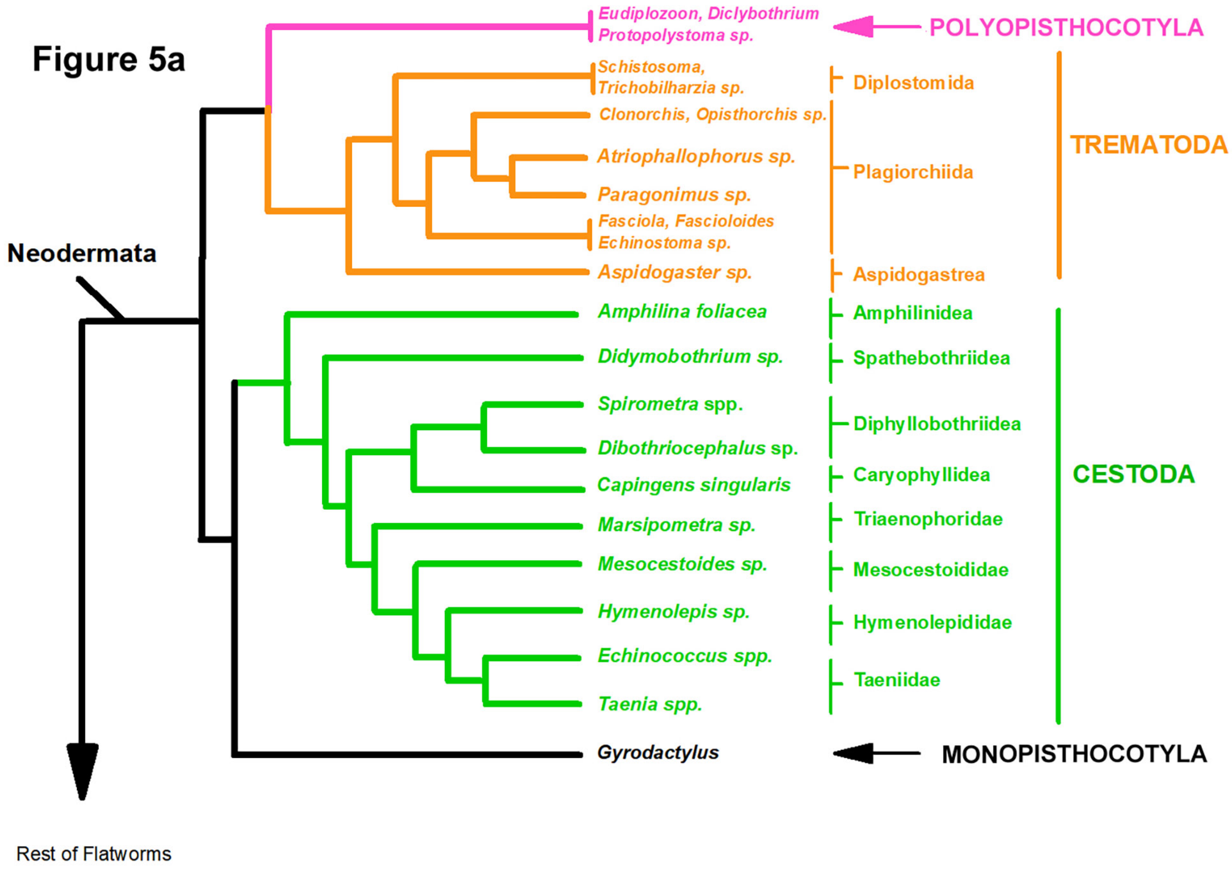

Figure 5A. Phylogeny based on 83 species of helminths of which 51 were parasites. Tree based on Bayesian inference algorithms showing a common ancestor of the Monopisthocotyla and the Cestoda while the Polyopisthocotyla shares a common ancestor with the Trematoda (see Brabec et al., 2023). Source: Adapted 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).

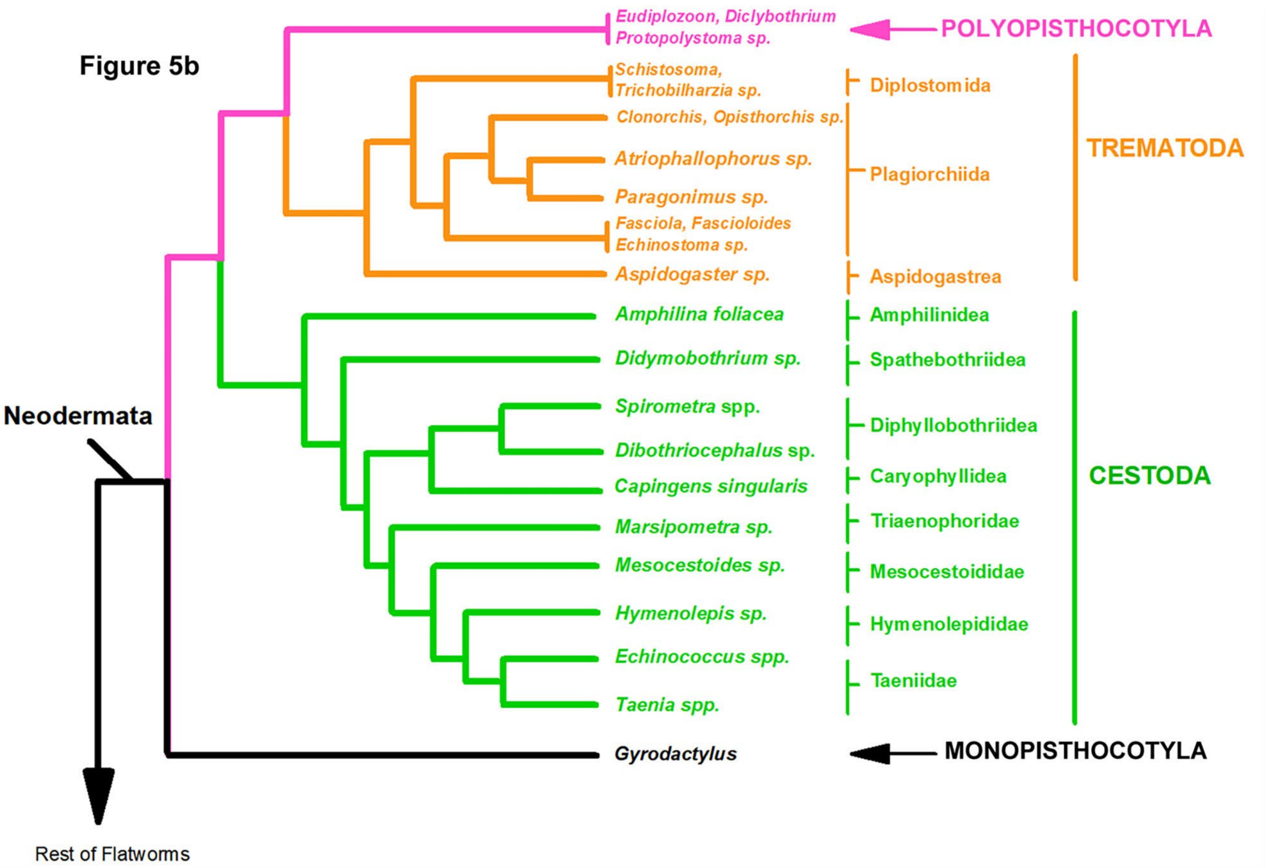

Figure 5B. Phylogeny based on 83 species of helminths of which 51 were parasites. Tree based on the maximum likelihood algorithm showing a common ancestor of the Polyopisthocotyla with the Trematoda and the Monopisthocotyla sharing a common ancestor with the rest of the Neodermata (see Brabec et al., 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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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

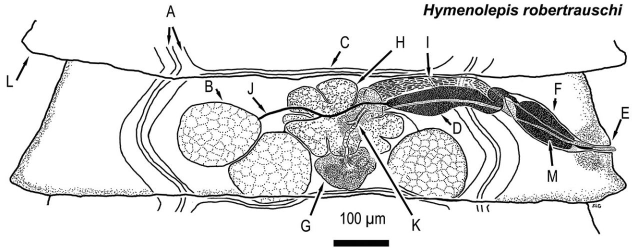

Figure 1. Mature proglottid (segment) of *Hymenolepis robertrauschi* from a grasshopper mouse (*Onychomys* sp.) collected in New Mexico, United States. A) Osmoregulatory canals, small canal is dorsal; wider canal is ventral; B) testis, here 3 are visible and are a characteristic of species of the genus *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.

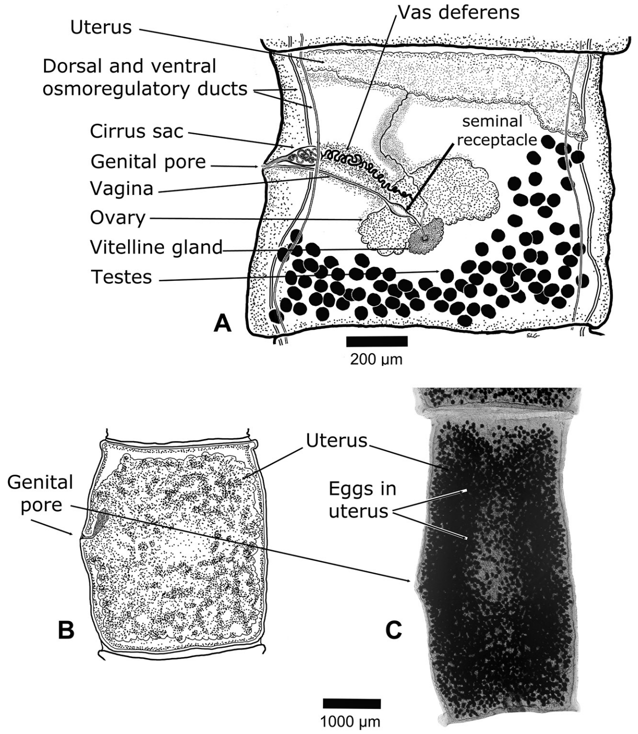

Figure 2. General structure of a craspedote tapeworm of the genus *Mathevotaenia* showing mature and gravid segments, also called proglottids. A) A fully mature proglottid showing male and female sex organs showing the longitudinal excretory ducts, testes, genital pore, cirrus sac, vitelline gland, lobed 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.

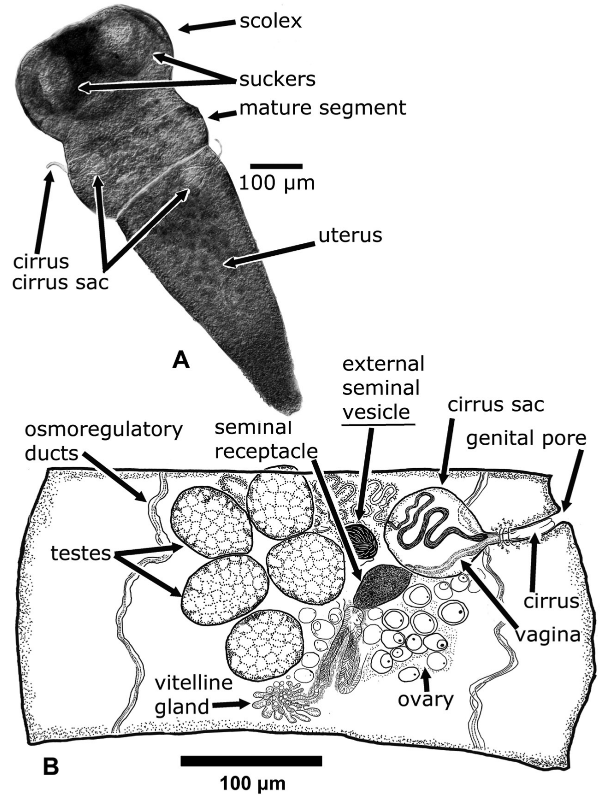

Figure 3. *Pritchardia boliviensis*. Known individuals of this species represent examples of a very small tapeworm, which as an adult has only a scolex and 3 discernible segments: One pre-mature segment, 1 mature segment, and 1 gravid segment. A) Photomicrograph of a whole animal; B) drawing of a mature 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.

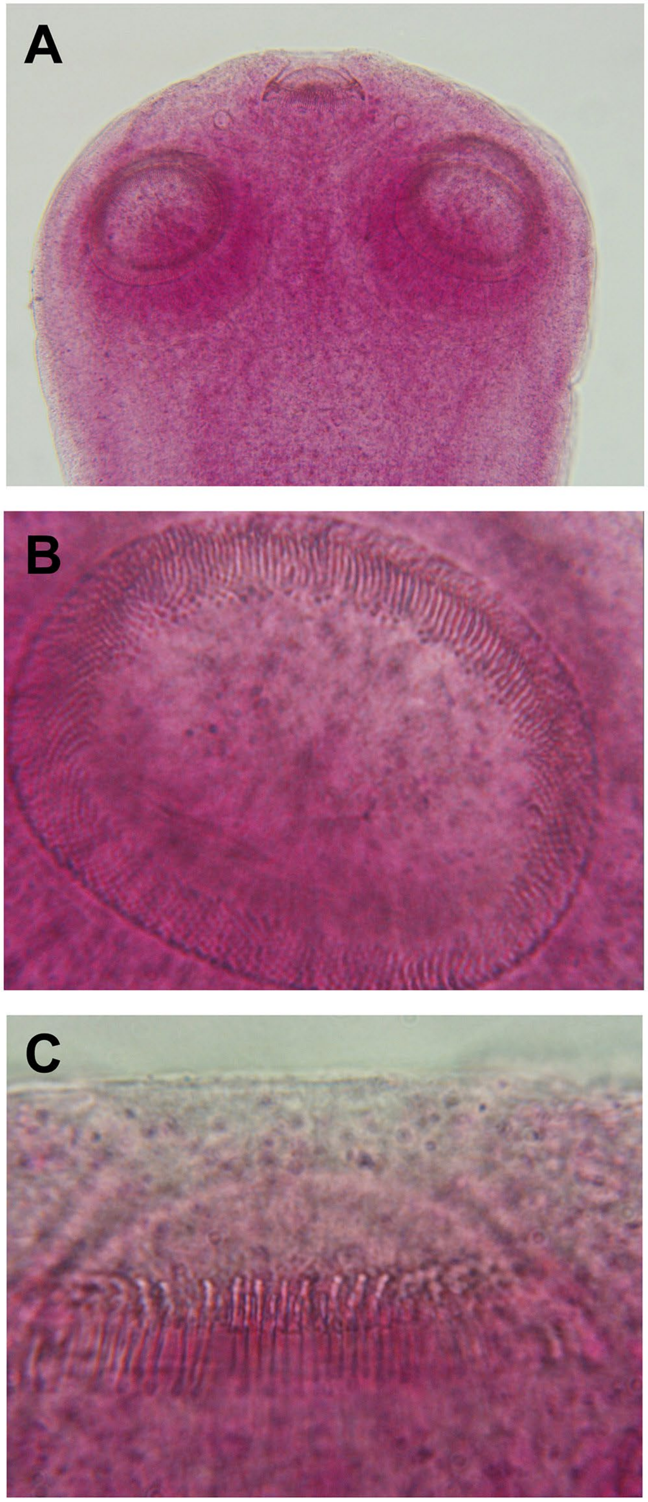

Figure 4. A) A species of *Raillietina* with hooks visible on the retracted rostellum and small hooklets visible on the suckers; B) a highly magnified view of one of the suckers showing the small hooks arranged around the margins of the sucker; C) a closer look at the hooks arranged around the rostellum 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.

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 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.

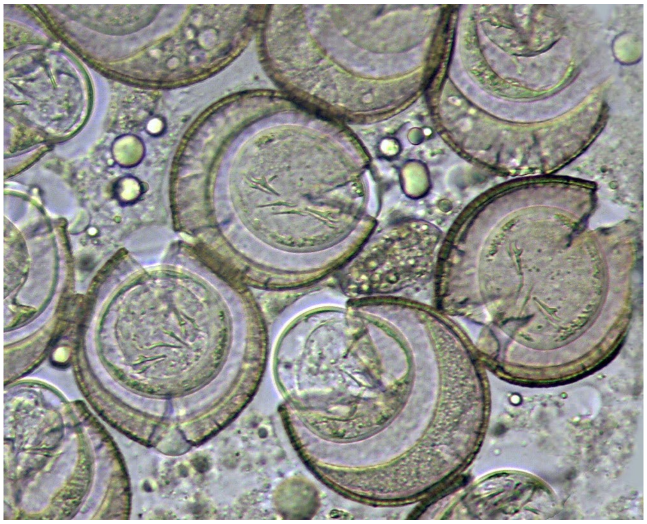

Figure 6. Eggs of *Hymenolepis weldensis* Gardner and Schmidt, 1988 from a Sandhills pocket gopher (*Geomys lutescens* Merriam 1890) from near Cedar Point Biological Station, near Ogallala, Nebraska, United States. The eggs were imaged after they were removed from the gravid uterus of a living tapeworm. 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,

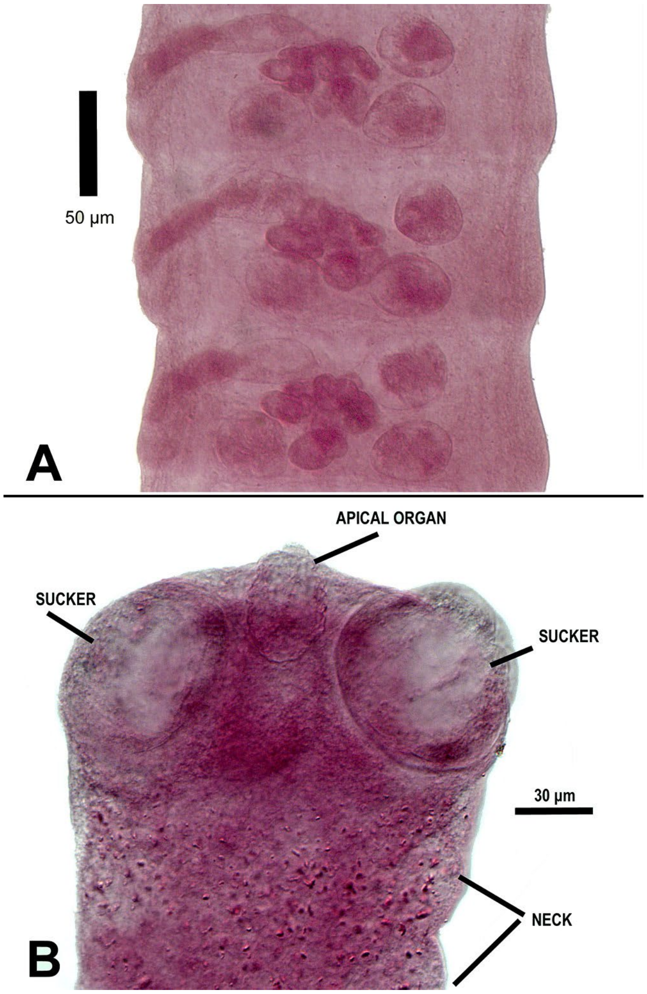

Figure 7. A) Example of a cyclophyllidean cestode in the family Hymenolepididae (*Hymenolepis tualatinensis* Gardner, 1985); B) scolex of the same specimen. Source: S. L. Gardner, HWML. License: CC BY.

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

doi:10.32873/unl.dc.ciap017
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*

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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 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.

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 2. Rostellar hooks and suckers of *Taenia kotlani* from a snow leopard. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

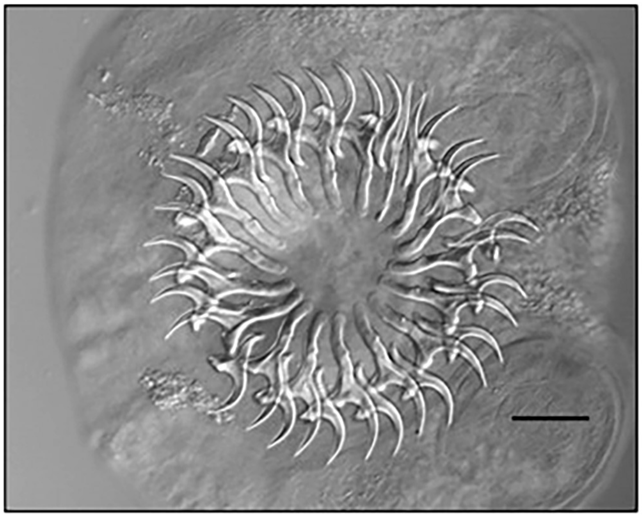

Figure 3. Rostellar hooks of *Taenia polyacantha*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

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* 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.

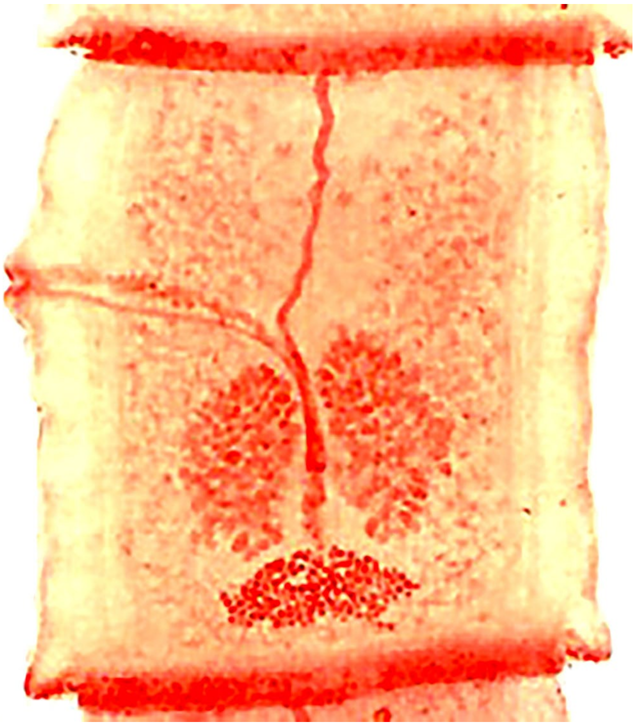

Figure 6. Mature proglottid of *Taenia crassiceps*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

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

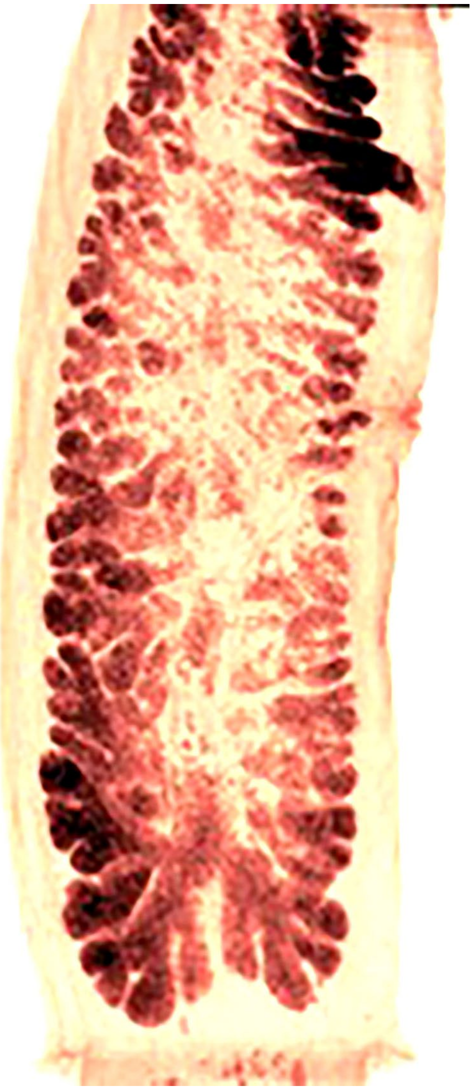

Figure 7. Gravid proglottid of *Taenia crassiceps*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

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).

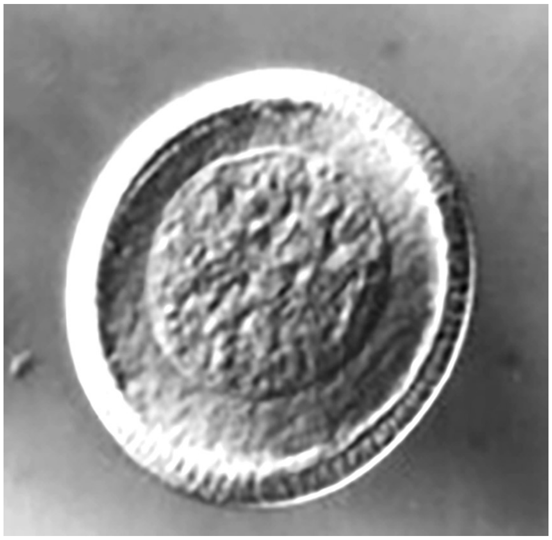

Figure 8. Egg of *Taenia kotlani*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

Figure 9. Egg of *Echinococcus multilocularis*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

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.

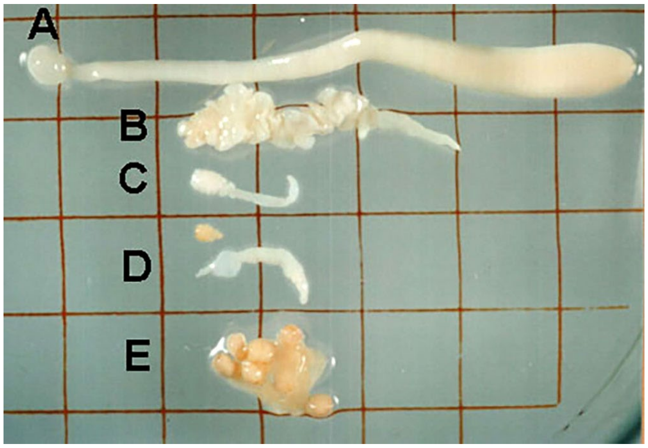

Figure 11. Different types of metacestode in *Taenia*. From top: A) Strobilocercus of *T. taeniaeformis*; B) armatetrathyridium of *T. martis*; C) *T. polyacantha*, tetrathyridium of *Mesocestoides* sp.; D) strobilocercus of *T. retracta*; E) polycephalic metacestode of *T. endothoracicus*. Source: S. Ganzorig and S. L. Gardner. License: CC BY.

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.

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 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

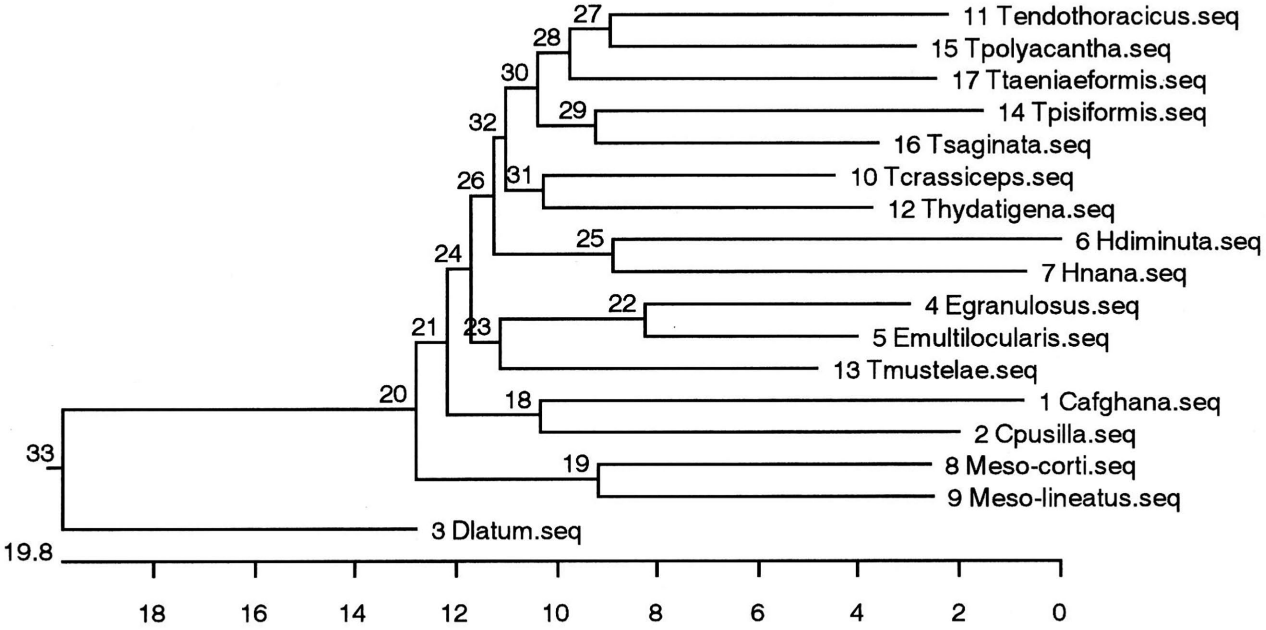

Figure 13. Phylogenetic tree of cyclophyllid cestodes constructed from neighbor joining (NJ) analysis of the mitochondrial *cox*1 gene partial nucleotide sequences. Source: S. Ganzorig. License: CC BY.

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.

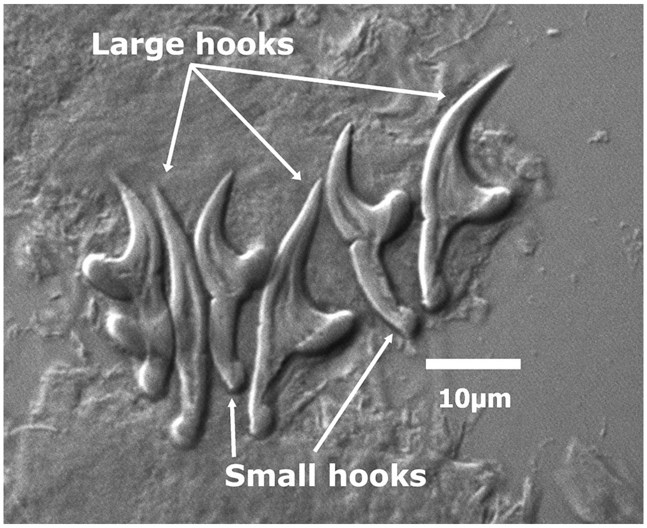

Figure 1. Hooks of *Echinococcus multilocularis* collected from the area near Har Us Lake, Hovd, Mongolia. Specimen number NK223782. Taeniids typically have 2 rows of hooks on the rostellum with 1 row consisting of smaller hooks and another row with larger hooks. Source: S. L. Gardner, HWML. License: CC BY.

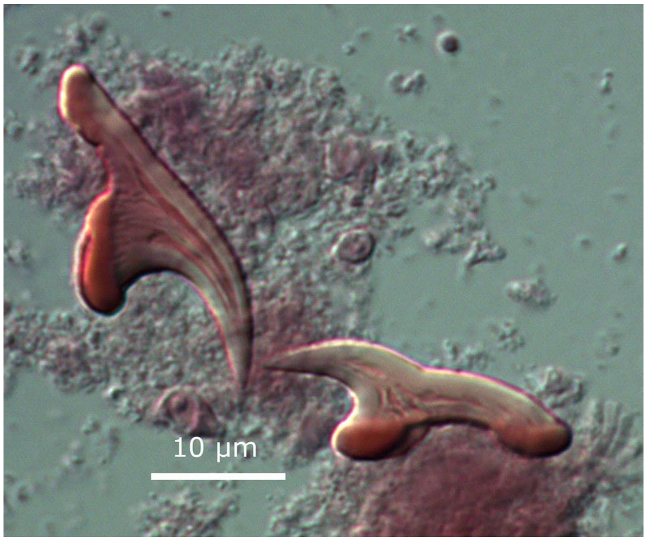

Figure 2. Two hooks (large and small) from a protoscolex of *Echinococcus multilocularis* from near Taos, New Mexico, United States from a deer mouse *Peromyscus maniculatus*. The specimen was cleared in lactophenol on a microscope slide under a number 1 coverslip. Using a small amount of pressure with 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

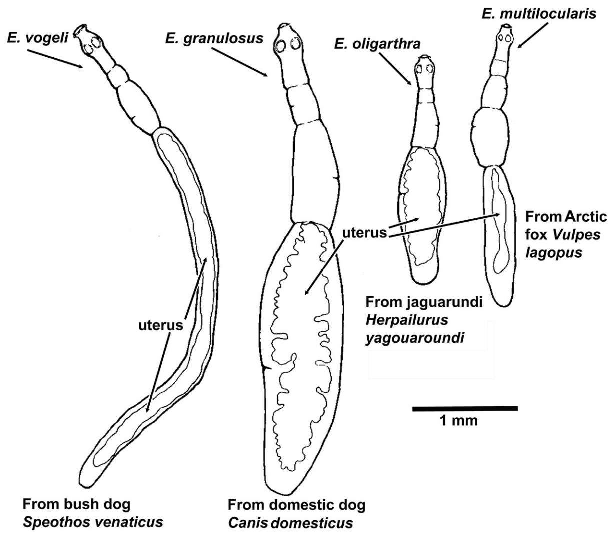

Figure 3. Comparisons among *Echinococcus vogeli*, *E. granulosus*, *E. oligarthra*, and *E. multilocularis*. Note the longer, thinner gravid proglottid in *E. vogeli* compared to the other species in the figure. The eggs are not shown in the uteri in this figure. Source: S. L. Gardner, HWML. License: CC BY.

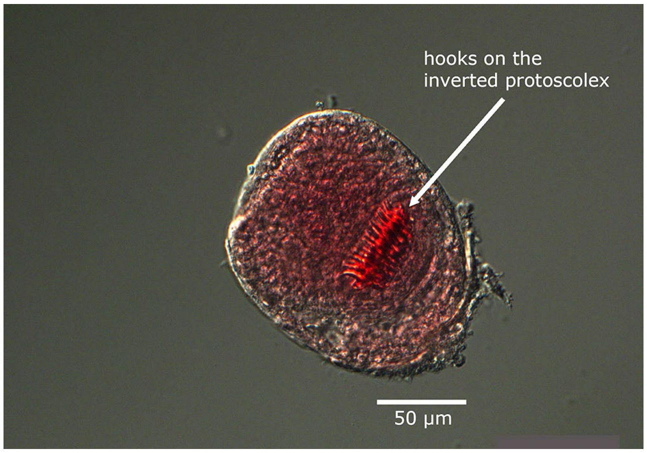

Figure 4. Protoscolex from the cyst of *Echinococcus multilocularis* obtained from a deer mouse *Peromyscus maniculatus* collected from near Taos, New Mexico, United States. The specimen was stained using Semichon's acetic carmine (which is the usual method). Source: A. T. Dursahinhan and S. L. Gardner, 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).

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.

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

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).

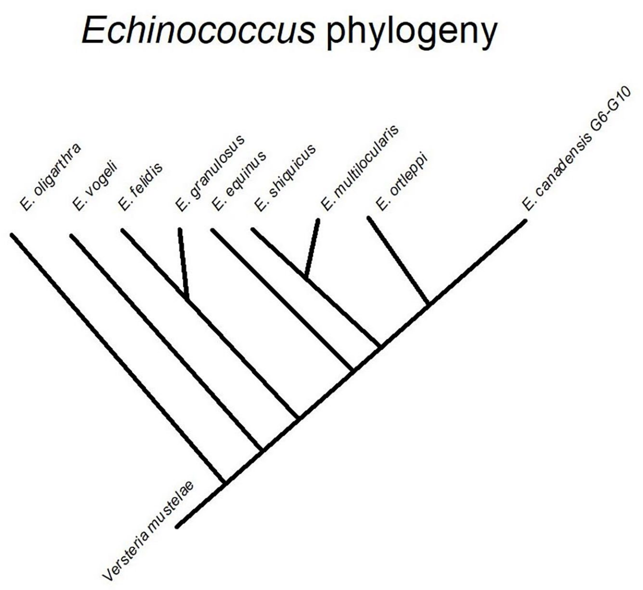

Figure 7. *Echinococcus* phylogeny. Estimated evolutionary relationships among all known species of *Echinococcus*. *Echinococcus canadensis* genotypes G6–G10 are shown as a single branch/species in this tree. The tree was based on a maximum likelihood analysis of mitochondrial genomes and nuclear protein-coding genes. Source: Adapted from Nakao et al., 2013b. License: CC BY-NC-SA 4.0.

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* (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.

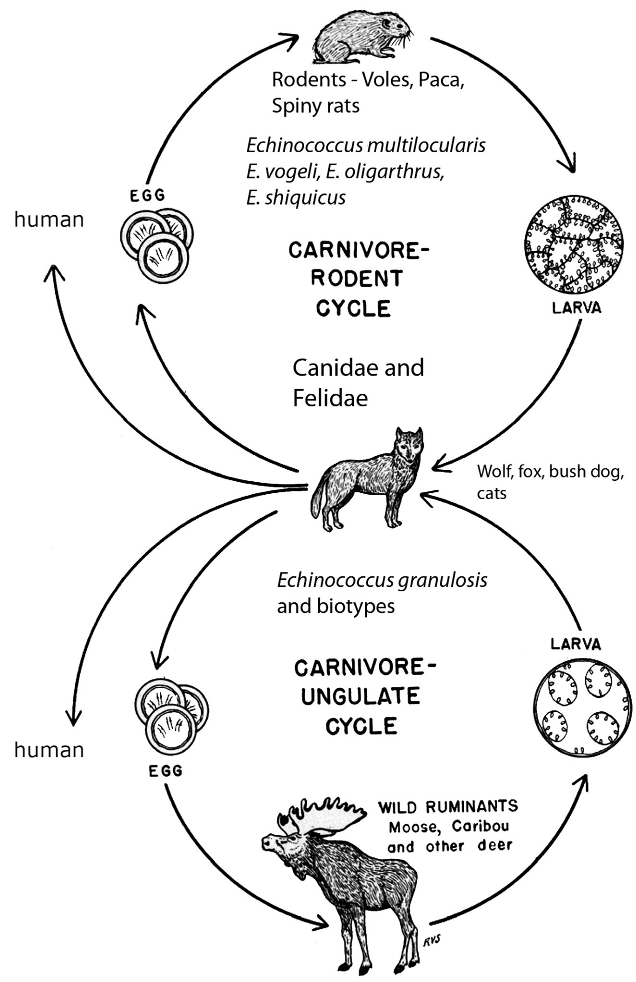

Figure 8. The general life cycle or life history of species of the genus *Echinococcus* showing the carnivore-rodent cycle and showing the carnivore-ungulate cycle. All *Echinococcus* species are not listed here. Source: S. L. Gardner, HWML. License: CC BY.

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

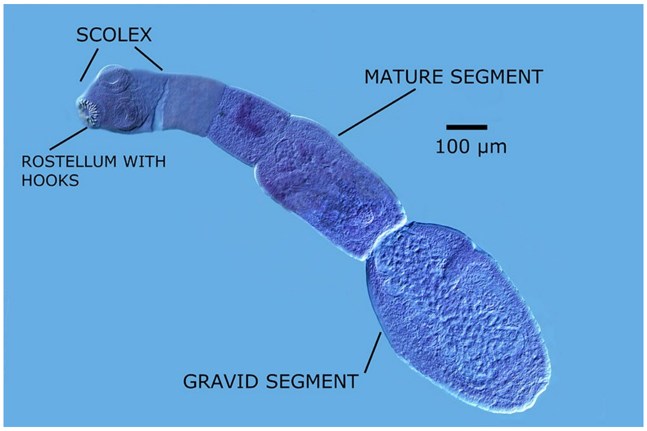

Figure 9. Mature specimen of *Echinococcus multilocularis* from Alaska, United States. Note that the posterior segment that would be full of eggs is missing in this specimen. Source: R. L. Rausch. License: CC BY.

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

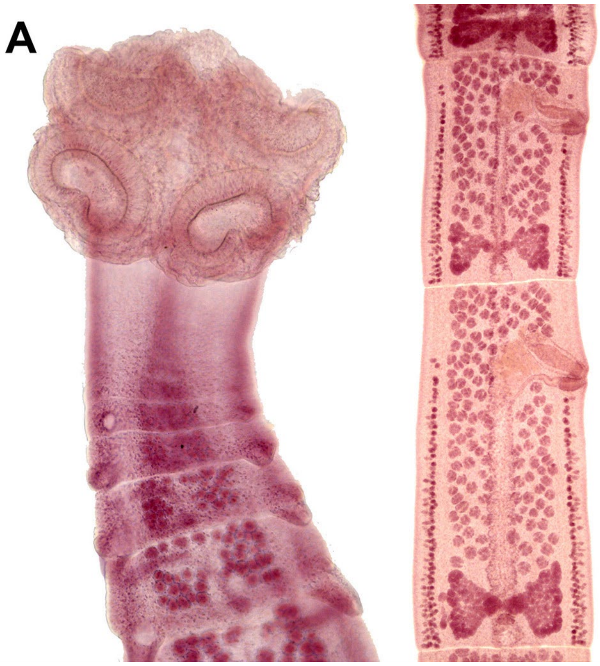

Figure 2. Adults from pimelodid catfishes in Peru (scolex and anterior proglottids of *Pseudocrepidobothrium eirasi* from *Phractocephalus hemioliopterus* and mature proglottids of *Proteocephalus sophiae* from *Paulicea luetkeni*. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

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).

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.

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.

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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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,

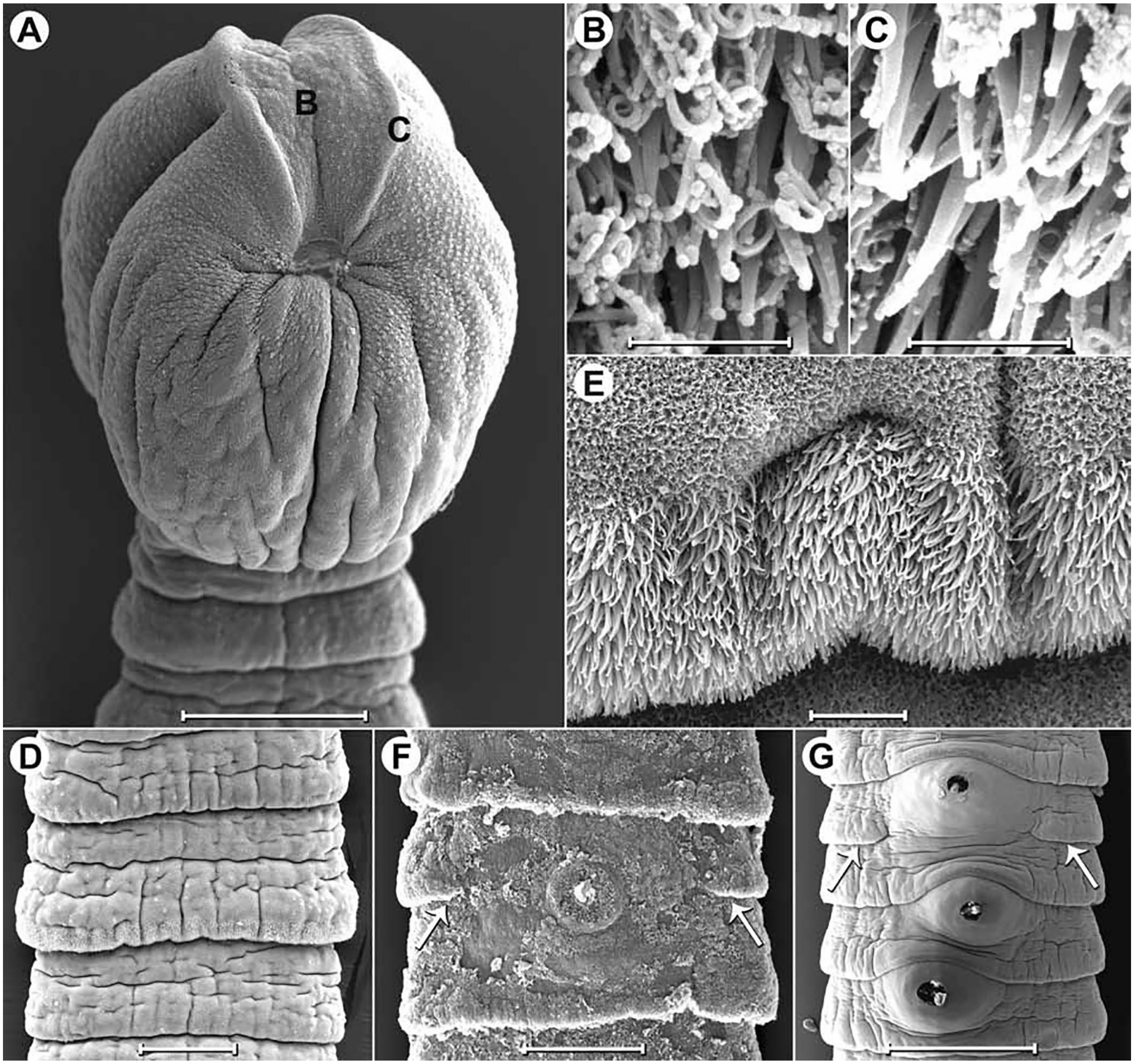

Figure 1. *Clestobothrium cristinae* from *Merluccius hubbsi*, scanning electron micrographs. A) Dorsoventral view of scolex showing tumuli; the surfaces of B and C are shown at high magnification; B) Central surface between lips; C) Marginal surface of lips; D) Piece of immature strobila; E) Surface 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

Tomáš Scholz and Roman Kuchta

- Phylum Platyhelminthes
  - Class Cestoda
    - Subclass Eucestoda
      - Order Diphyllobothriidea

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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 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

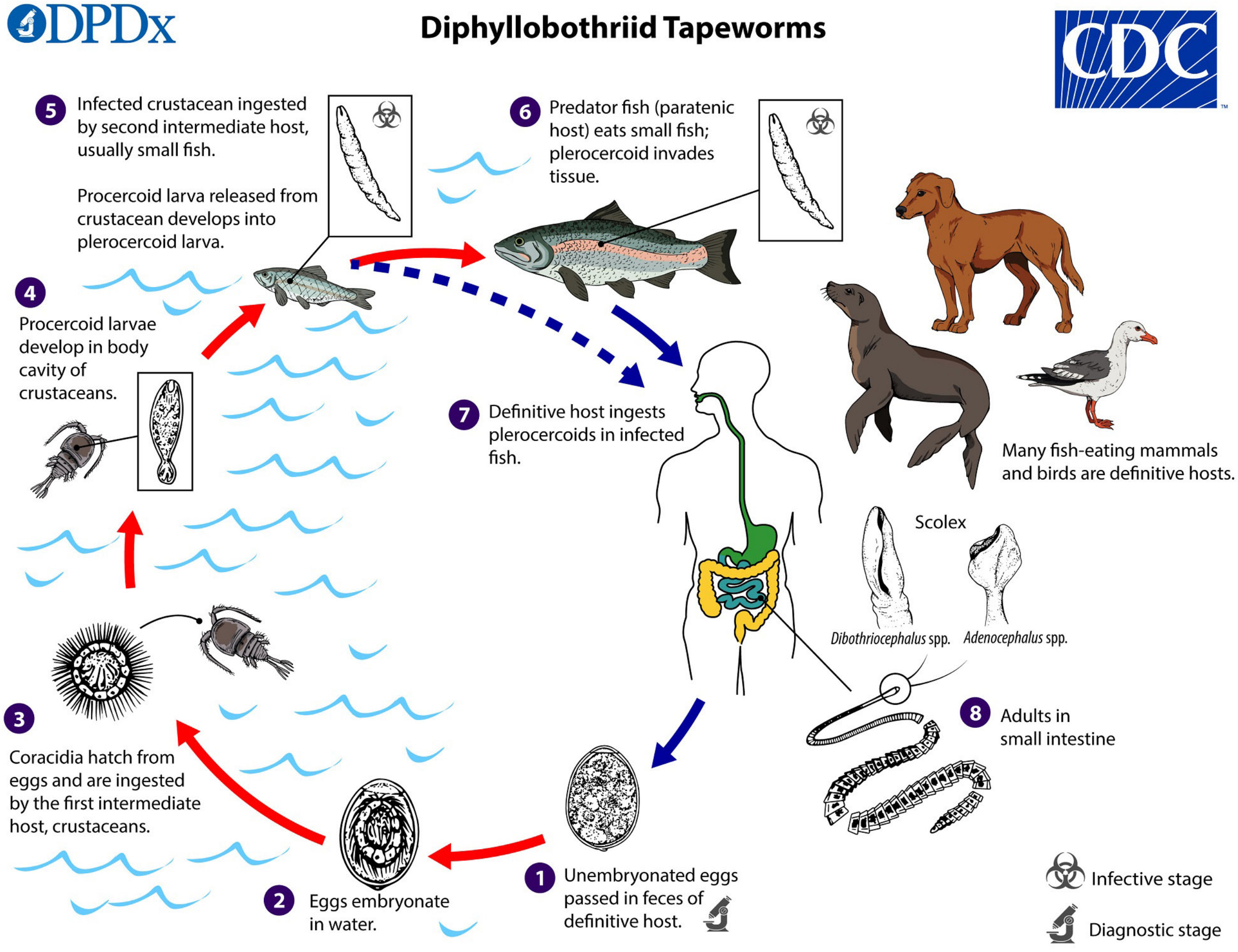

Figure 2.

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).

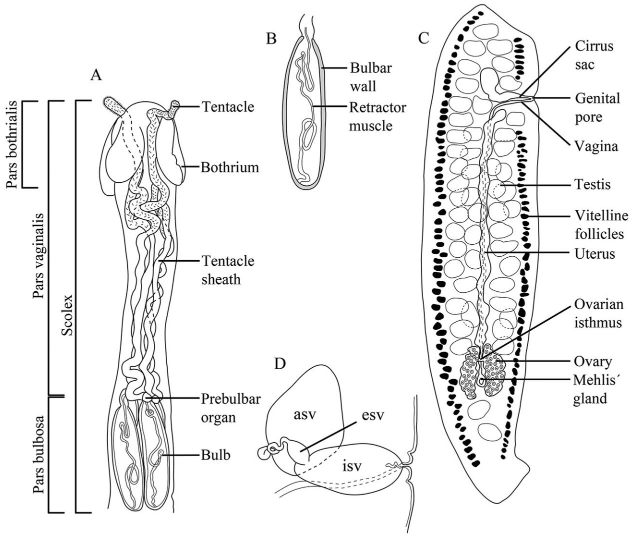

Figure 1. General morphology of a trypanorhynch: A) Scolex; B) Bulb; C) Mature proglottid; D) Terminal genitalia. Abbreviations: asv = accessory seminal vesicle; esv = external seminal vesicle; isv = internal seminal vesicle. Source: Modified from Beveridge and Justine, 2006. License: CC BY-NC-SA 4.0.

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.

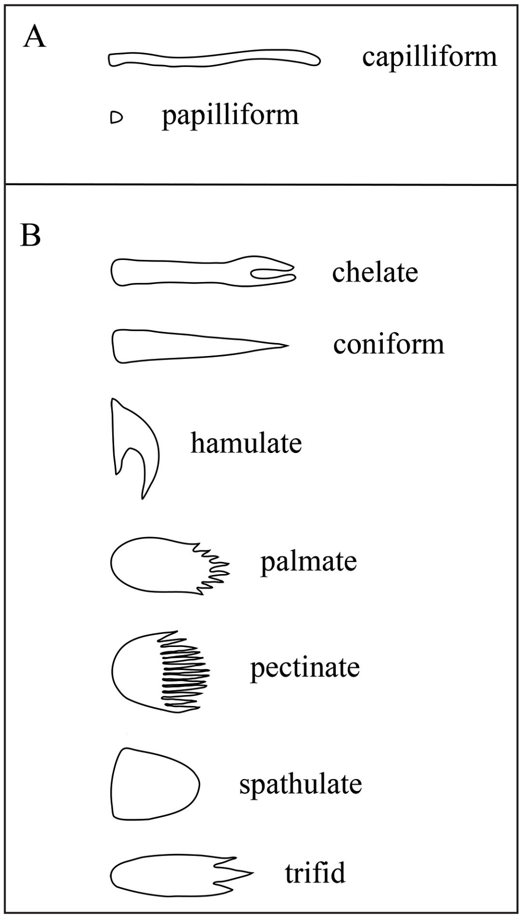

Figure 2. Schematic representation of some ultrastructural features of the scolex reported for trypanorhynchs. **Microtriches** with basal widths ≤ 200 nm are considered to be **filitriches** (A); in contrast, microtriches with basal widths > 200 nm are considered to be **spinitriches** (B). Source: Modified from Chervy, 2009. License: CC BY-NC-SA 4.0.

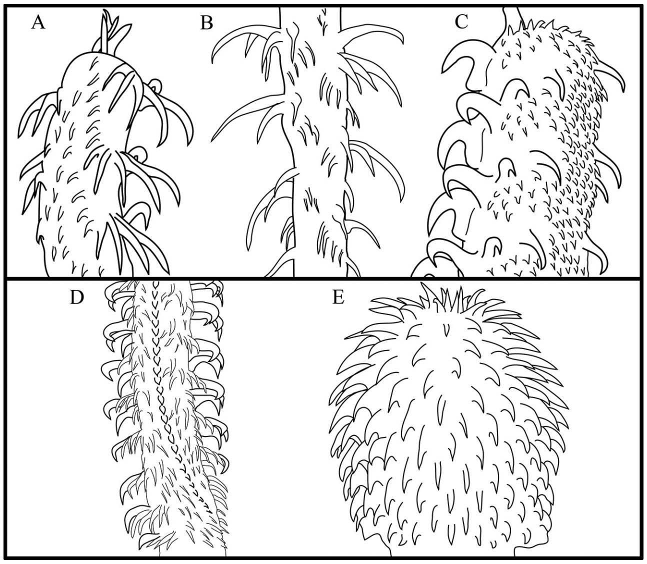

Figure 3. Armature patterns of tentacles of some trypanorhynchs. A) Typical heteroacanthous tentacular armature; B) Atypical heteroacanthous tentacular armature; C) Multiatypical heteroacanthous tentacular armature; D) Poeciloacanthous tentacular armature; E) Homeocanthous tentacular armature. Source: 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

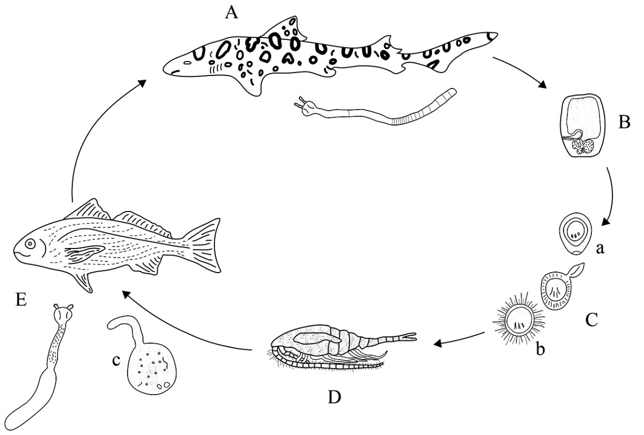

Figure 4. Life cycle of *Lacistorhynchus dollfusi* Beveridge and Sakanari, 1987. A) Adult trypanorhynch in the spiral valve of *Triakis semifasciata* Girard, 1855 (leopard shark); B) Gravid proglottids pass out with the feces and release eggs into the water; C) Coracidium larva (b) hatch from operculate 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.

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

(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 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,

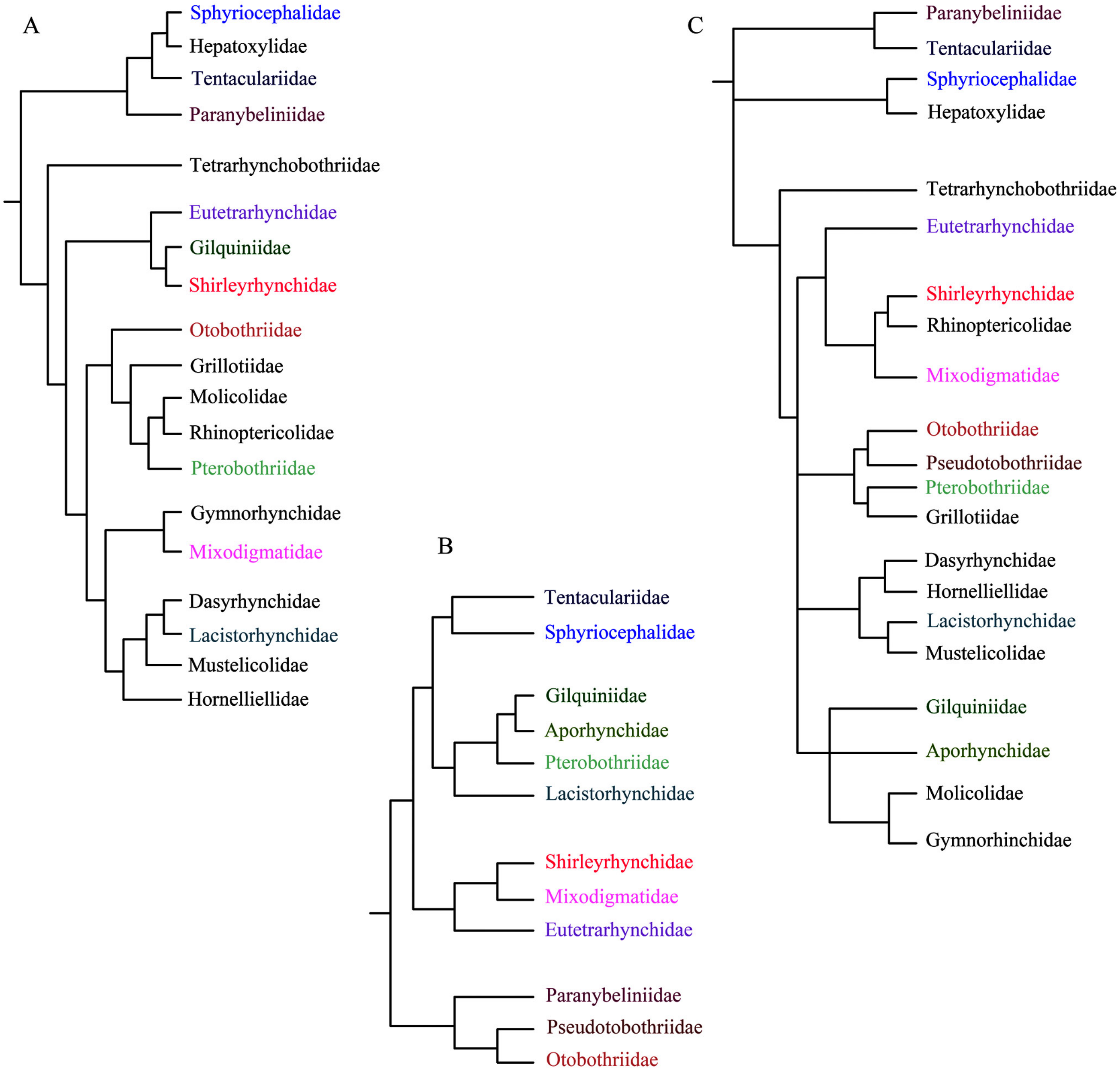

Figure 5. Classification of the order Trypanorhyncha to the family level using morphological characters. A) Hypothesis by Campbell and Beveridge, 1994; B) Hypothesis by Palm, 1997; C) Hypothesis by Beveridge et al., 1999. Source: Modified from Beveridge et al., 1999. License: CC BY-NC-SA 4.0.

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.

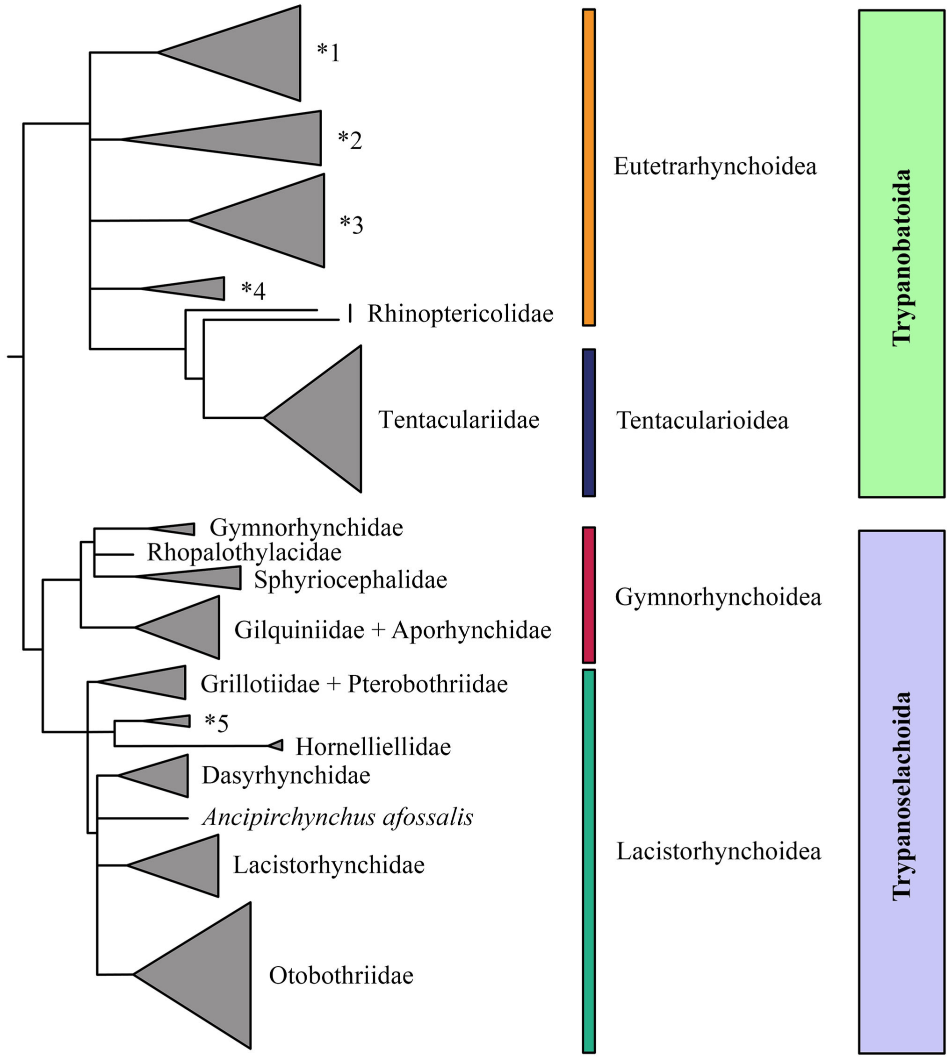

Figure 6. Schematic molecular phylogeny of Trypanorhyncha with recognized suborders, superfamilies, and families. Note: The clades marked with an asterisk * followed by a number are new clades for which there is no existing a name for the family. Source: Modified from Beveridge et al., 2017. License: 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

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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

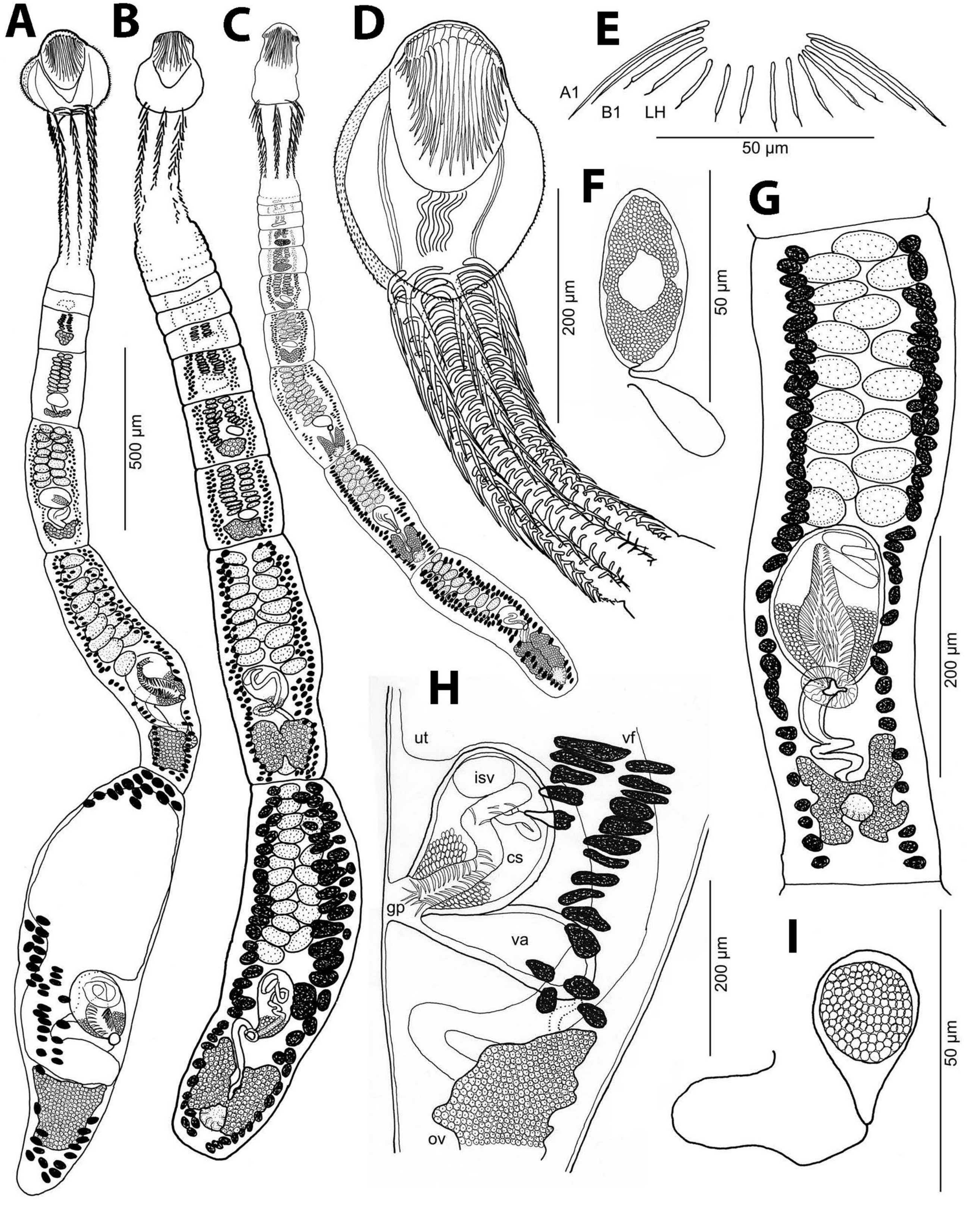

Figure 1. Line drawings. A, D–H) *Echinobothrium nataliae* A) Whole worm; D) scolex; E) L lateral hooklets; F) egg. G) mature proglottid; H) detail of terminal genitalia, lateral view; B) *Echinobothrium reginae*, whole worm; C, I) *Echinobothrium vojtai*; C) whole worm; I) egg. Abbreviations: A1) First 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

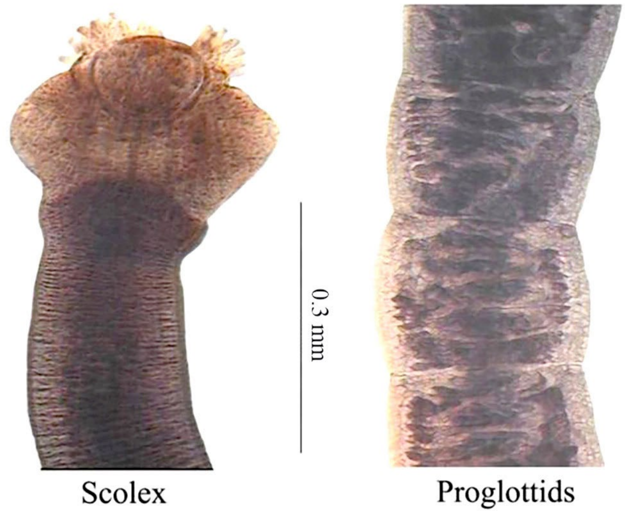

Figure 1. Scolex and proglottids of *Polypocephalus moretonensis* Butler, 1987, holotype specimen from the Queensland Museum, South Brisbane, Queensland, Australia. See https://www.gbif.org/occurrence/1066763010 for more information about this specimen. Source: Queensland Museum, 2023. License: CC BY.

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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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

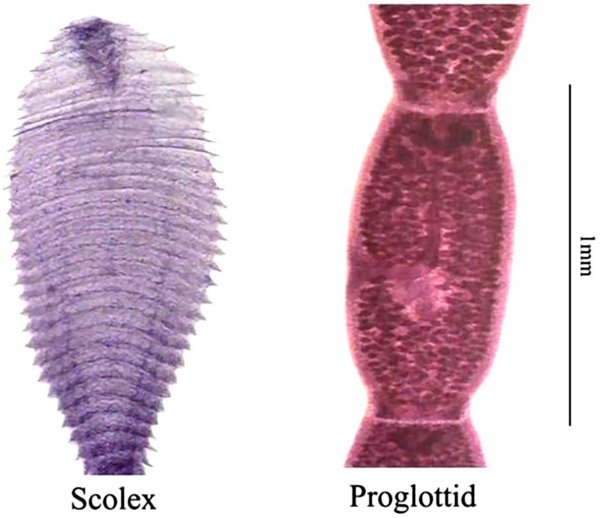

Figure 1. Scolex and proglottids of *Litobothrium amischensis* Caira & Runkle, 1993, holotype specimen from the Queensland Museum, South Brisbane, Queensland, Australia. See https://www.gbif.org/occurrence/1066761304 for more information about this specimen. Source: Queensland Museum, 2023. License: 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
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      Order Phyllobothriidea

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Chapter 28

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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

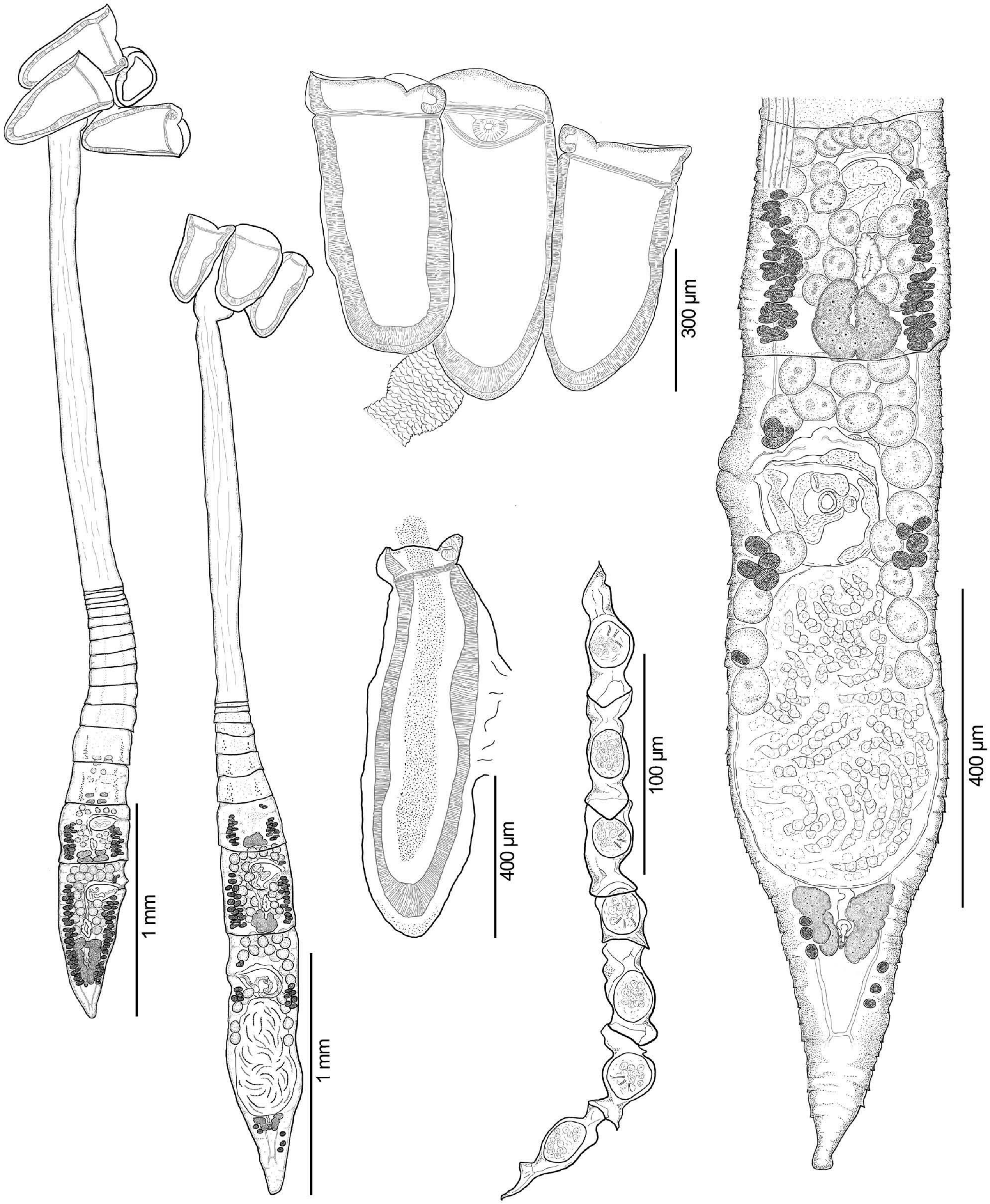

Figure 1. *Guidus francoi* sp. n. from *Bathyraja magellanica* (Philippi), line drawings. A) Entire mature worm (holotype MACN-Pa No. 739); B) entire gravid worm (paratype MACN-Pa No. 746/6); C) scolex (paratype MACN-Pa No. 743); D) bothridium attached to host tissue, muscular bothridial sphinchter contracted (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.

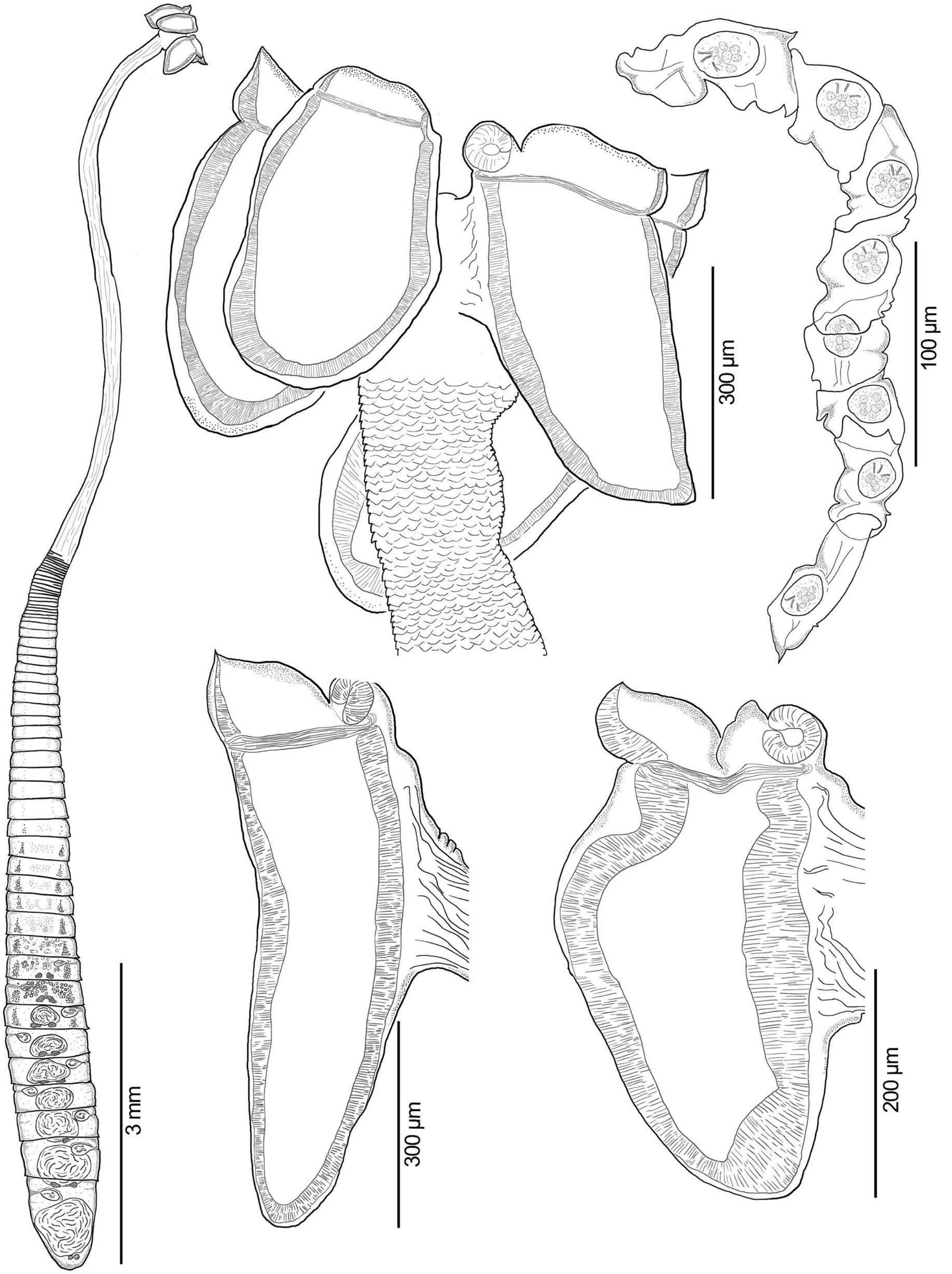

Figure 2. *Guidus magellanicus* from *Bathyraja magellanica* (Philippi), line drawings. A) Entire gravid worm (holotype MACN-Pa No. 747); B) scolex (holotype MACN-Pa No. 747); C) cocoon; D) bothridium, muscular sphincter relaxed (paratype MACN-Pa No. 748/2); E) bothridium, muscular sphincter contracted (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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EUCESTODA

Rhinebothriidea Healy et al., 2009 (Order)

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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

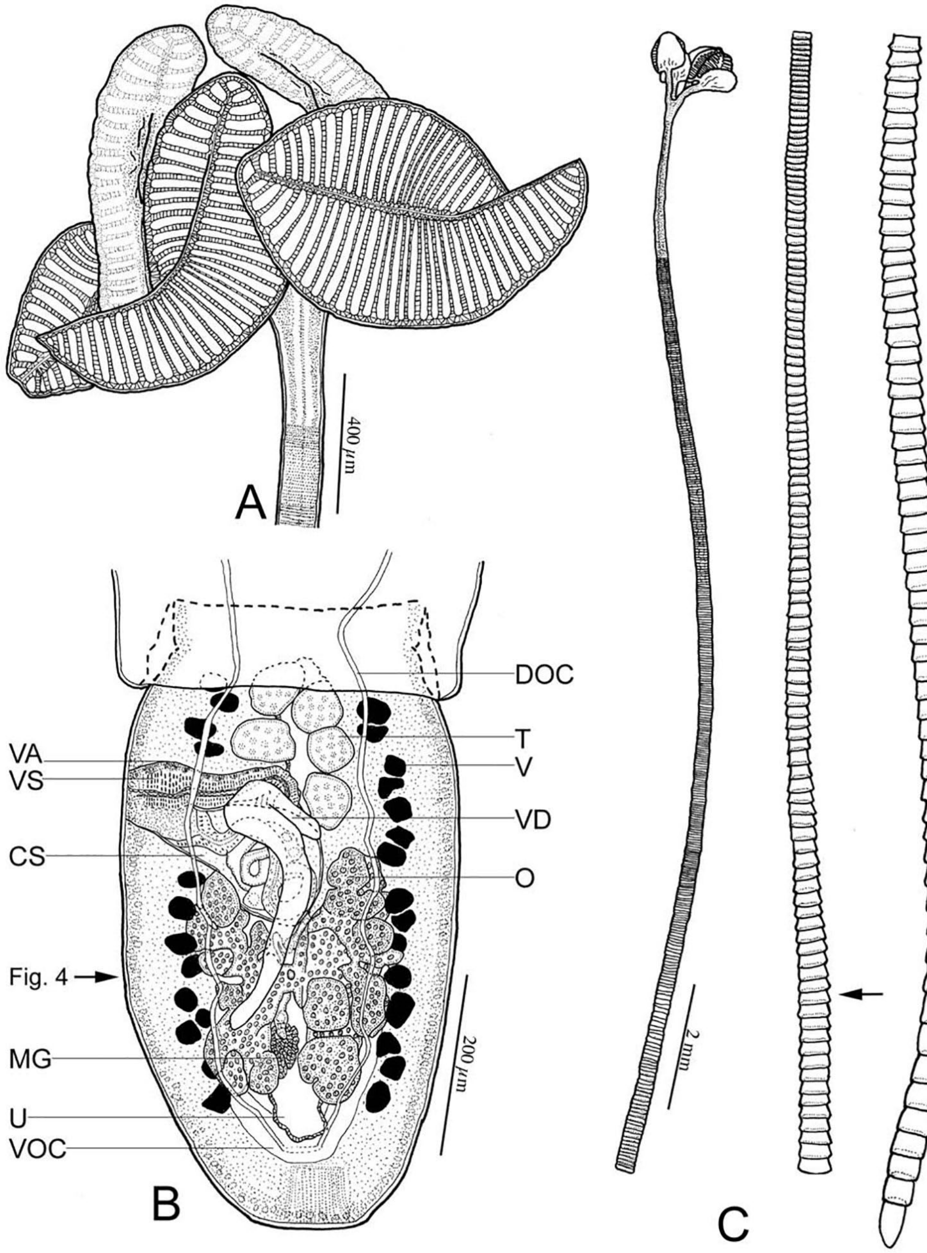

Figure 1. Line drawings of *Rhinebothrium paratrygoni* Rego & Dias, 1976 collected from the type locality. A) Scolex of voucher (MZUSP 6214); B) terminal mature proglottid of voucher (MZUSP 6214). The vas deferens is above the cirrus sac. The arrow indicates the location of the section shown in the portion 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.

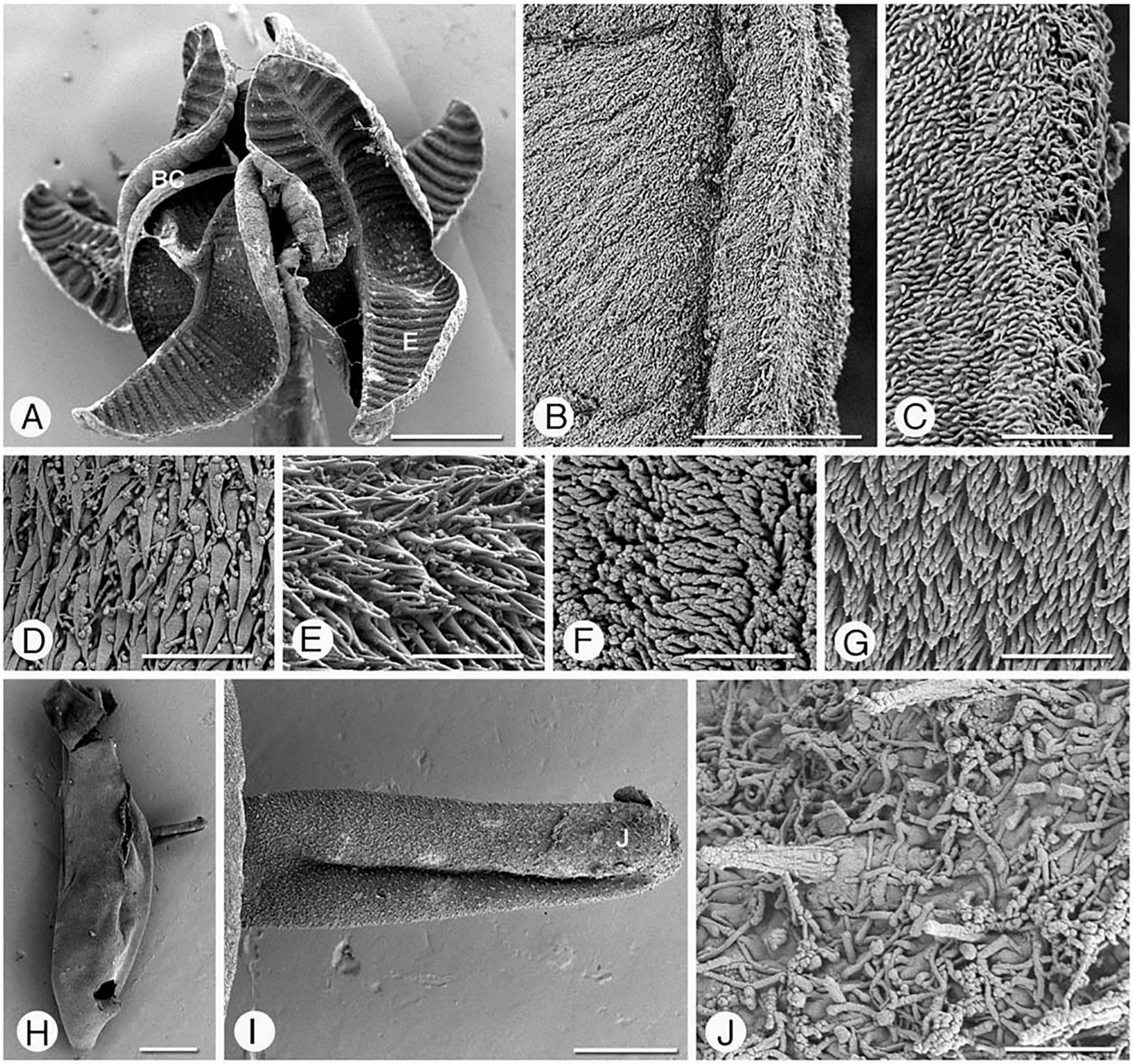

Figure 2. Scanning electron micrographs of *Rhinebothrium paratrygoni*. Scolex, Figures A–G. A) Scolex; B) small letter indicates the locations of details shown in B–C and E. Proximal surface of the rim of the bothridium; C) proximal bothridial surface adjacent to the bothridial rim; D) proximal bothridial 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)

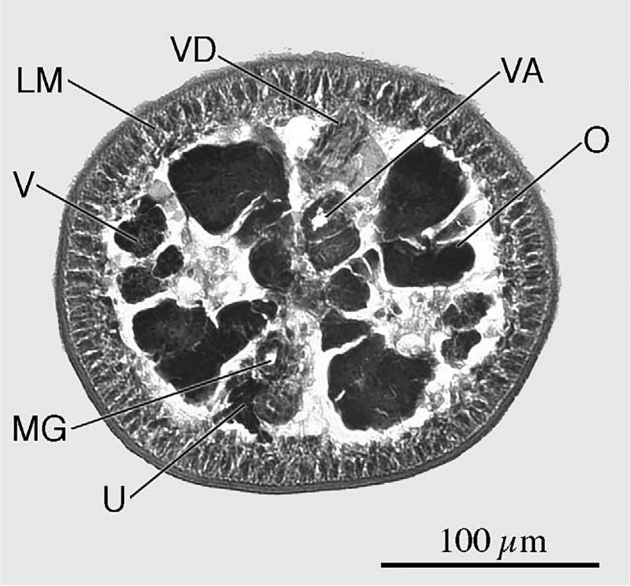

Figure 3. Cross section through a mature proglottid of *Rhinebothrium paratrygoni* at the level of the ovarian isthmus. Abbreviations: LM) Longitudinal muscles; MG) Mehlis' gland; O) ovary; U) uterus; V) vitellaria; VA) vagina; VD) vas deferens. Source: Reyda and Marques, 2011. License: CC BY.

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 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

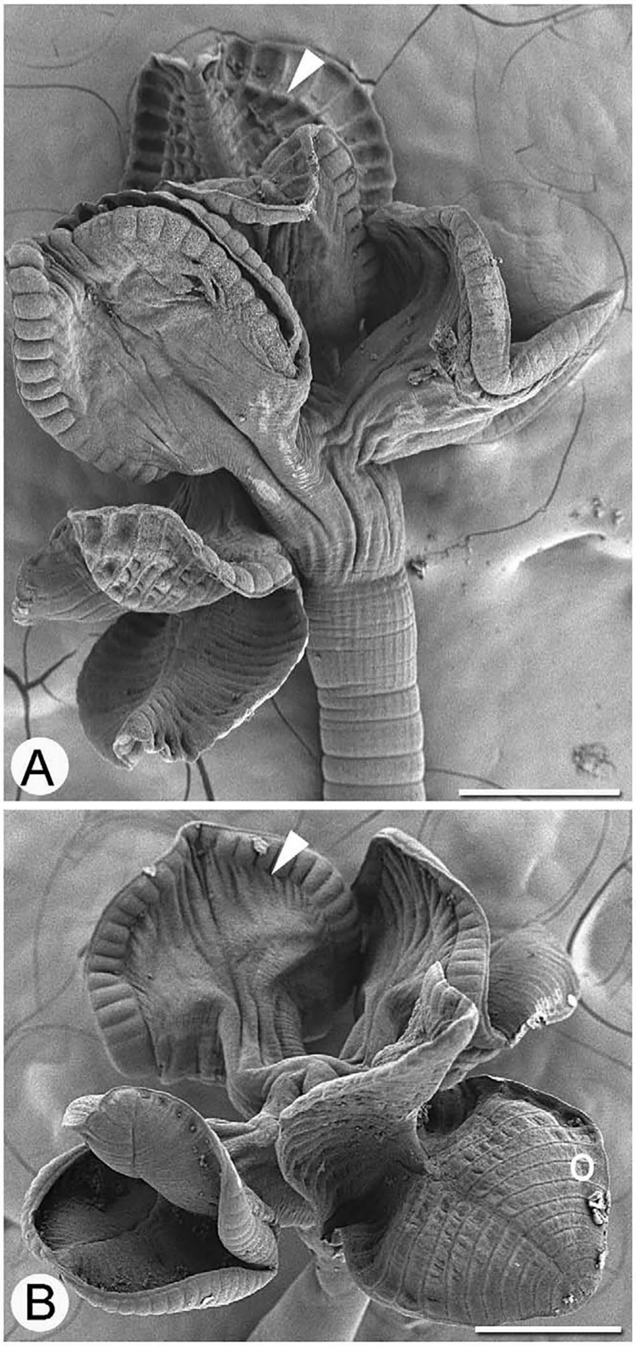

Figure 4. Scoleces of *Rhinebothrium copianullum*. A) Scolex in which marginal longitudinal septa are visible, indicated by the white arrow; B) scolex in which marginal longitudinal septa are visible on the proximal bothridial surface, indicated by the white arrow. The white circle indicates the position of the marginal longitudinal septum on the distal surface. Scale bar: A–B = 200 mm. Source: Reyda and Marques, 2011. License: CC BY.

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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Relics of “Tetraphyllidea” van Beneden, 1850 (Order)

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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.

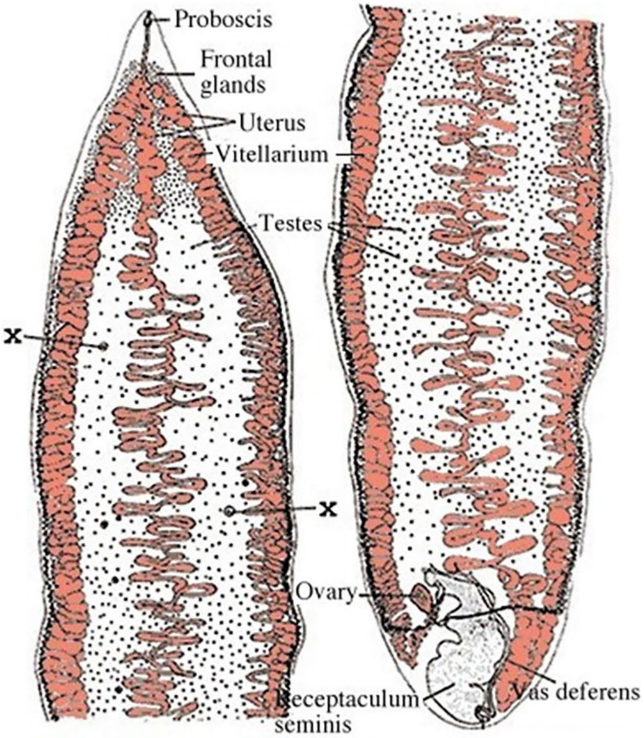

Figure 2. *Austramphilina elongata*, whole mount. X = bodies of unknown function. Source: K. Rohde. License: CC BY-NC-SA 4.0.

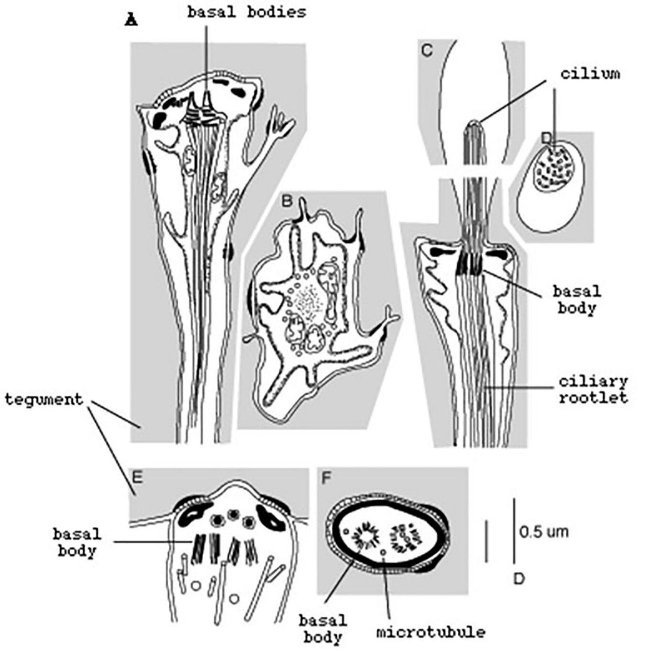

Figure 3. *Austramphilina elongata*, receptors of adult. Source: Adapted from Rohde and Watson, 1990b. License: CC BY-NC-SA 4.0.

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).

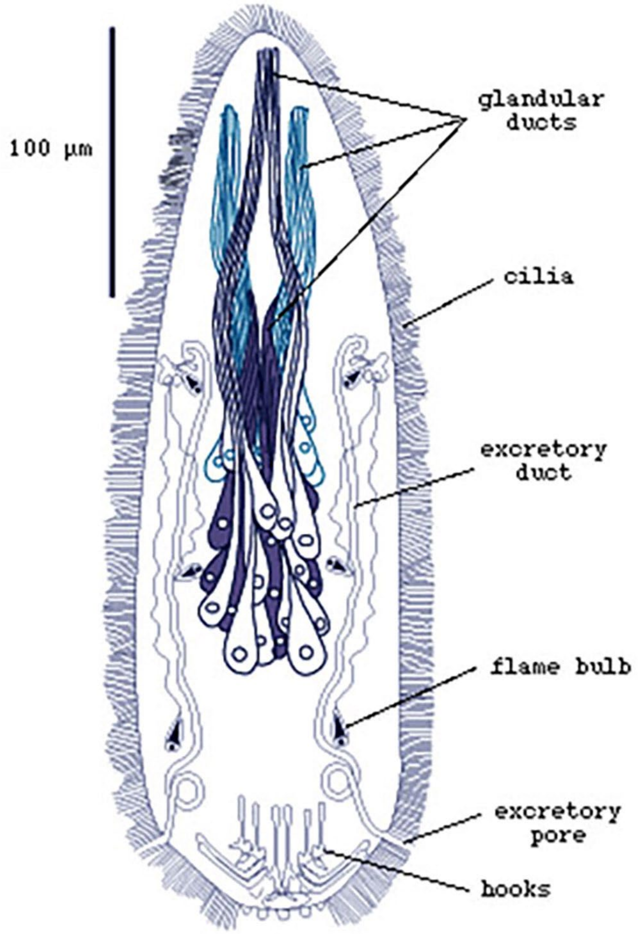

Figure 4. *Austramphilina elongata* larva. Note the bundles of secretory glands opening near the anterior end, the protonephridial system with 3 flame bulbs on each side opening near the posterior end, and the 10 posterior hooks. Source: Adapted from K. Rohde, 1986. License: CC BY-NC-SA 4.0.

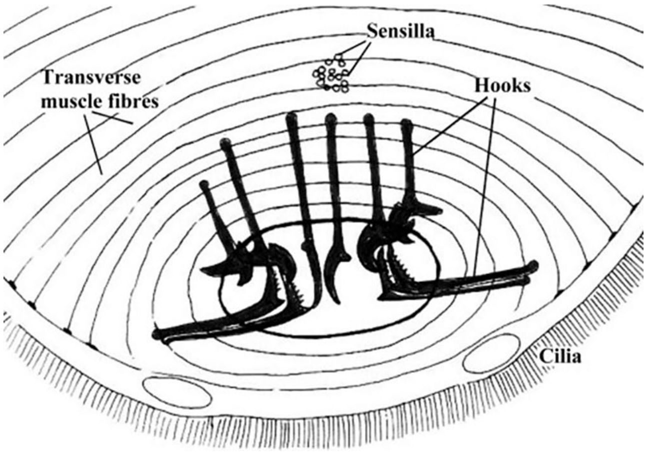

Figure 5. Posterior end of a larval *Austramphilina elongata*. Note the cluster of sensilla, transverse muscle bands, ciliated epidermis, and 5 pairs of hooks of 3 types. Source: K. Rohde. License: CC BY-NC-SA 4.0.

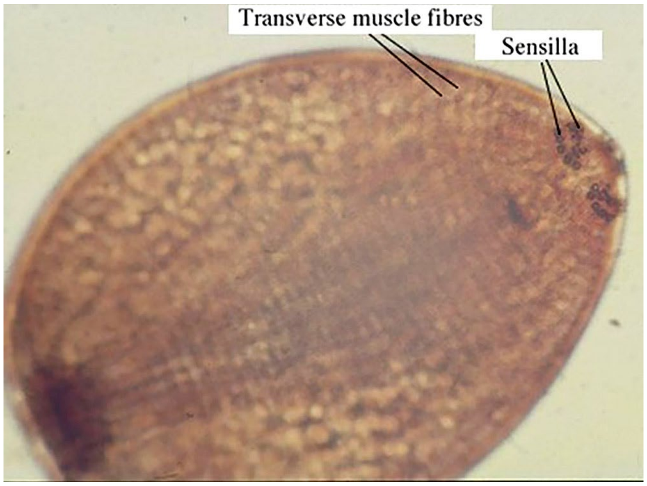

Figure 6. Larva of *Austramphilina elongata* impregnated with silver. Note the transverse muscle bands and receptors (sensilla). Source: K. Rohde. License: CC BY-NC-SA 4.0.

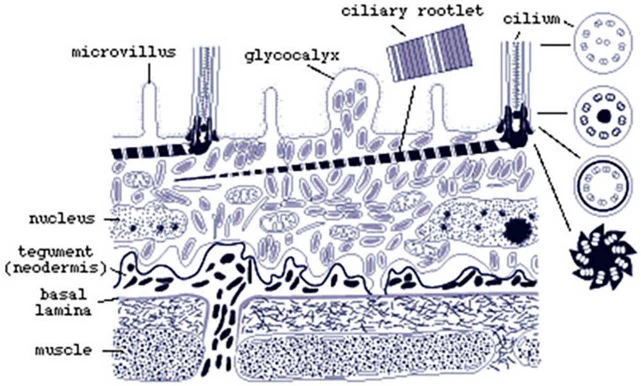

Figure 7. Larval *Austramphilina elongata*, diagram of electron-microscopic structure of surface layers. Note larval syncytial and ciliated epidermis at the surface, based on the tegument (neodermis) that has insunk (below the surface) nuclei (only the process leading to 1 nucleus is illustrated). Source: K. Rohde. License: CC BY-NC-SA 4.0.

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

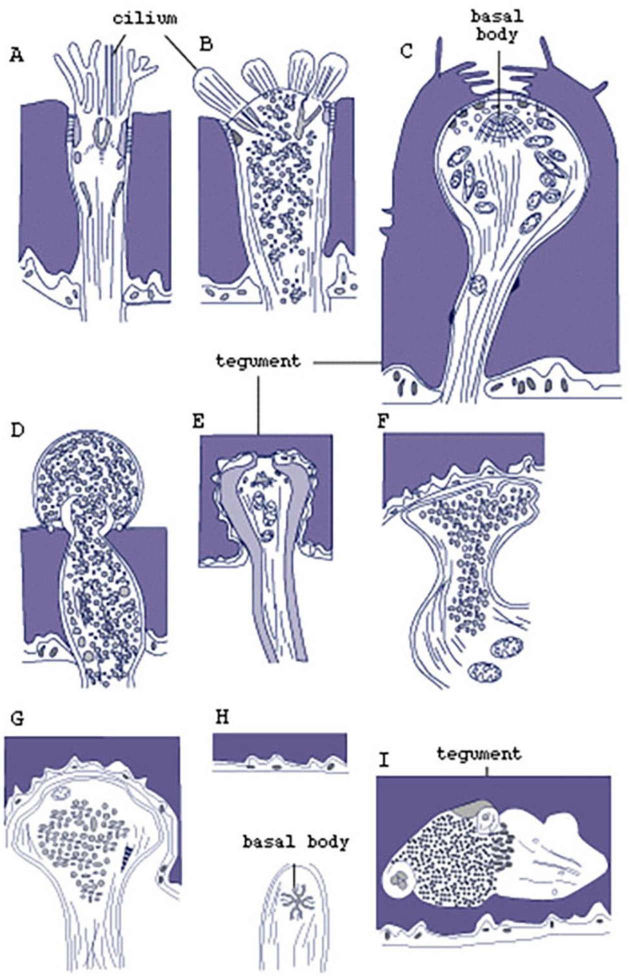

Figure 8. *Austramphilina elongata,* diagrams of larval receptors as seen under the transmission electron microscope. Source: Adapted from Rohde et al., 1986a. License: CC BY-NC-SA 4.0.

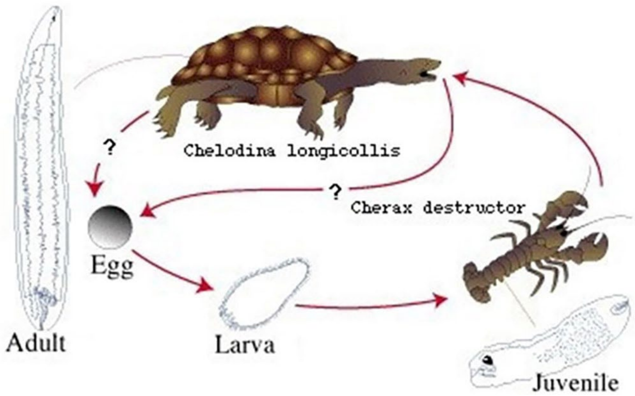

Figure 9. Life cycle of *Austramphilina elongata*. Note: Escape route of egg from turtle is unknown. Source: K. Rohde. License: CC BY-NC-SA 4.0.

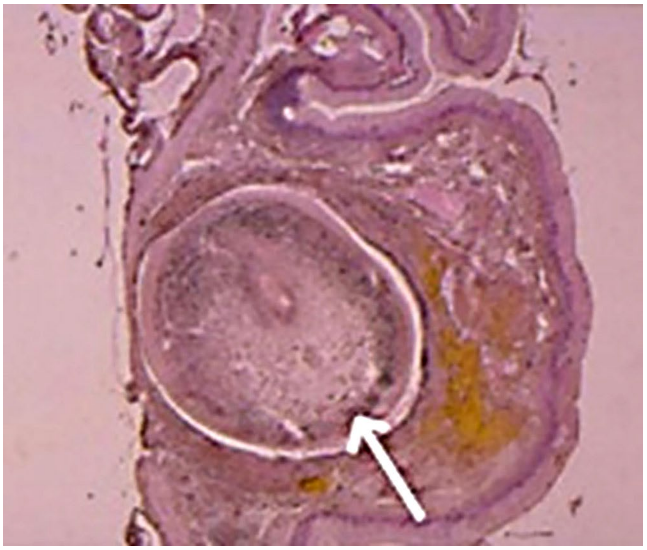

Figure 10. Section though the esophageal wall of a turtle, *Chelodina longicollis,* showing a penetrating *Austramphilina* juvenile (arrow). Source: K. Rohde. License: CC BY-NC-SA 4.0.

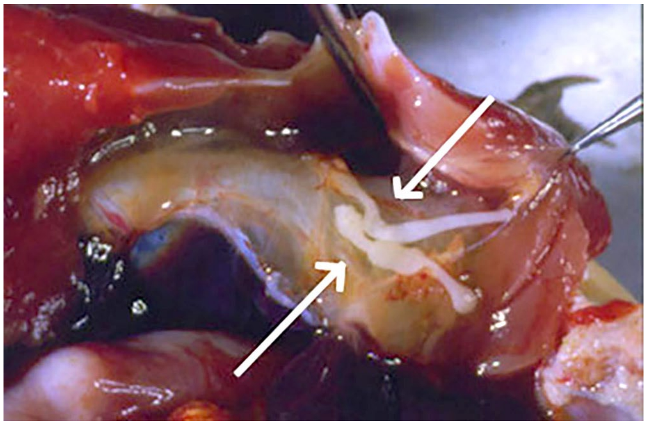

Figure 11. Two juvenile *Austramphilina* specimens (arrows) migrating along the trachea towards the body cavity of a turtle. Source: K. Rohde. License: CC BY-NC-SA 4.0.

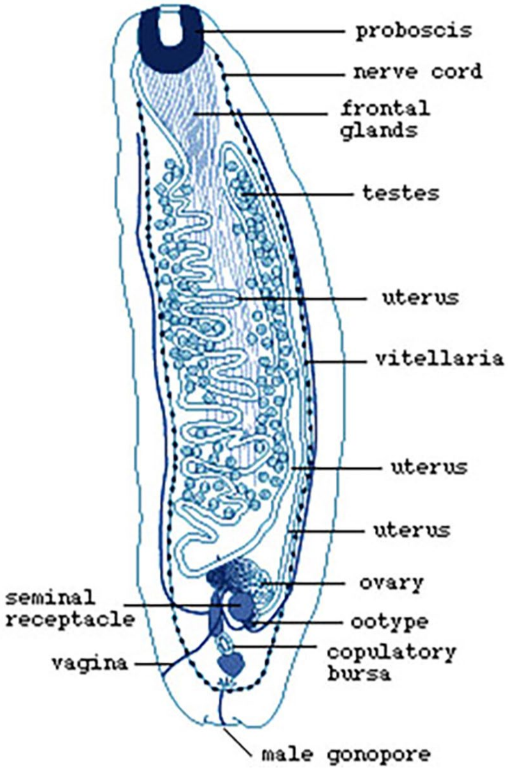

Figure 12. *Amphilina foliacea,* adult. Source: Adapted from Dubinina, 1982. License: CC BY-NC-SA 4.0.

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 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).

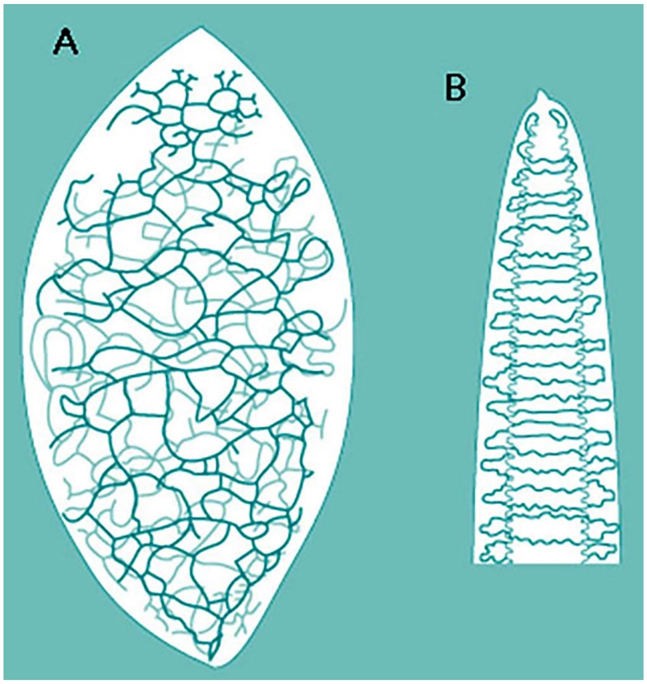

Figure 13. Protonephridial canal system of *Amphilina foliacea* (A) and of *Gephyrolina paragonopora* (B). Adapted from Dubinina, 1982. License: CC BY-NC-SA 4.0.

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.

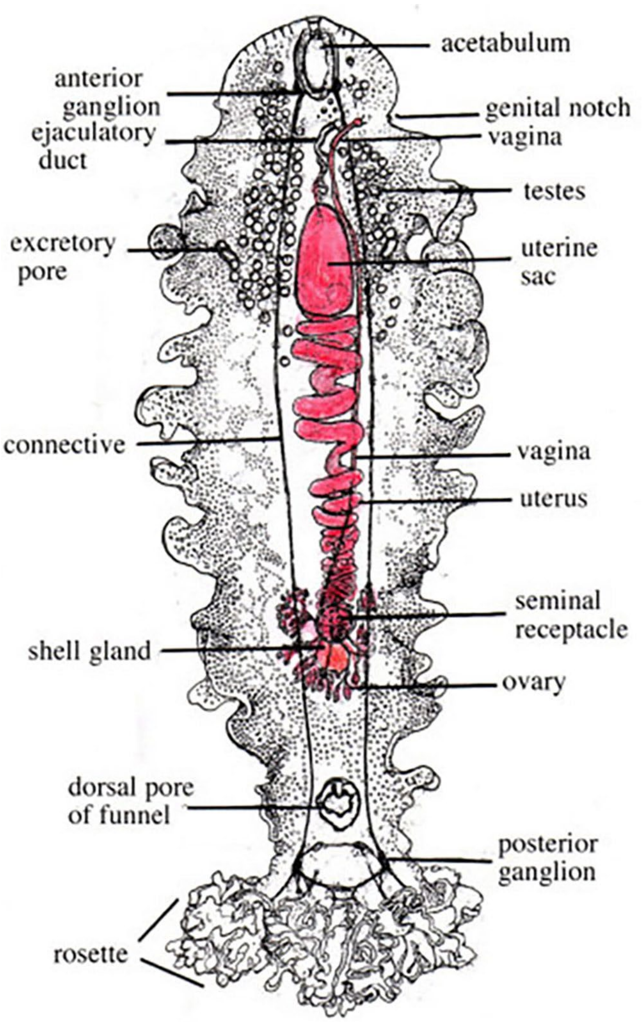

Figure 2. Adult *Gyrocotyle fimbriata*, dorsal view. Female reproductive system except vitellaria drawn red. Source: Adapted from Lynch (1945), Cheng (1986), and other sources. License: CC BY-NC-SA 4.0.

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.

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 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**

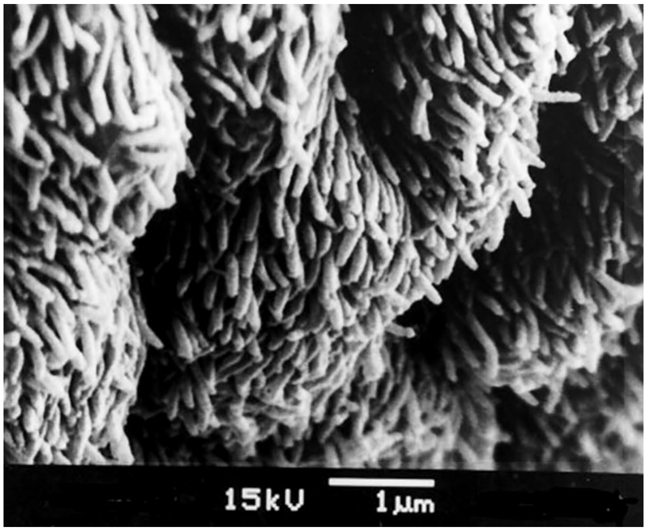

Figure 4. Scanning electron micrograph of the neodermis of *Gyrocotyle* sp., probably *G. rugosa,* from the holocephalan *Callorhinchus milii* in Tasmania, Australia. Note the numerous microvilli. Source: K. Rohde. License: CC BY-NC-SA 4.0.

(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).

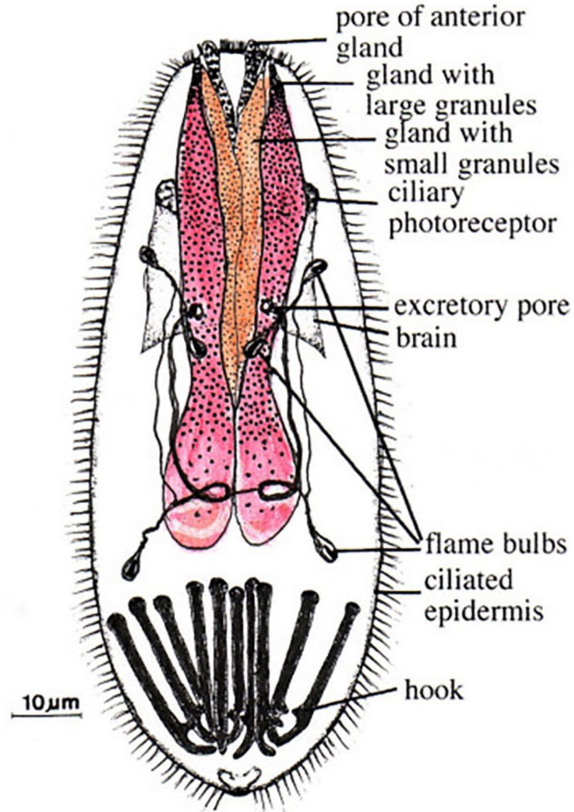

Figure 5. Lycophora larva of *Gyrocotyle urna*. Adapted from Xylander (1997c; 1990; 1991; 2001; 2006b) and Rohde (1994). License: CC BY-NC-SA 4.0.

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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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

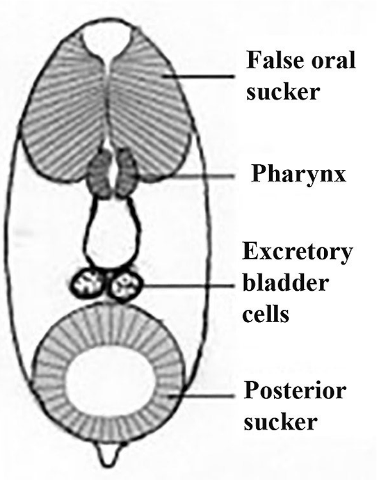

Figure 1. Larva of *Lobatostoma manteri*. Note the **false anterior sucker**, the **pharynx**, the **blind ending cecum**, and the 2 **excretory bladder cells**. The posterior end is drawn out into a short appendage of unknown function. Source: K. Rohde. License: CC BY-NC-SA 4.0.

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.

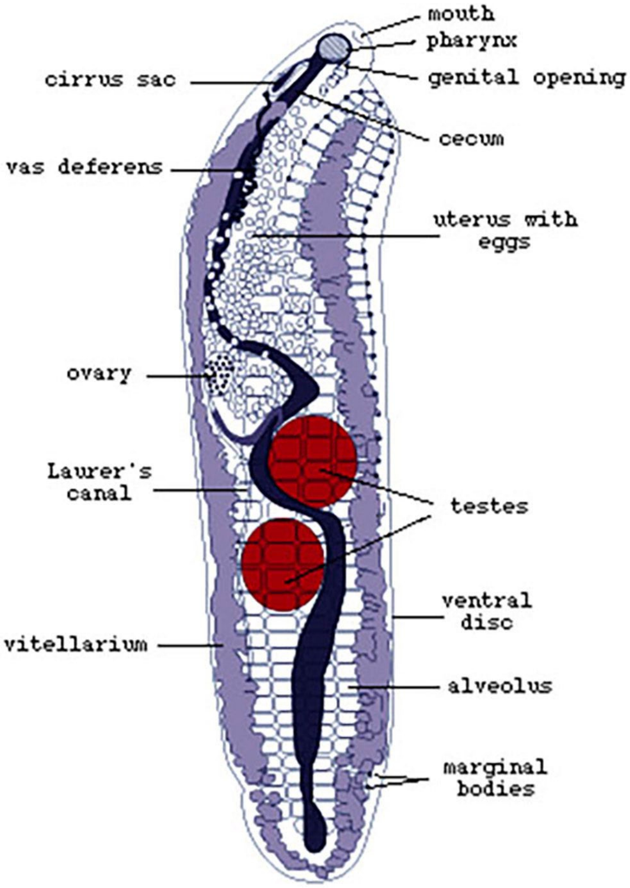

Figure 5. Adult *Multicotyle purvisi*. Note the single and blind ending **cecum** (**intestine**), the **ventral** (**adhesive**) **disc** consisting of 4 rows of **alveoli**, the **male genital system** consisting of 2 large **testes** opening into the **vas deferens** (**sperm duct**) and the **terminal 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).

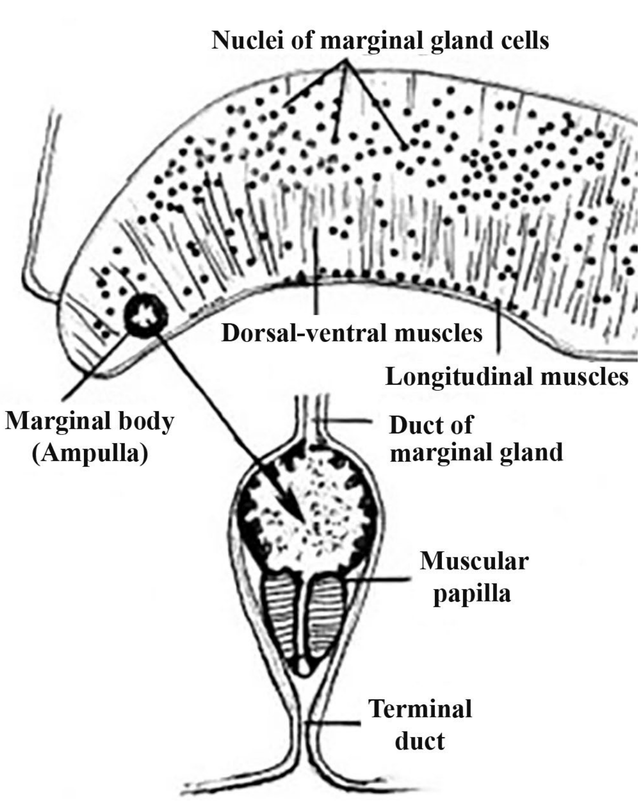

Figure 6. Cross section through the lateral part of the **adhesive disc** of *Lobatostoma manteri* showing the **nuclei** and the **terminal ampulla**, **papilla**, and **terminal duct** of a **marginal gland**. Source: K. Rohde. License: CC BY-NC-SA 4.0.

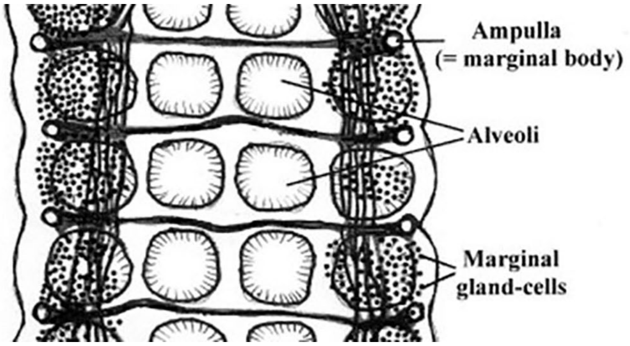

Figure 7. Ventral view of part of the ventral disc of *Lobatostoma manteri* showing the marginal glands and bodies connected by longitudinal and transverse ducts. Source: K. Rohde. License: CC BY-NC-SA 4.0.

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**

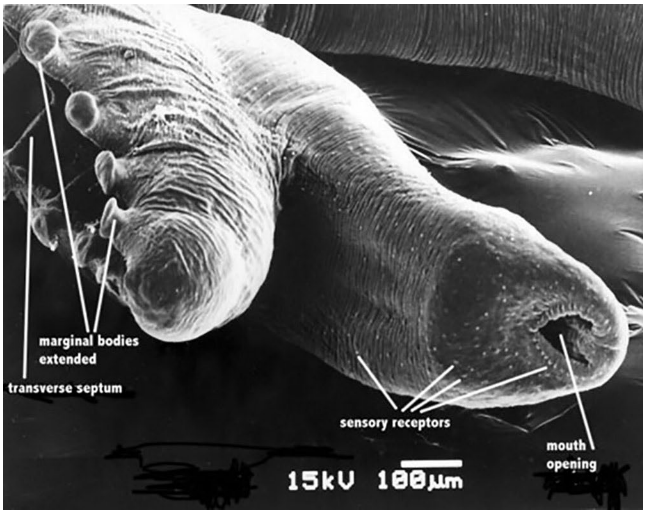

Figure 8. Scanning electron micrograph of anterior end of *Multicalyx elegans*. Source: K. Rohde. License: CC BY-NC-SA 4.0.

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;

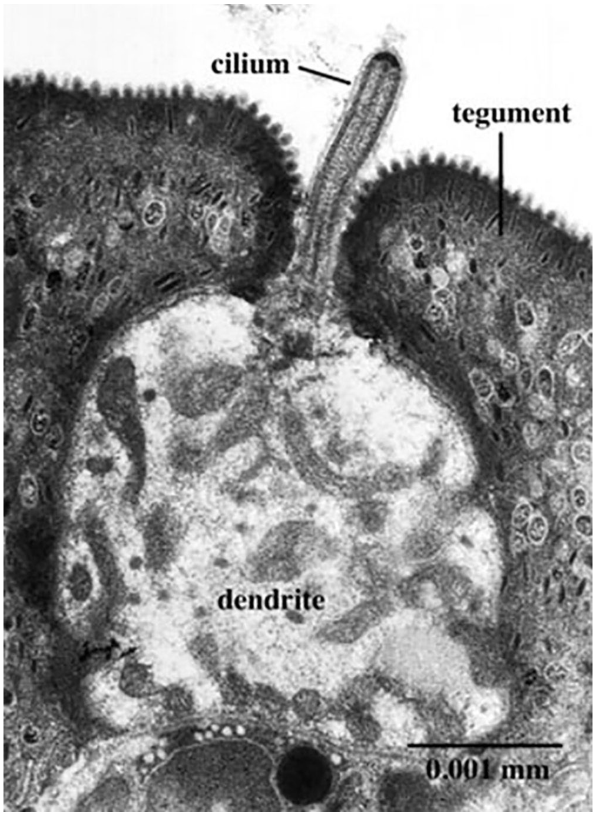

Figure 9. Ciliated surface receptor of *Lobatostoma manteri*. A single cilium arises from the terminal dendritic swelling. Source: K. Rohde. License: CC BY-NC-SA 4.0.

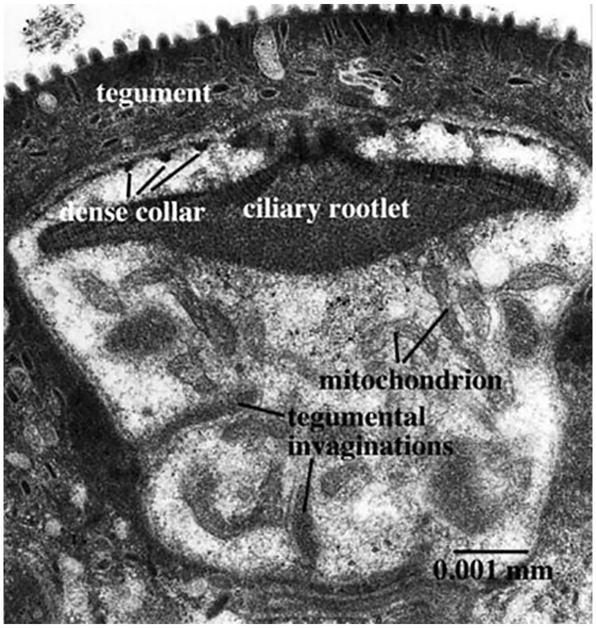

Figure 10. The receptor of *Lobatostoma manteri* without a free cilium, but with an expanded ciliary rootlet. Also note the sections through the dense collars around the upper part of the dendrite. Source: K. Rohde. License: CC BY-NC-SA 4.0.

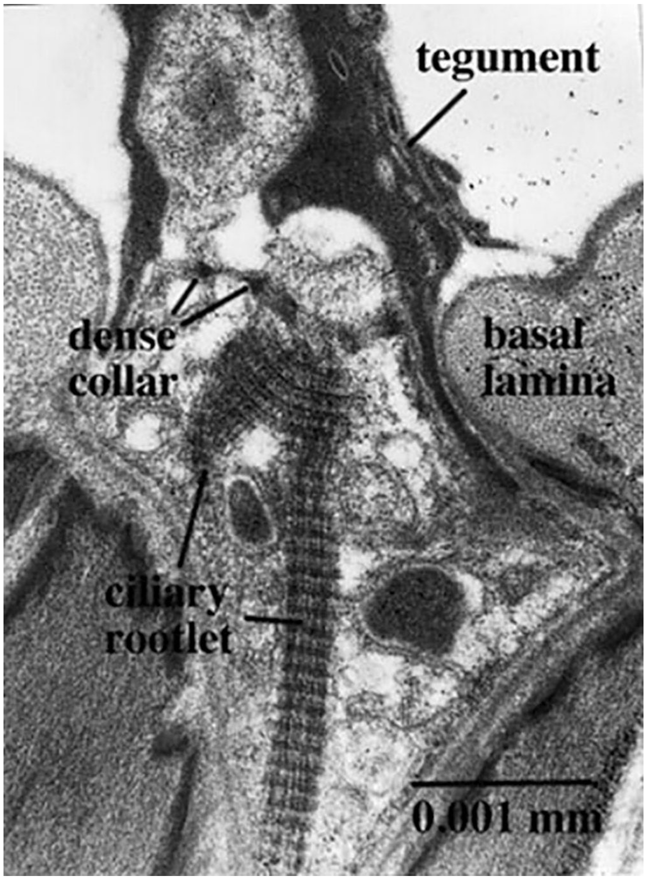

Figure 11. Non-ciliate receptor of *Lobatostoma manteri* located in a deep pit, with branching ciliary rootlet. Source: K. Rohde. License: CC BY-NC-SA 4.0.

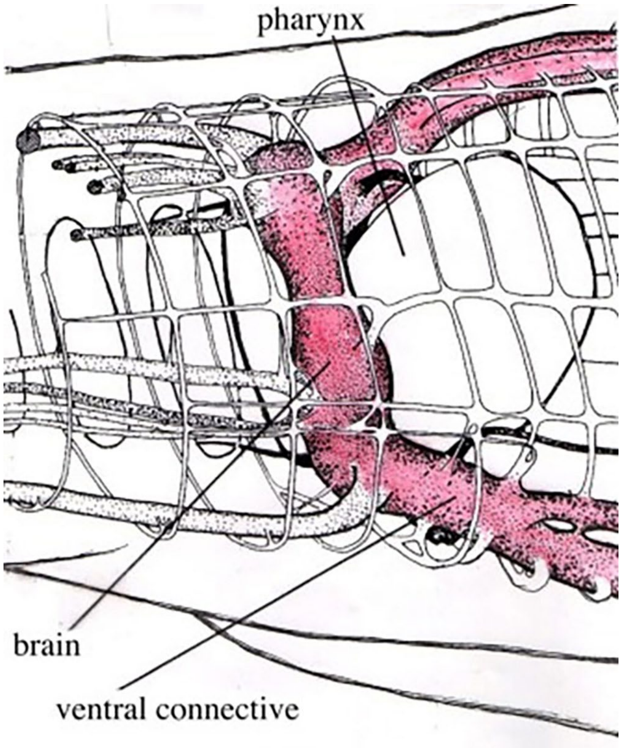

Figure 12. **Nervous system** of *Multicotyle purvisi* in the anterior part of the body. Some nerves on the right-hand side of the worm have been cut in order to show the arrangement of the nerves in cross section. Note the 2 rings of commissures, and the very large brain (**cerebral commissure**) and **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.

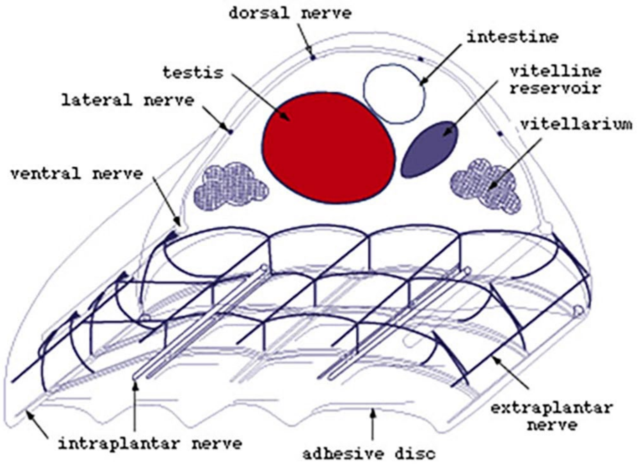

Figure 13. **Nervous system** of *Multicotyle purvisi* in the middle part of the body, showing a typical arrangement of connectives and commissures in the dorsal part of the body, and an intricate pattern of nerves innervating the **ventral** (**adhesive**) **disc**. Source: K. Rohde. License: CC BY-NC-SA 4.0.

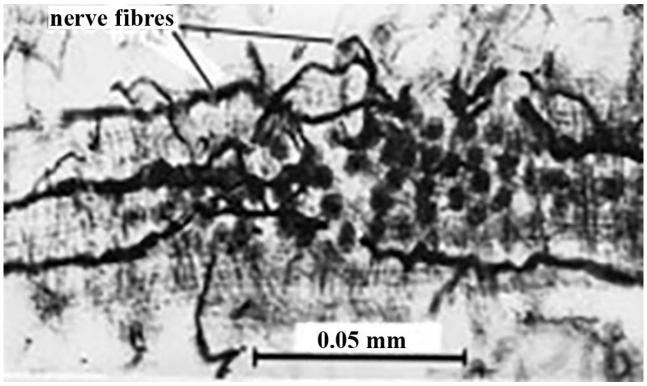

Figure 14. **Nerve plexus** around the intestine of *Multicotyle purvisi*. Section impregnated with silver as seen under the light microscope. Source: K. Rohde. License: CC BY-NC-SA 4.0.

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).

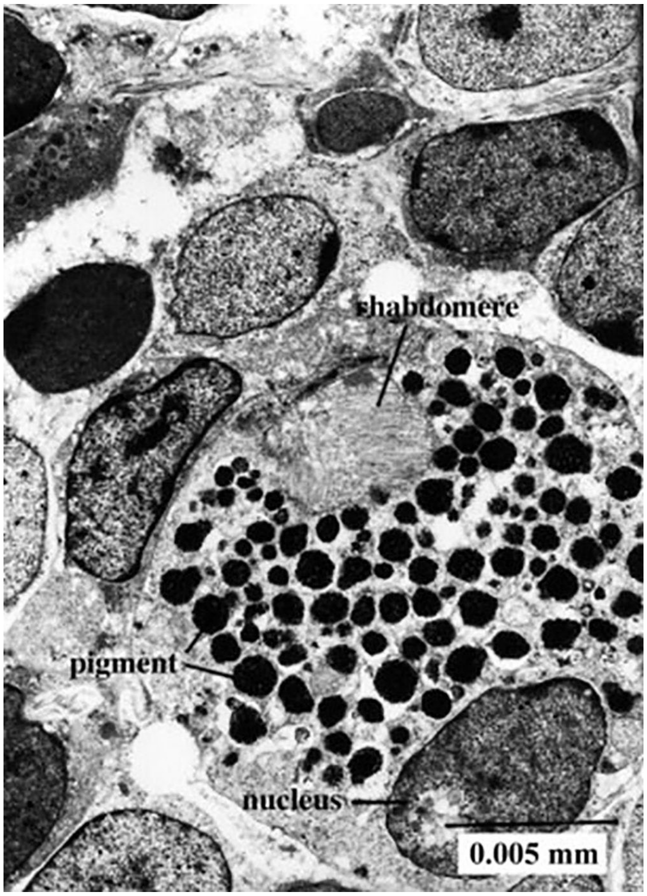

Figure 15. Transmission electron micrograph of ocellus (eye) of larval *Multicotyle purvisi*. Note the pigment cell with pigment granules, nucleus of pigment cell, and rhabdomere (light-sensitive dendritic endings). Source: K. Rohde. License: CC BY-NC-SA 4.0.

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

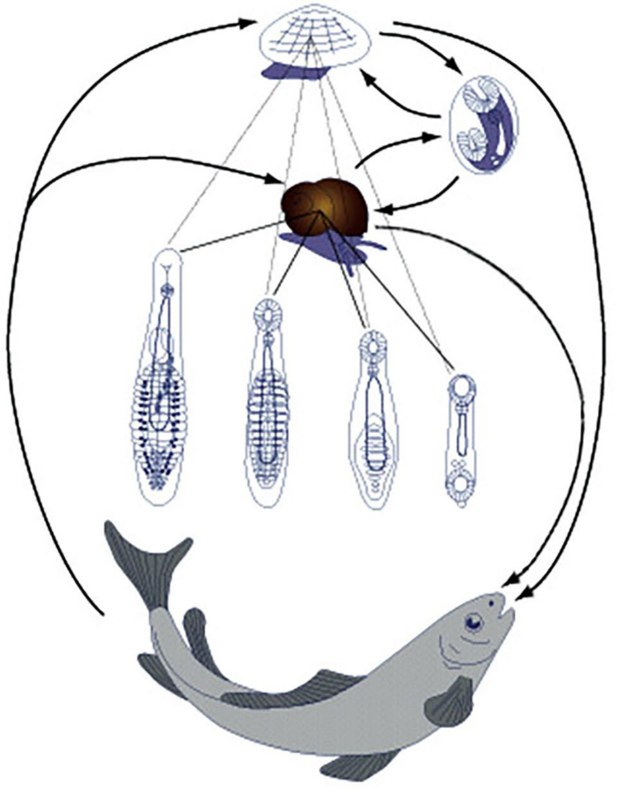

Figure 16. Life cycle of *Aspidogaster conchicola*. Only molluscs (freshwater bivalves or snails) are necessary for the completion of the life cycle. Adult worms produce eggs in which a non-ciliated larva with an anterior and posterior sucker develops. There are conflicting reports on how molluscs become 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.

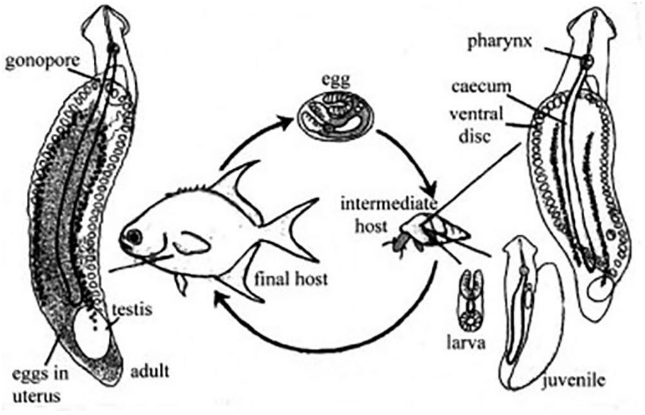

Figure 17. The life cycle of *Lobatostoma manteri*. Adults live in the small intestine of the marine teleost fish *Trachinotus blochi* (family Carangidae). They possess fully mature male and female reproductive systems with 1 large posterior testis, and a uterus filled with eggs which are shed through 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.

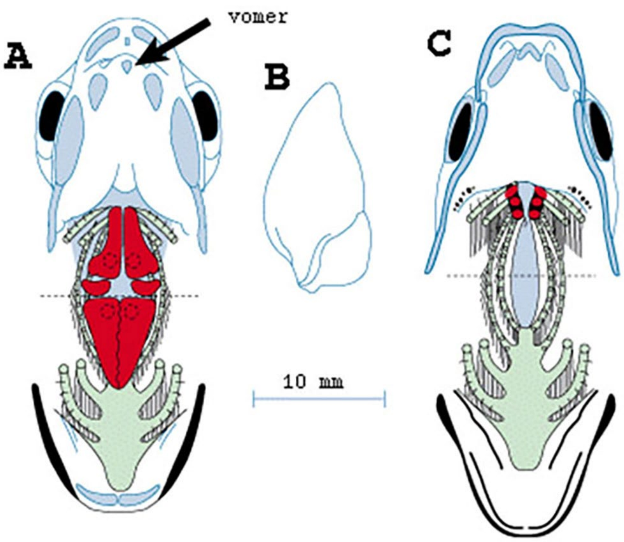

Figure 18. The head of juvenile *Trachinotus blochi* (A) opened along the dotted line. Snails (B) are crushed between the pharyngeal plates (red) which are moved by strongly developed muscles responsible for the peculiarly shaped head of the fish species (snub-nosed dart). The pointed vomer (arrow) prevents 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.

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*,

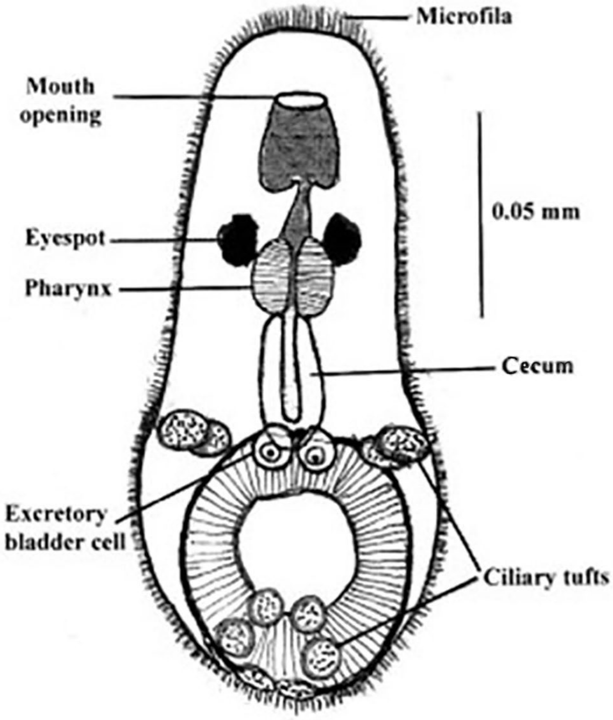

Figure 19. Larva of *Multicotyle purvisi*. Note the anterior mouth not surrounded by an anterior sucker, followed by the pharynx and cecum. As in the larva of *Lobatostoma manteri* (see Figure 1), there are a posterior sucker and 2 excretory bladder cells. In addition, the surface is covered by a thick 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.

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.

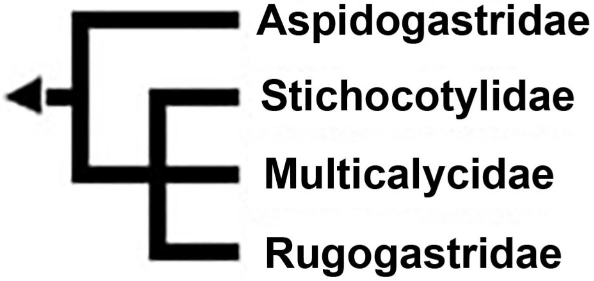

Figure 20. The likely relationship of the aspidogastrean families with each other based on morphology and hosts. Source: K. Rohde. License: CC BY.

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 °C 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.

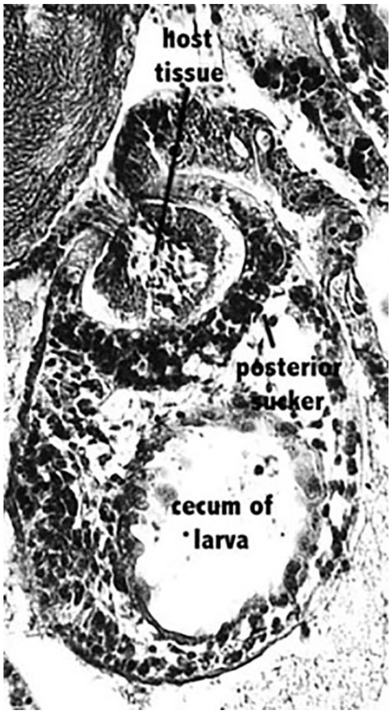

Figure 21. Young larva of *Lobatostoma manteri* (47–49 days old) feeding on the digestive gland of an experimentally infected *Cerithium moniliferum*. Note the posterior sucker around some host tissue. Source: K. Rohde. License: CC BY-NC-SA 4.0.

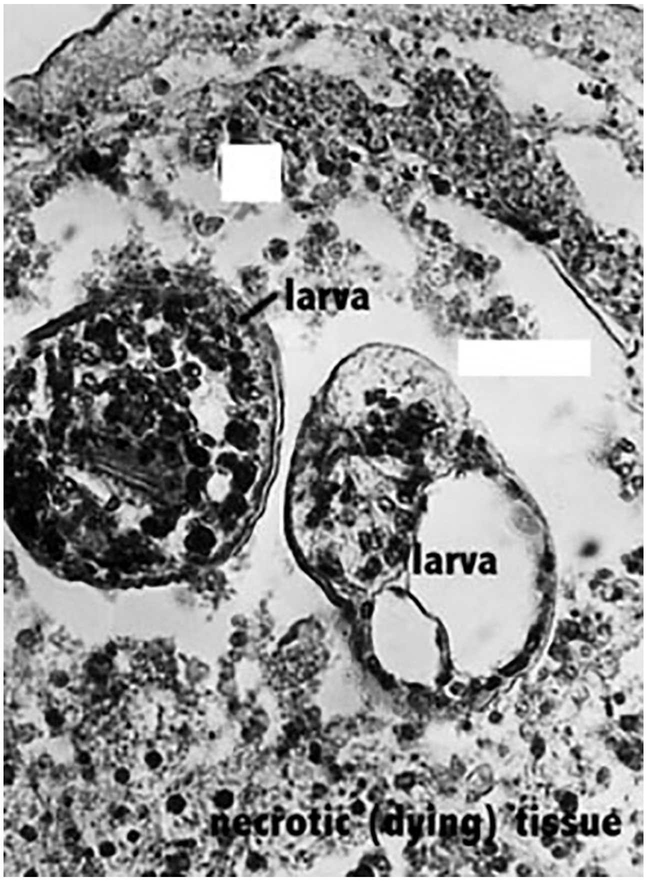

Figure 22. Two larvae (65–67 days old) of *Lobatostoma manteri* in the digestive gland of an experimentally-infected *Cerithium moniliferum*. Note the functional glandular tissue replaced by necrotic (dead and dying) cells. Source: K. Rohde. License: CC BY-NC-SA 4.0.

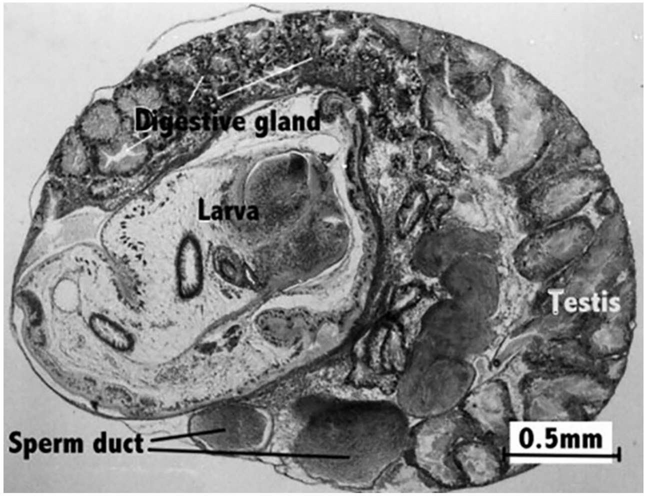

Figure 23. *Cerithium moniliferum* with one large juvenile *Lobatostoma manteri* ("larva") in the digestive gland. Source: K. Rohde. License: CC BY-NC-SA 4.0.

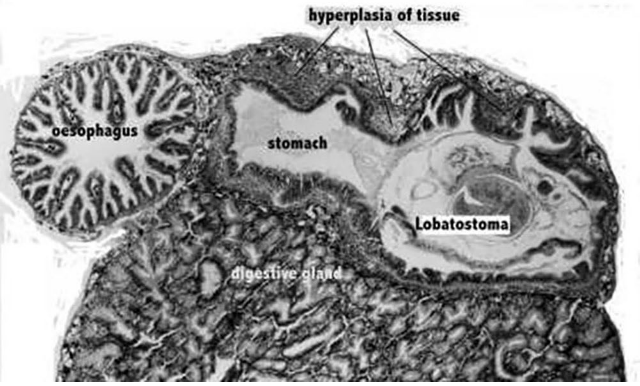

Figure 24. Large juvenile of *Lobatostoma manteri* in the stomach of naturally infected *Peristernia australiensis*. Note the enlarged subepithelial tissue (hyperplasia), that is, fibrosis (abnormally thick connective tissue) around parts of the stomach. Source: K. Rohde. License: CC BY-NC-SA 4.0.

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.

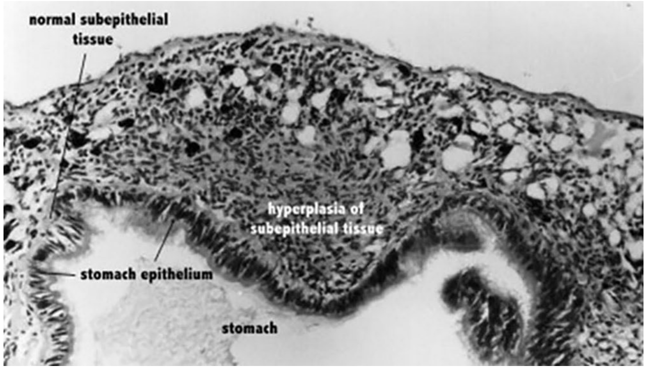

Figure 25. Another view of the *Lobatostoma manteri* specimen in the stomach of naturally infected *Peristernia australiensis* shown in Figure 24. Source: K. Rohde. License: CC BY-NC-SA 4.0.

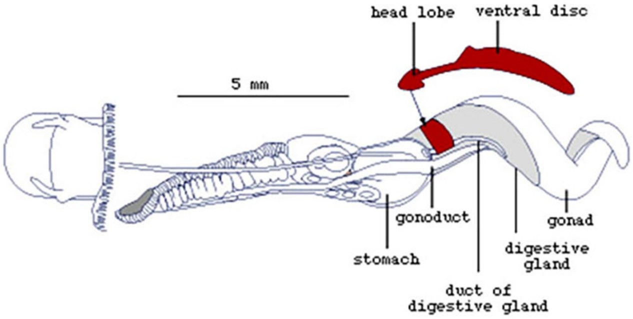

Figure 26. *Peristernia australiensis* infected with *Lobatostoma manteri.* This is an enlarged portion of Figure 25. Source: K. Rohde. License: CC BY-NC-SA 4.0.

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).

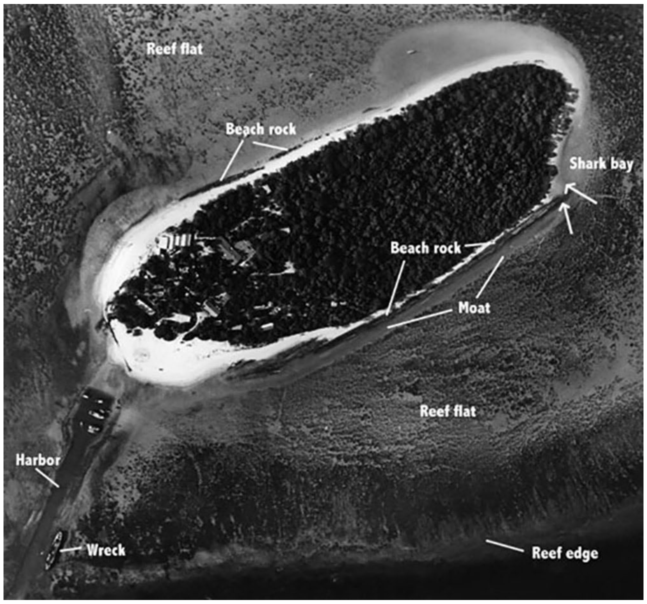

Figure 27. The distribution and infection of snails with *Lobatostoma manteri* at Heron Island at the southern end of the Great Barrier Reef in January–April 1971. Note: Beach rock around much of the island, the harbor and the moat (shallow channel) extending from the harbor area towards Shark Bay. At 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/

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DIGENEA, DIPLOSTOMIDA

Introduction to Diplostomida Olson et al., 2003 (Order)

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Phylum Platyhelminthes
  Class Trematoda
    Subclass Digenea
      Order Diplostomida

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Chapter 34

Introduction to Diplostomida Olson et al., 2003 (Order)

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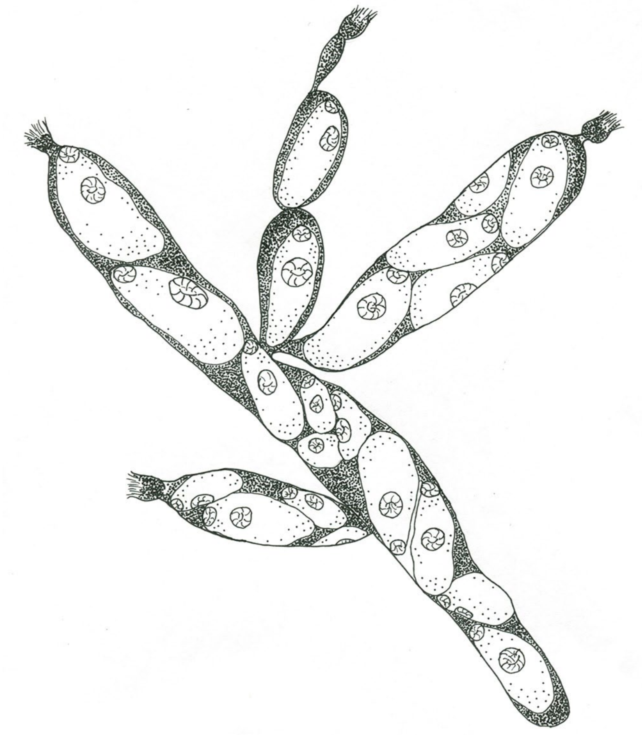

Figure 1. Branched sporocyst of Brachylaimoidea. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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).

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).

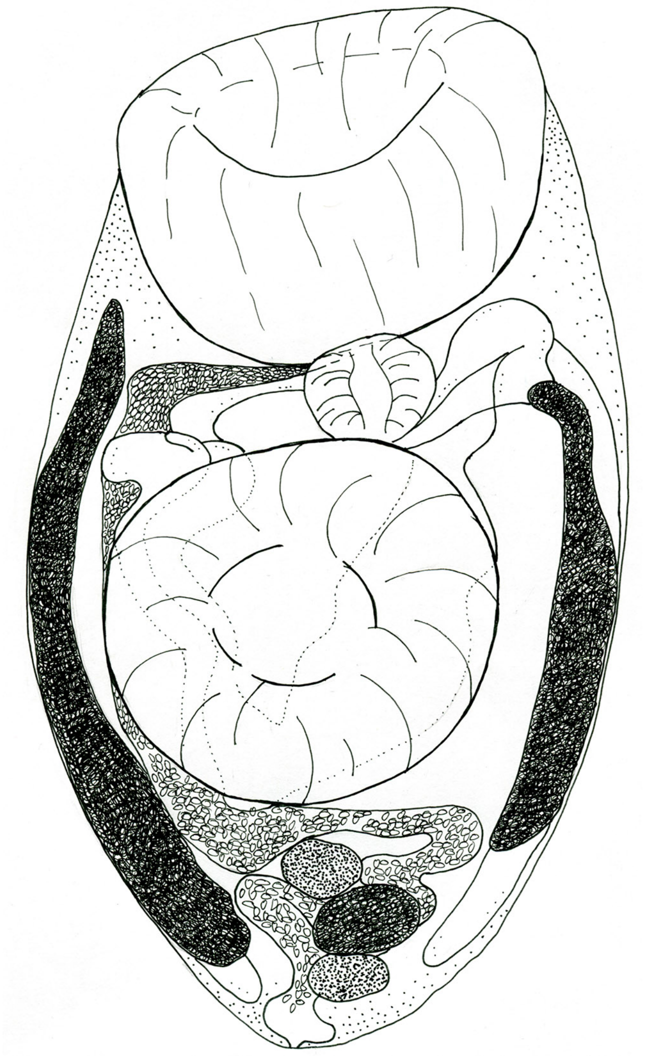

Figure 2. General scheme of an adult specimen of *Urogonimus* sp. (Leucochloridiidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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,

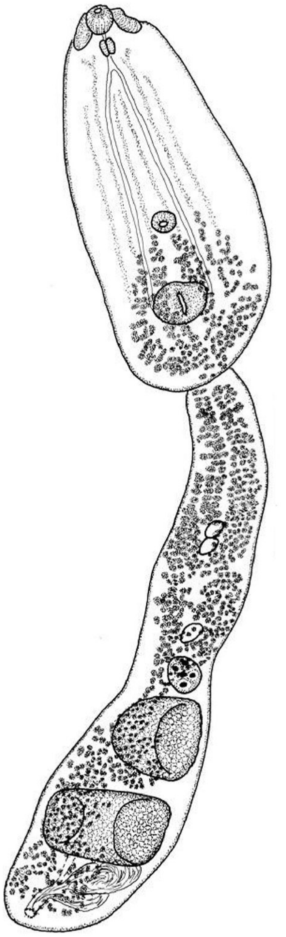

Figure 3. Adult specimen of *Diplostomum pseudospathaceum* (family Diplostomidae). Source: Pérez del Olmo et al., 2014. License: CC BY 4.0.

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

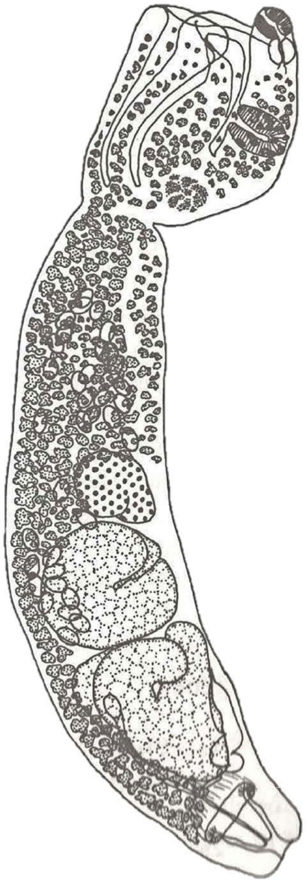

Figure 4. Adult specimen of *Strigea falconis* (family Strigeidae). Source: Dubois, 1968. License: Public domain.

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 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.

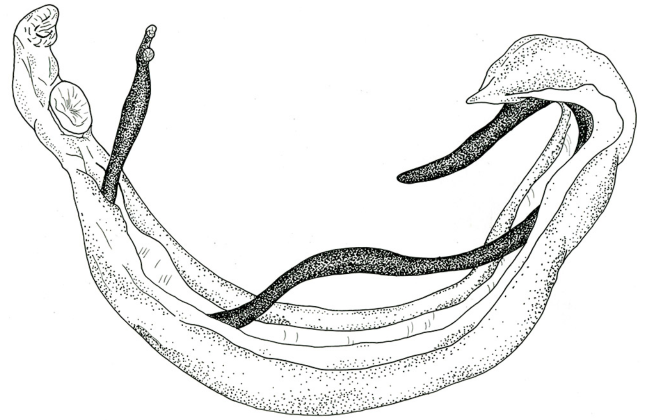

Figure 5. Adults of *Schistosoma* spp. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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

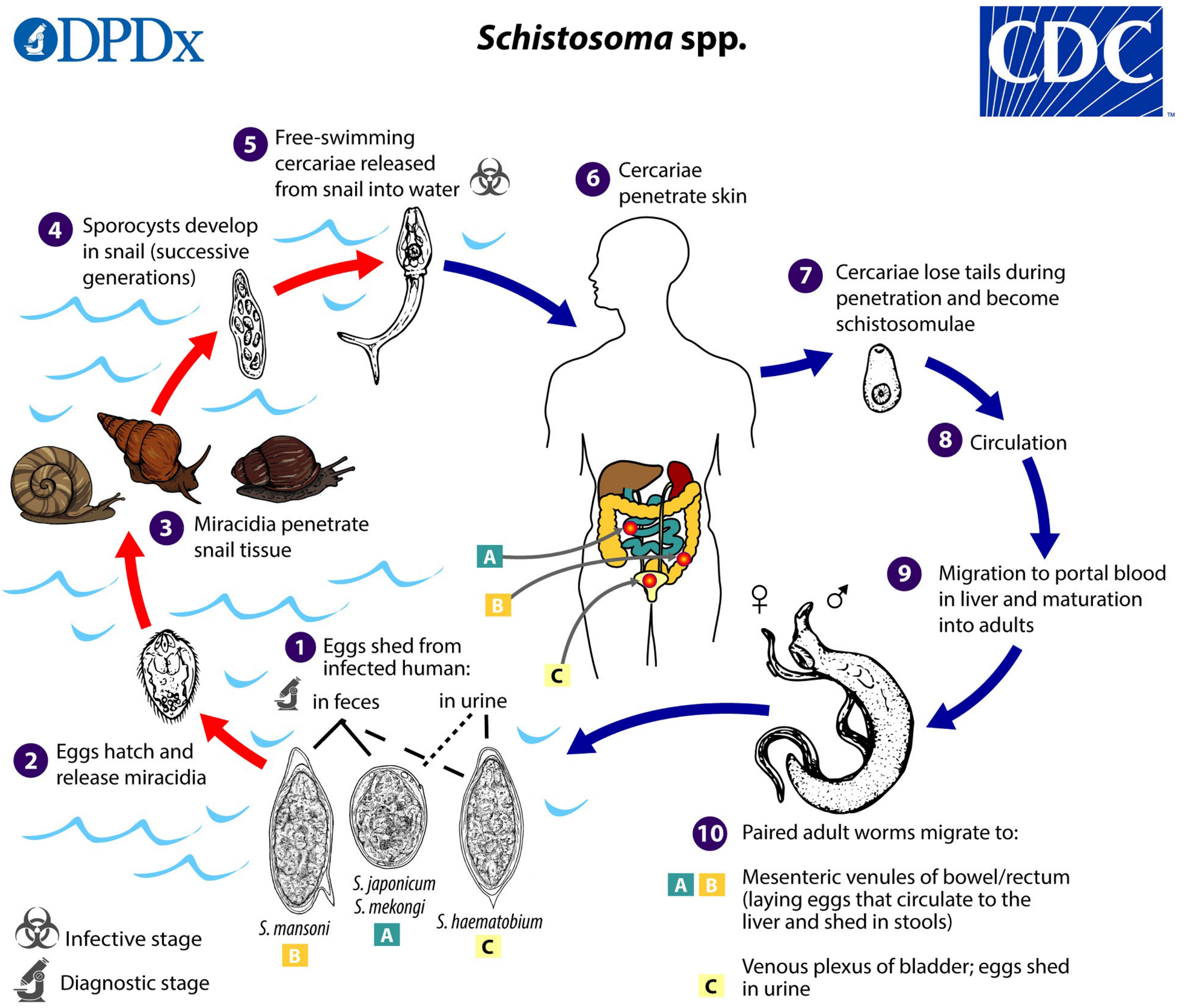

Figure 6. *Schistosoma* eggs are eliminated with feces or urine, depending on species (1). Under appropriate conditions the eggs hatch and release miracidia (2), which swim and penetrate specific snail intermediate hosts (3). The stages in the snail include 2 generations of sporocysts (4) and the production 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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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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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).

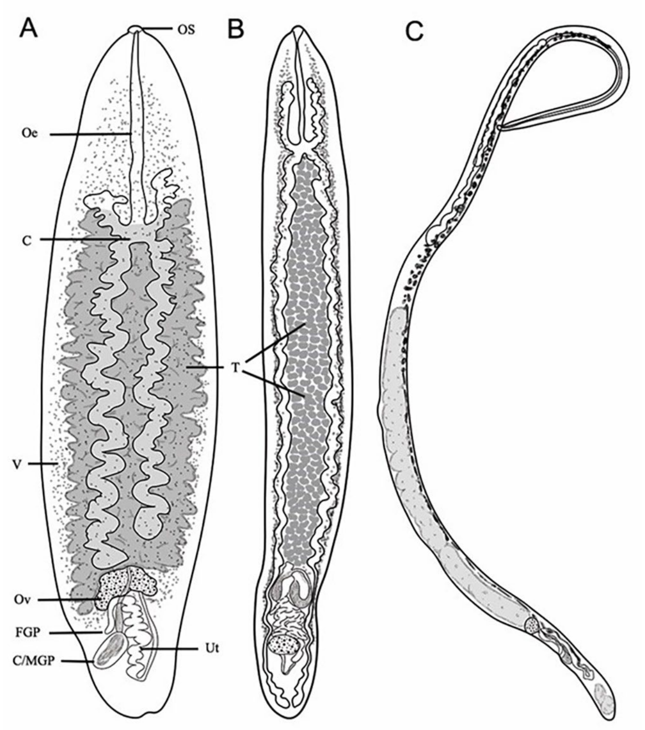

Figure 1: Selected aporocotylid species demonstrating their general body plans. A) *Psettarium pandora* Yong et al., 2018 (after Yong et al., 2018), a good example of a typical aporocotylid; B) *Aporocotyle simplex* Odhner, 1900 (after Thulin, 1980), showing a similar body plan but possessing multiple 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).

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).

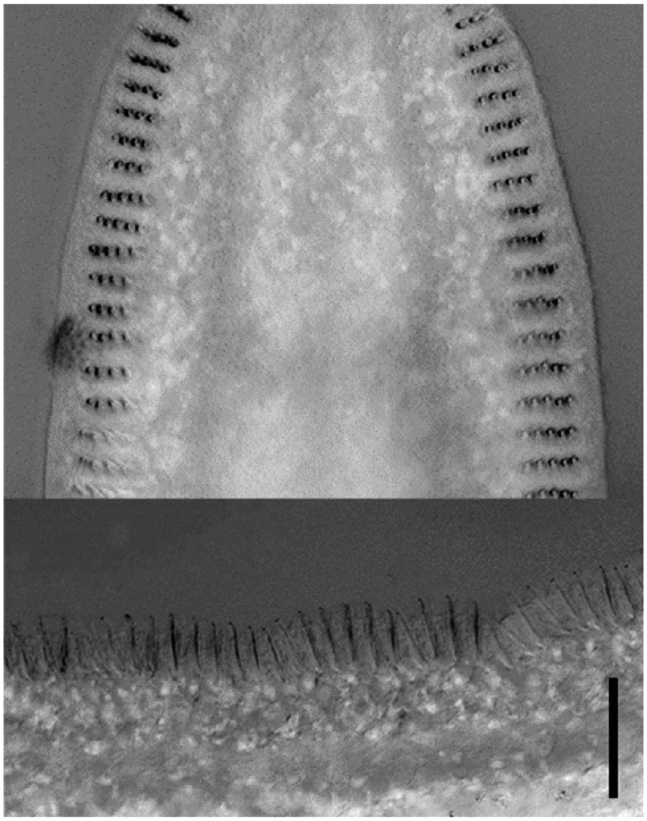

Figure 2: Ventral (top) and lateral (bottom) views of the tegumental spines of aporocotylids, as exemplified by *Cardicola suni* Yong et al., 2016. Note the hooked ends of the spines (seen most clearly in lateral view), and their arrangement in regular rows along the ventrolateral body margins. Scale bar: 20 µm. Source: R. Q.-Y. Yong. License: CC BY-NC-SA 4.0.

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

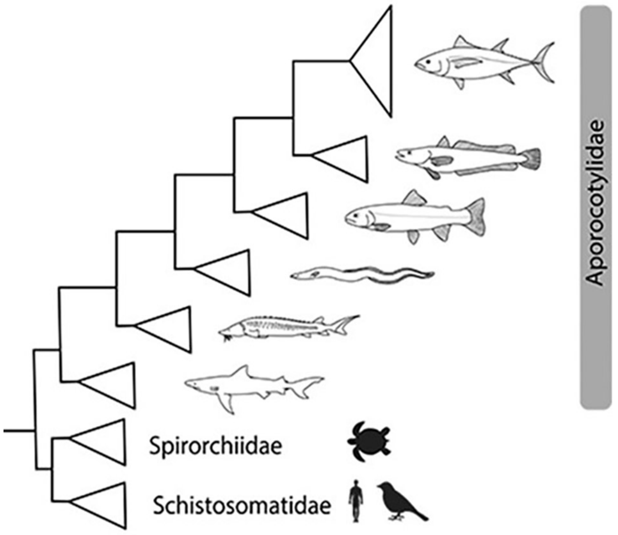

Figure 3: A simplified phylogenetic tree of the Aporocotylidae showing its relationships to the 2 other schistosomatoid families, the Schistosomatidae (blood flukes of mammals and birds) and the Spirorchiidae (blood flukes of reptiles), and cophyly with progressively evolutionarily-advanced groups of 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.

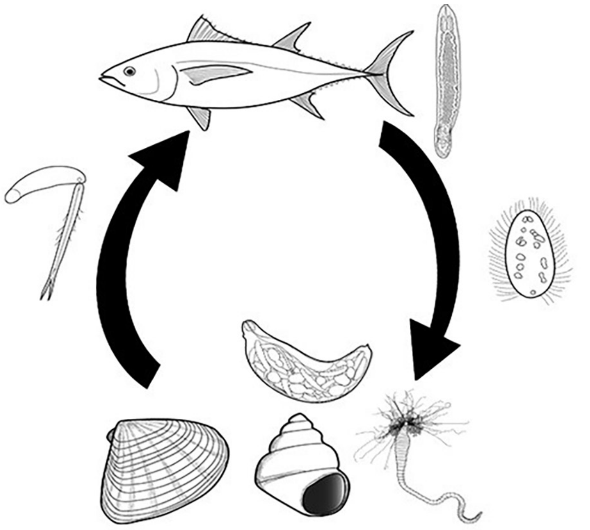

Figure 4: A generalized life cycle for aporocotylids. Adult worms live in the fish host and produce eggs (top); miracidial larvae (right) hatch, leave the fish and infect either a bivalve (bottom left; chondrichthyan-infecting species), gastropod (bottom middle; freshwater teleost-infecting species), 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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36

DIGENEA, PLAGIORCHIIDA

Introduction to Plagiorchiida La Rue, 1957 (Order)

Rafael Toledo, Bernard Fried, and Lucrecia Acosta Soto

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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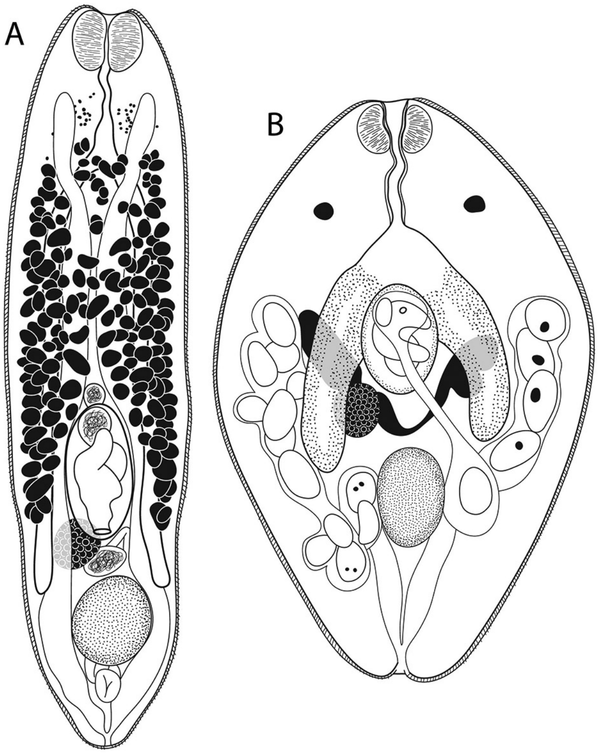

Figure 1. Sexual adult worms of family Bivesiculidae. All species lack oral and ventral suckers but have a well-developed pharynx. Species of the genus *Paucivitellosus* are unique within the family in having a highly reduced vitellarium and eggs that embryonate and grow in utero. A) *Bivesicula claviformis*. 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.

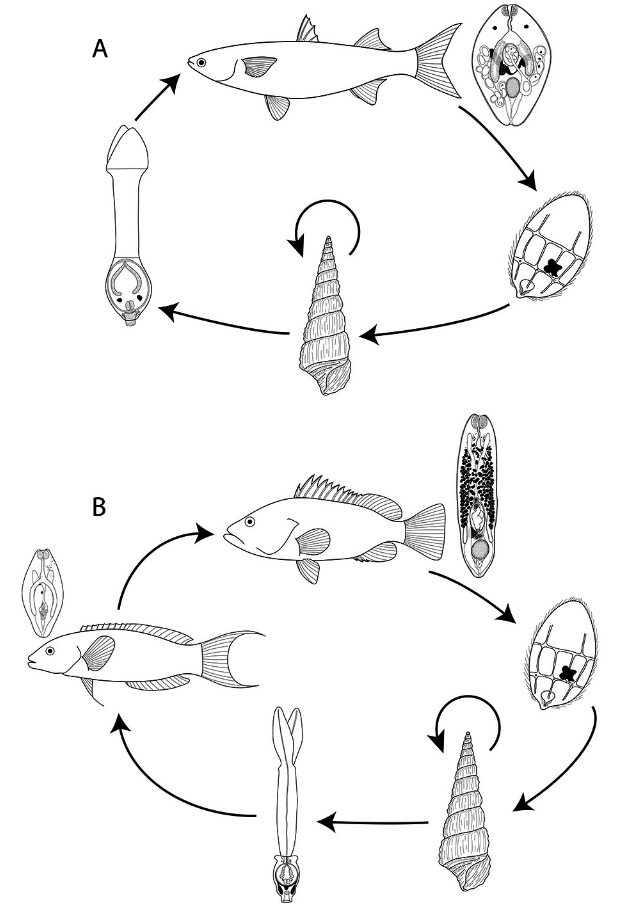

Figure 2. Life cycle of Bivesiculidae. A) *Paucivitellosus fragilis*; cercaria is ingested directly by definitive host. B) *Bivesicula claviformis*; cercaria is ingested by small fish, develops into a juvenile in the gut, and is then ingested with its host by a larger fish-eating grouper. The worms never 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.

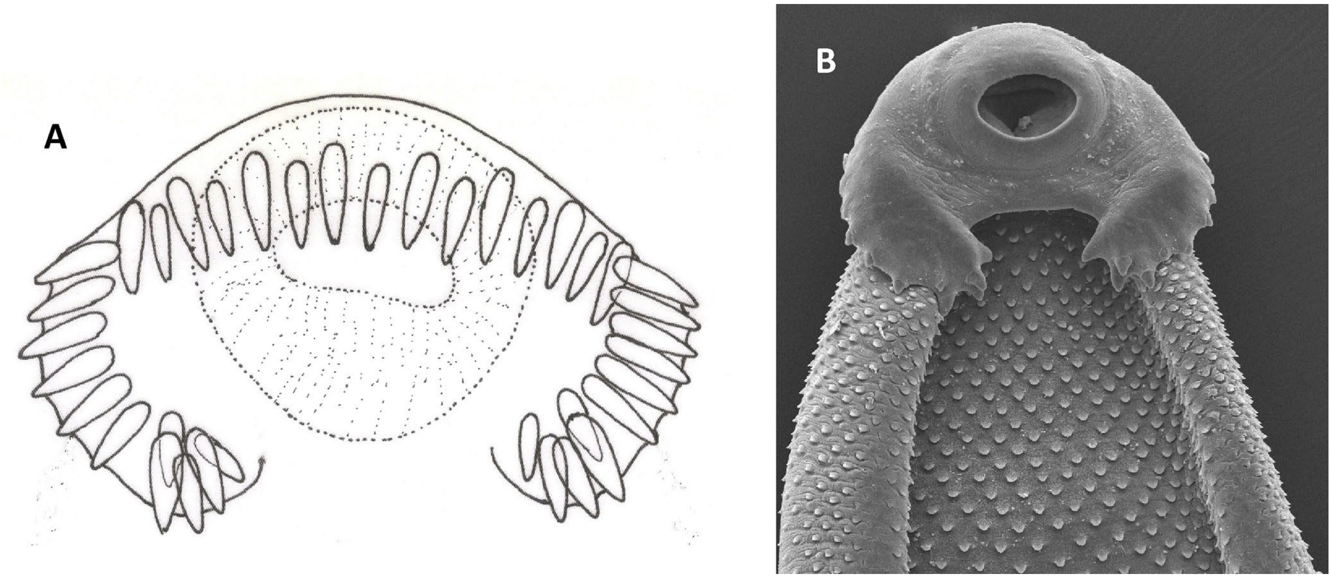

Figure 1. A) Cephalic collar of spines of *Echinostoma* sp. (Echinostomatidae) arranged in a double row (original); B) SEM microphotography of the forebody of *Echinostoma* sp. (Echinostomatidae) showing the cephalic collar of spines. Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 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

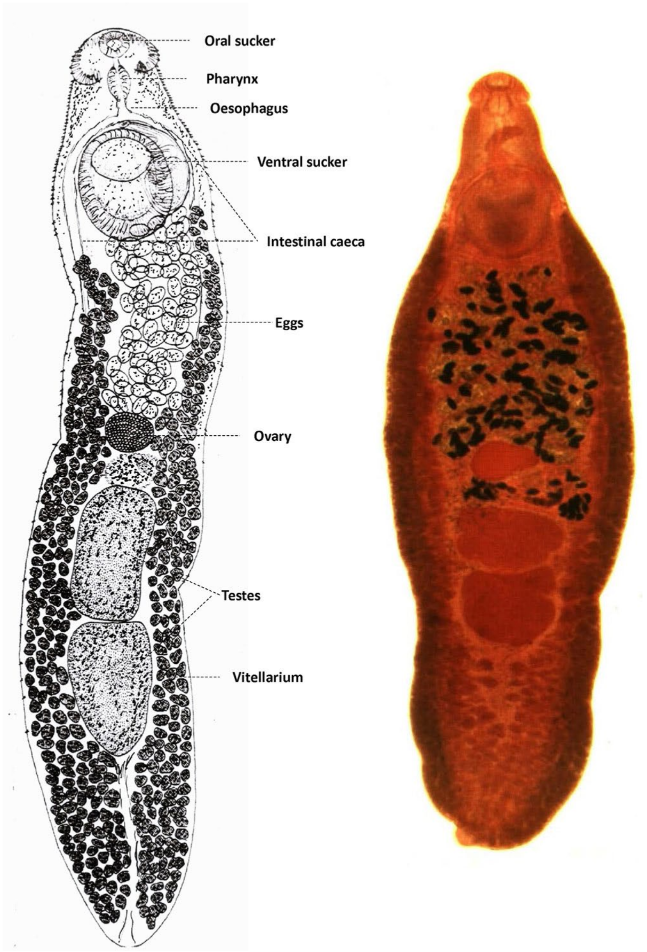

Figure 2. A) Adult *Echinostoma* sp. (Echinostomatidae); B) Adult specimen of *E. caproni* (Echinostomatidae) stained with Grenacher's borax carmin. Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

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

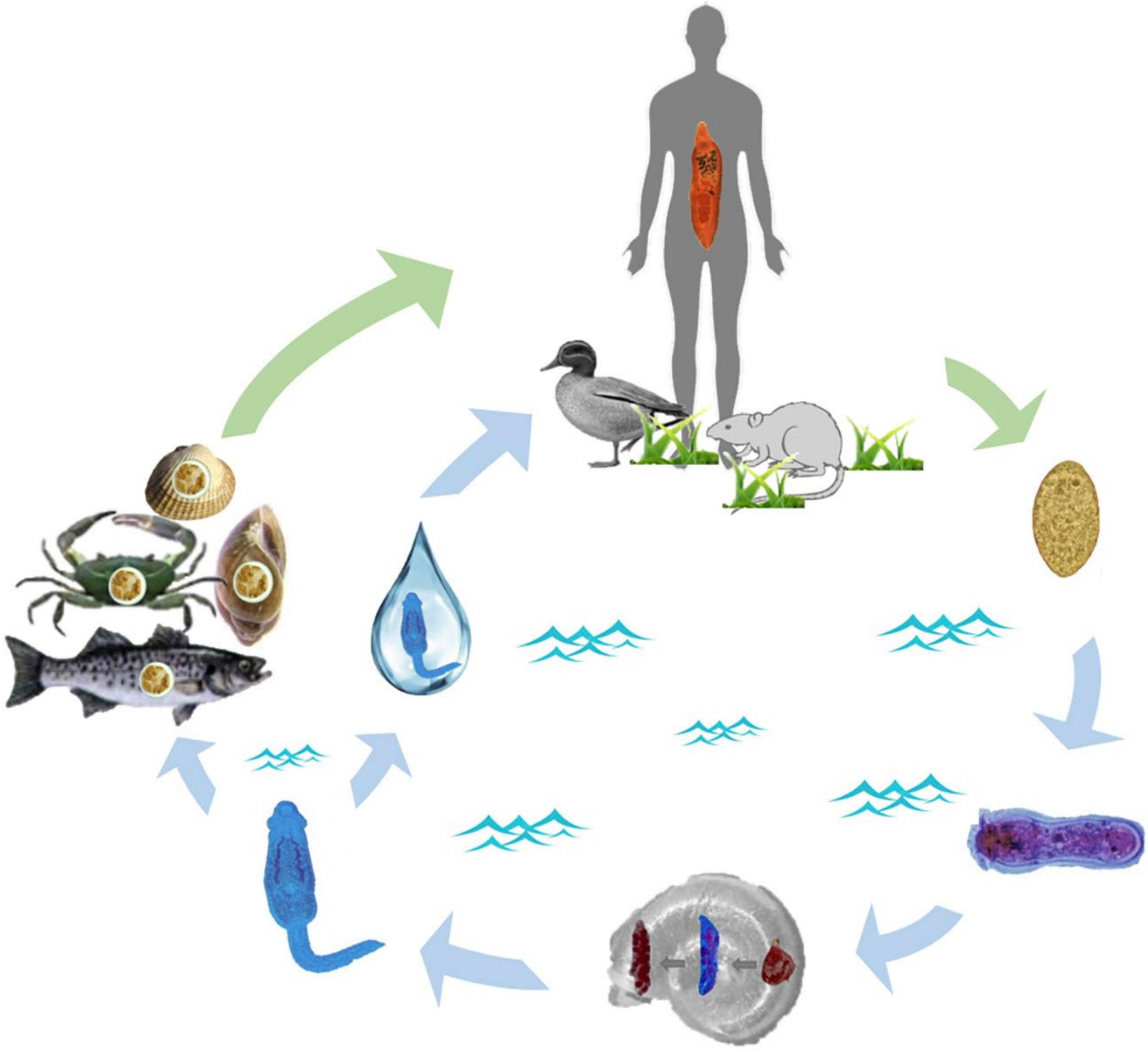

Figure 3. Generalized life cycle of echinostomes: Adult worms inhabiting the small intestine of several vertebrate hosts, including humans; eggs are voided with host feces; miracidia hatch in freshwater and actively infect the snail first intermediate host; intramolluscan stages, that is, sporocysts, 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).

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).

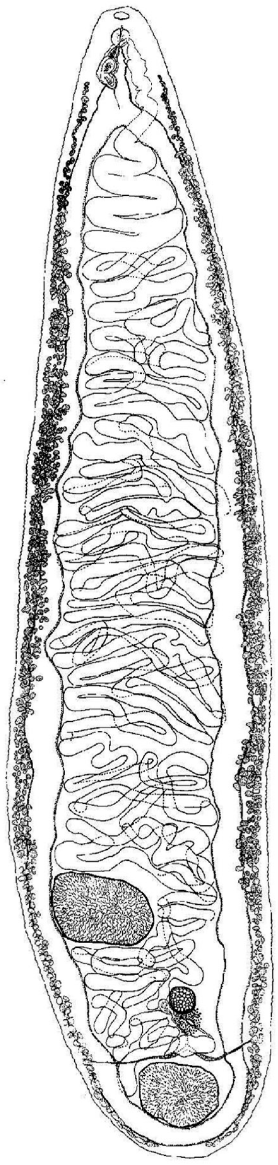

Figure 4. Adult specimen of *Cyclocoelum obscurum* (Cyclocoelidae). Source: Lamothe-Argumedo and Orozco-Flores, 2000. License: CC BY.

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).

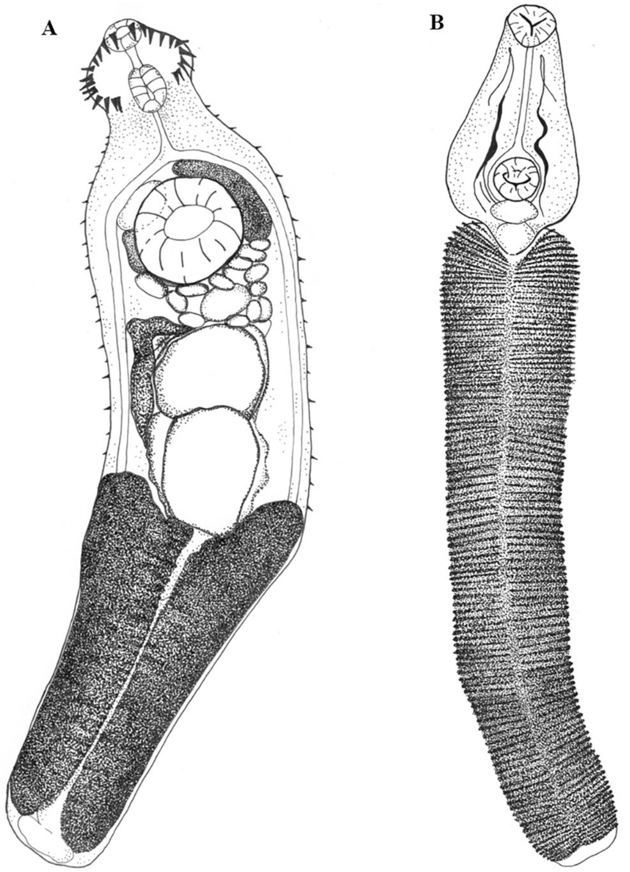

Figure 5. A) Adult specimen of *Stephanoprora* sp. (Echinochasmidae); and (B) cercariae magnacauda of *Stephanoprora* sp. (Echinochasmidae). Source: R. Toledo, B. Fried, and L. Acosta Soto. License: CC BY-NC-SA 4.0.

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

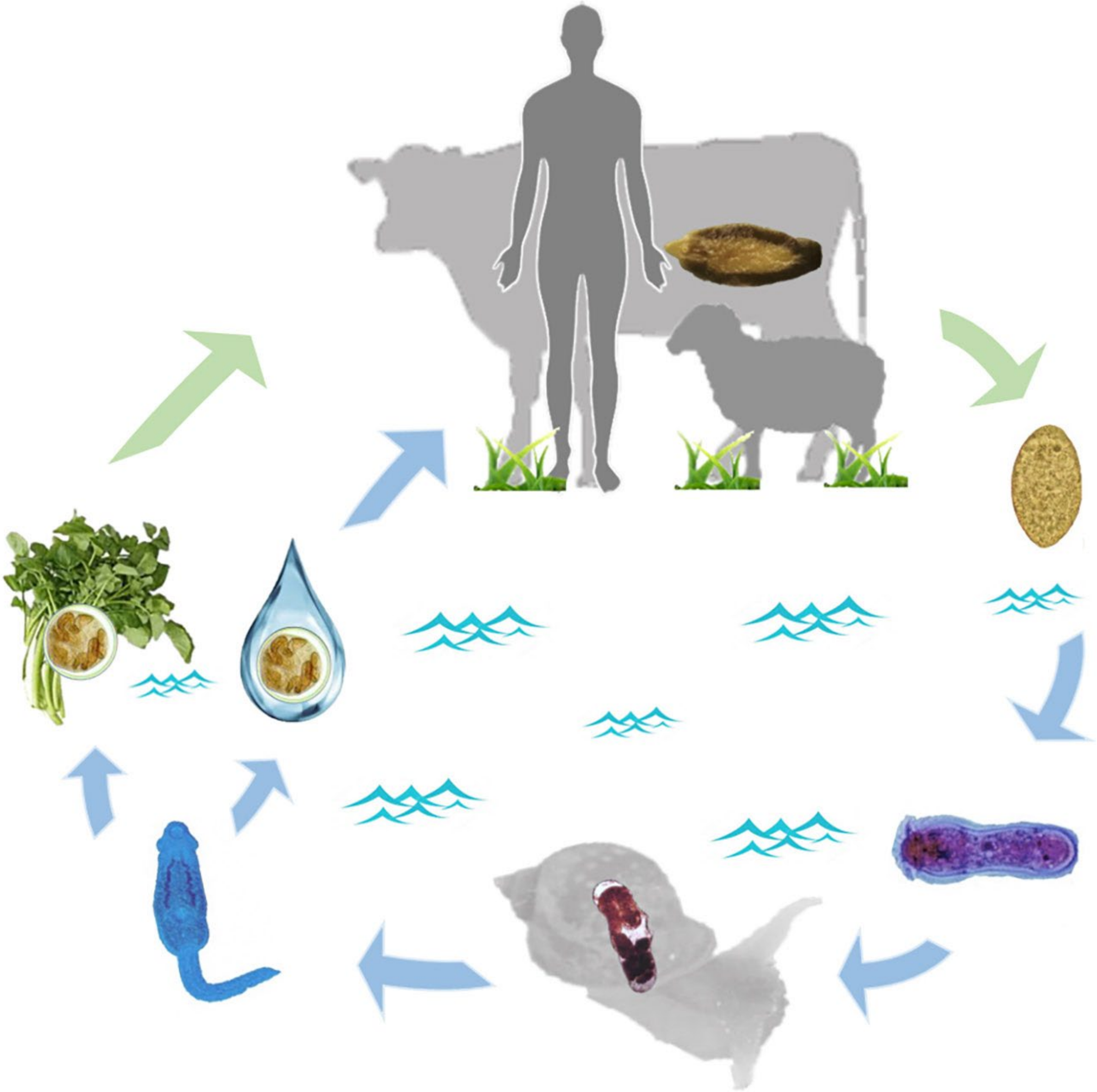

Figure 7. Generalized life cycle of *Fasciola* spp. (Fasciolidae): adult worms inhabiting the biliary ducts of several vertebrate hosts, including humans; eggs are voided with host feces; miracidia hatch in freshwater and actively infect the snail first intermediate host (Lymnaeidae); intramolluscan 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.*

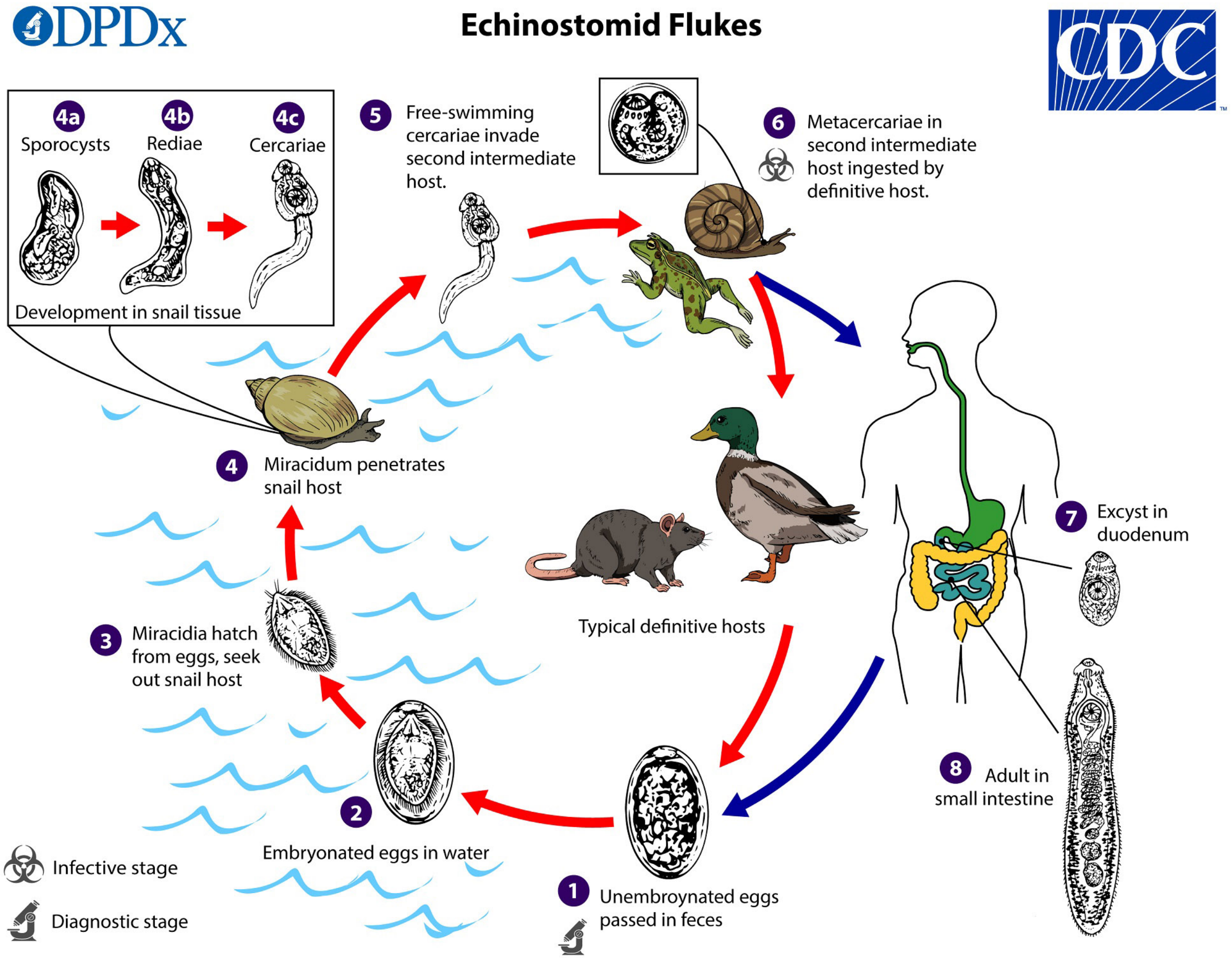

Figure 8. Life cycle of echinostomatid trematodes (flukes). Like many trematodes, echinostomid flukes undergo a multi-host (indirect) life cycle. Unembryonated eggs are passed in feces of infected definitive hosts (1) and develop in water (2). Miracidia usually take about 3 weeks to mature before hatching (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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DIGENEA, PLAGIORCHIIDA

Haplosplanchnata (Suborder): Two Hosts with Half the Guts

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Phylum Platyhelminthes
  Class Trematoda
    Subclass Digenea
      Order Plagiorchiida
        Suborder Haplosplanchnata

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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

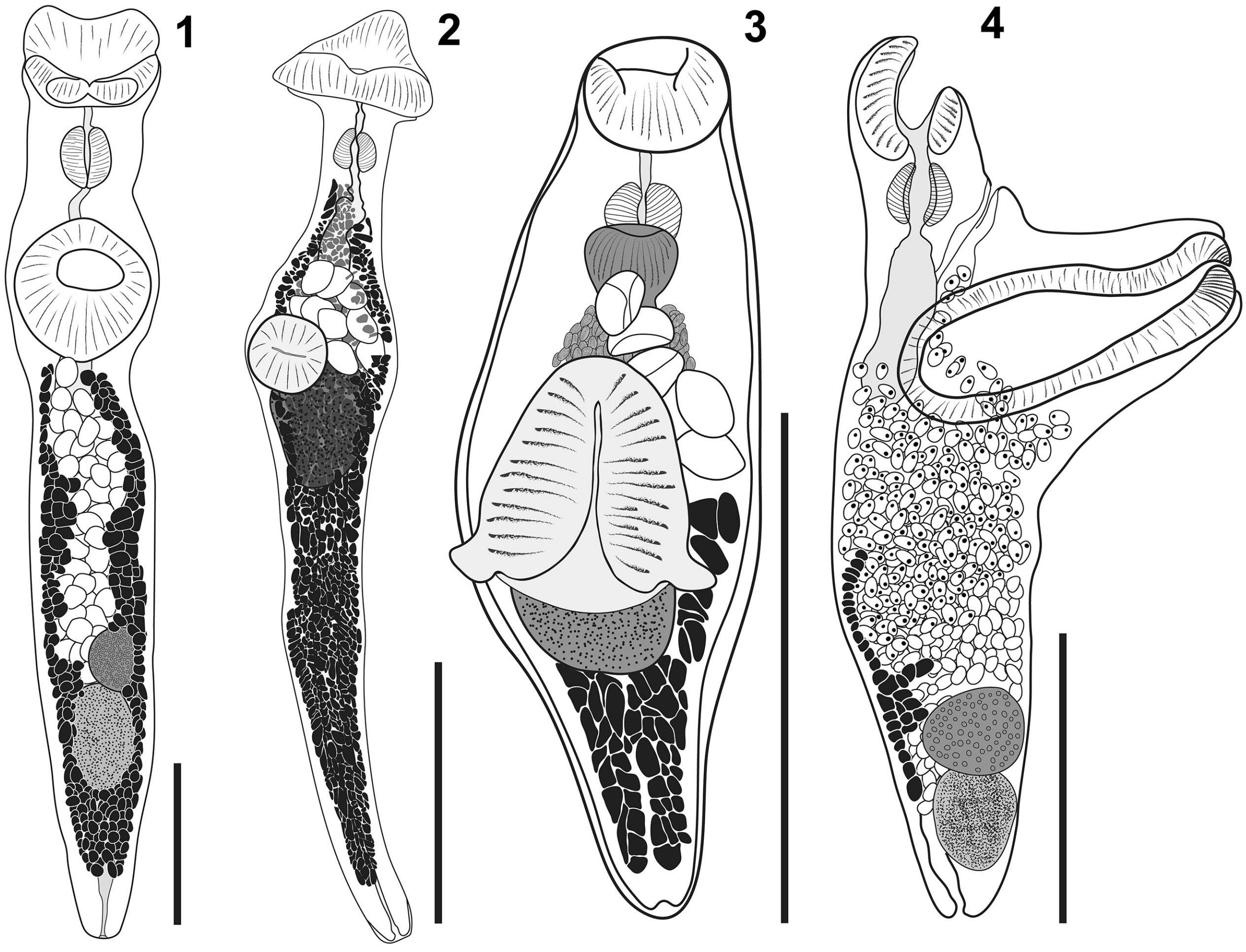

Figure 1. Representative species of the Haplosplanchnata. 1*) Hymenocotta mulli*. 2) *Trigonocephalotrema hipparchi*. 3) *Schikhobalotrema huffmani*. 4) *Haplosplanchnus pachysomus*. The single caecum is obscured in all except for *H. pachysomus*; this is frequently the case because of the densely packed 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

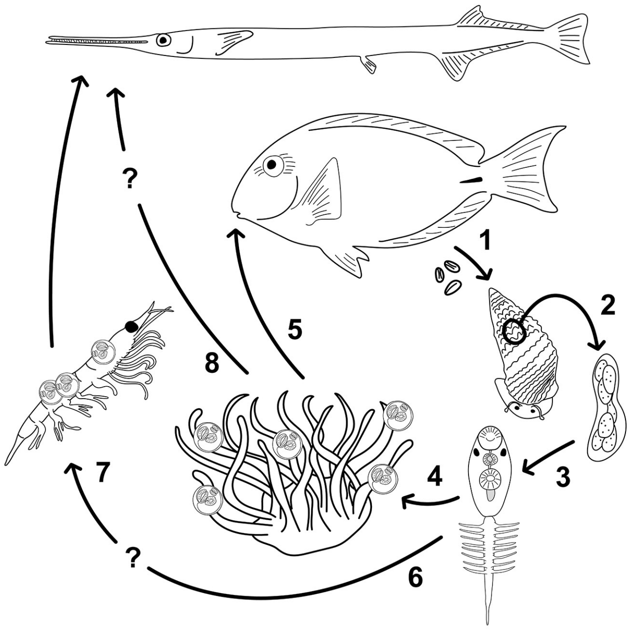

Figure 2. The haplosplanchnid life cycle. 1) Adult trematodes lay eggs, which pass into the environment with the host's waste. 2) Miracidium hatches from egg, infects snail intermediate host. Miracidium transforms to mother sporocyst; mother sporocyst produces daughter sporocysts. 3). Sporocysts produce 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)

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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

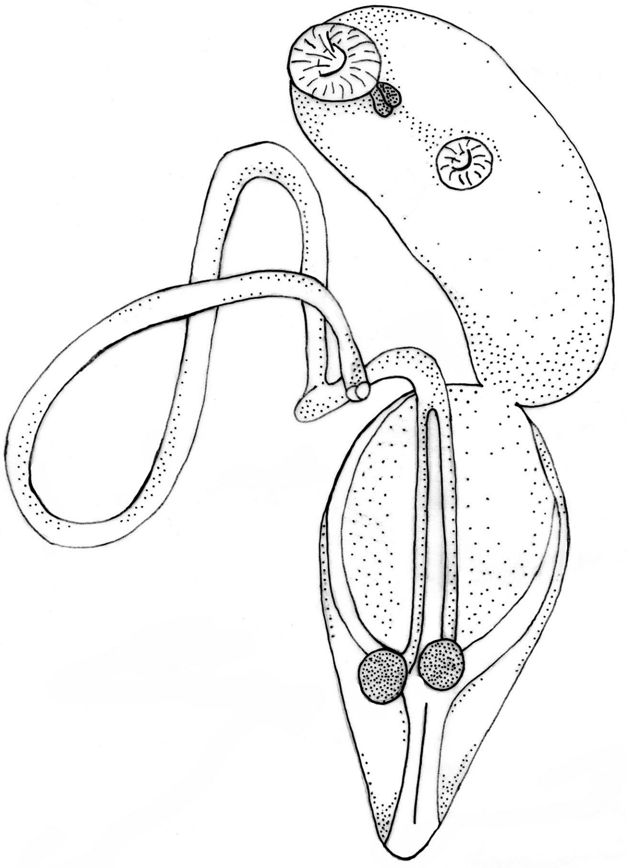

Figure 1. General scheme of a cystophorous cercariae. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

(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).

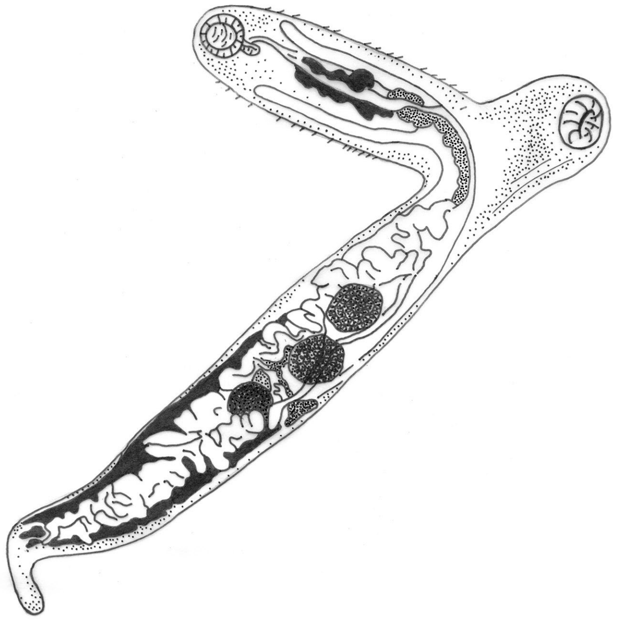

Figure 2. General scheme of an adult specimen of *Accacoelium* sp. (Accacoeliidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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 (Glomericirrinae, 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).

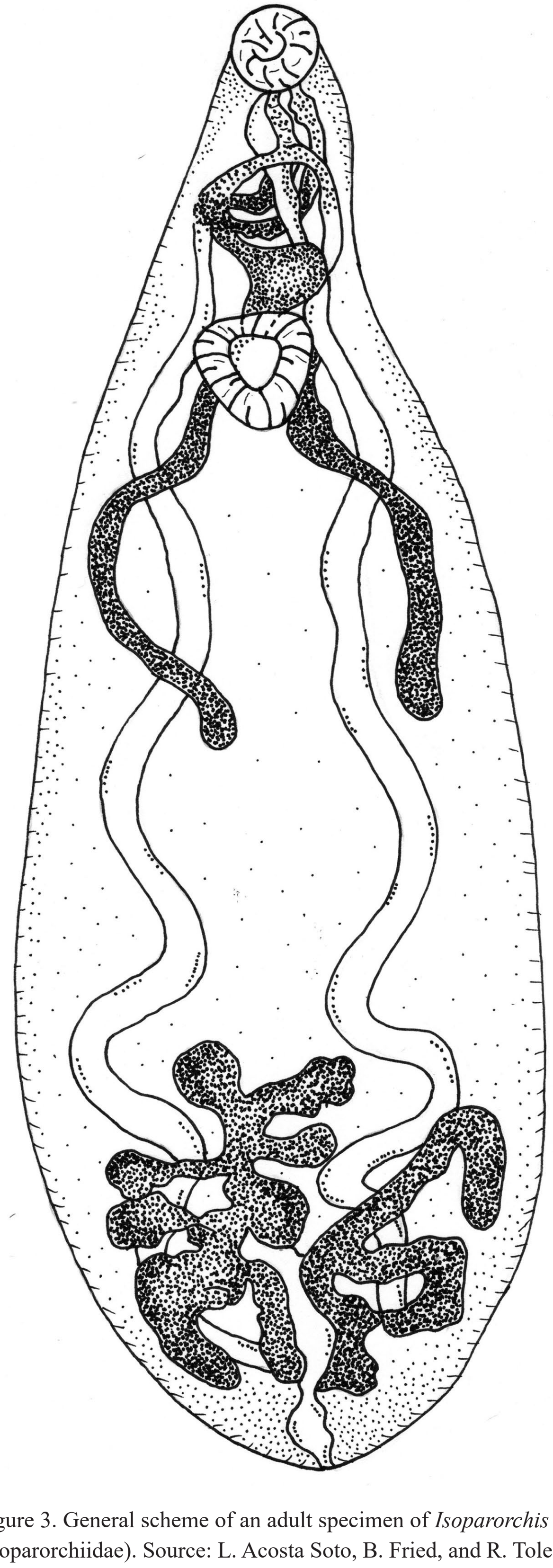

Figure 3. General scheme of an adult specimen of *Isoparorchis* sp. (Isoparorchiidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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).

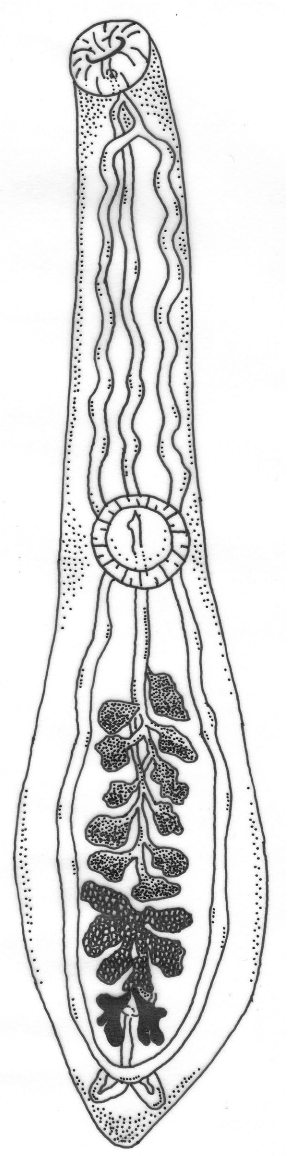

Figure 4. General scheme of an adult specimen of *Syncoelium* sp. (Syncoeliidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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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DIGENEA, PLAGIORCHIIDA

Monorchiata (Suborder): Two Families Separated by Salinity

Nicholas Q.-X. Wee

Phylum Platyhelminthes
  Class Trematoda
    Subclass Digenea
      Order Plagiorchiida
        Suborder Monorchiata

doi:10.32873/unl.dc.ciap041
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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.

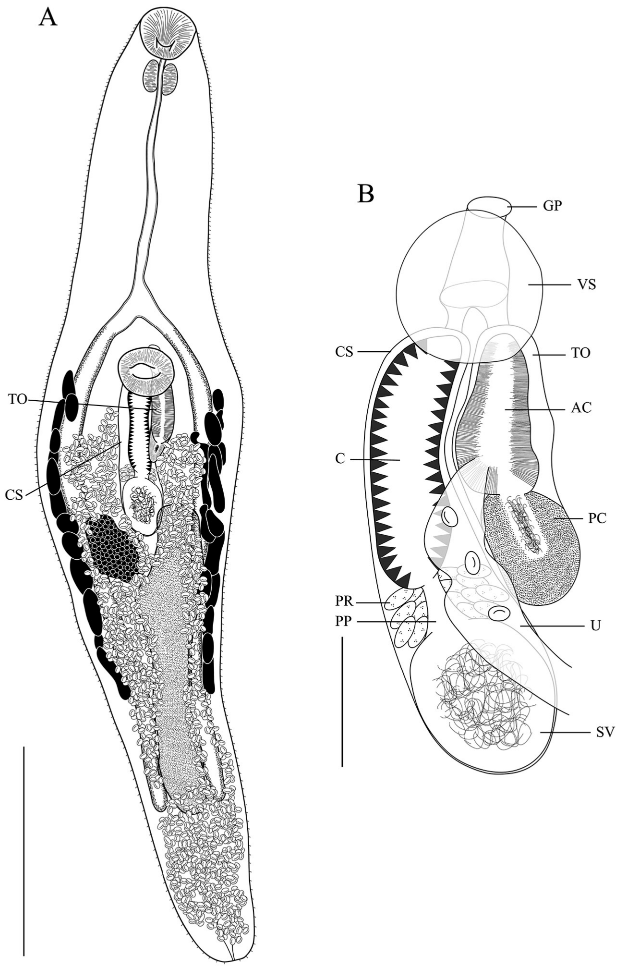

Figure 1. A typical monorchiid, *Parachrisomon delicatus* (Manter & Pritchard, 1964) Madhavi, 2008. A) Whole worm, ventral view; B) Terminal genitalia, ventral view. Abbreviations: AC: Anterior chamber; C: Cirrus; CS: Cirrus sac; GP: Genital pore; PC: Posterior chamber; PP: Pars prostatica; PR: Prostatic 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).

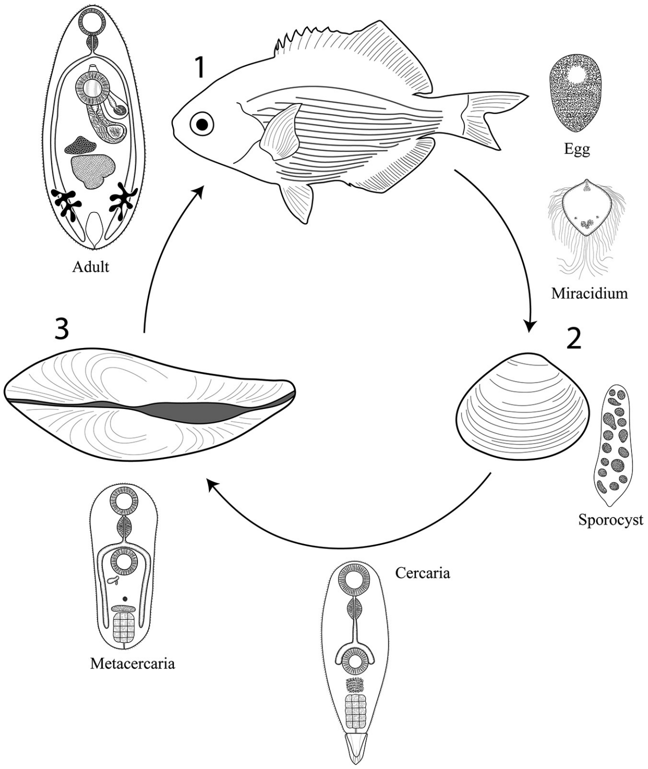

Figure 2. Life cycle of *Telolecithus pugetensis* Lloyd & Guberlet, 1932 (Monorchiidae). Source: modified from DeMartini and Pratt, 1964. License: CC BY-NC-SA 4.0.

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

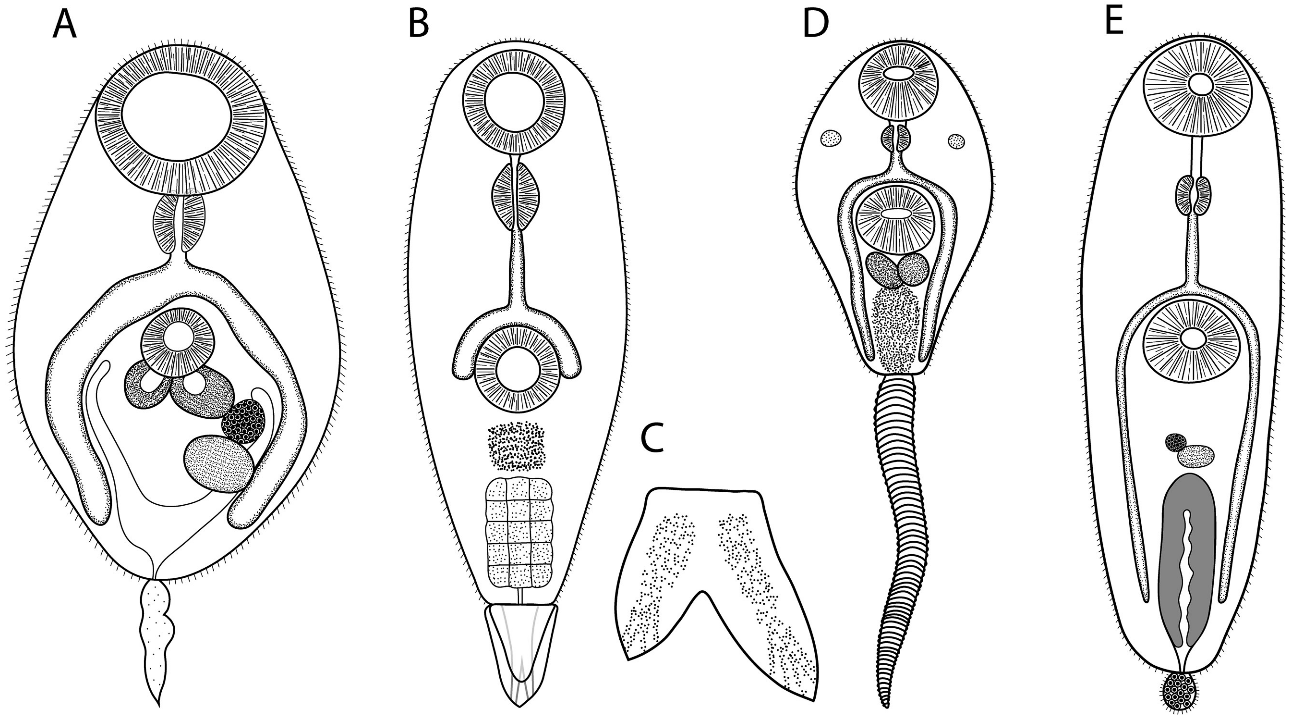

Figure 3. Monorchiid cercariae showing different body forms. A) *Monorchis parvus* Looss, 1902; B) *Telolecithus pugetensis* Lloyd & Guberlet, 1932; C) Bifurcated tail of *T. pugetensis* cercariae; D) *Paratimonia gobii* Prévot & Bartoli, 1967; E) *Proctotrema bartolii* Carballo, Laurenti & Cremonte, 2011. Source: Adapted from Bartoli et al., 2000; DeMartini and Pratt, 1964; Maillard, 1975; Gilardoni et al., 2013. License: CC BY-NC-SA 4.0.

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.

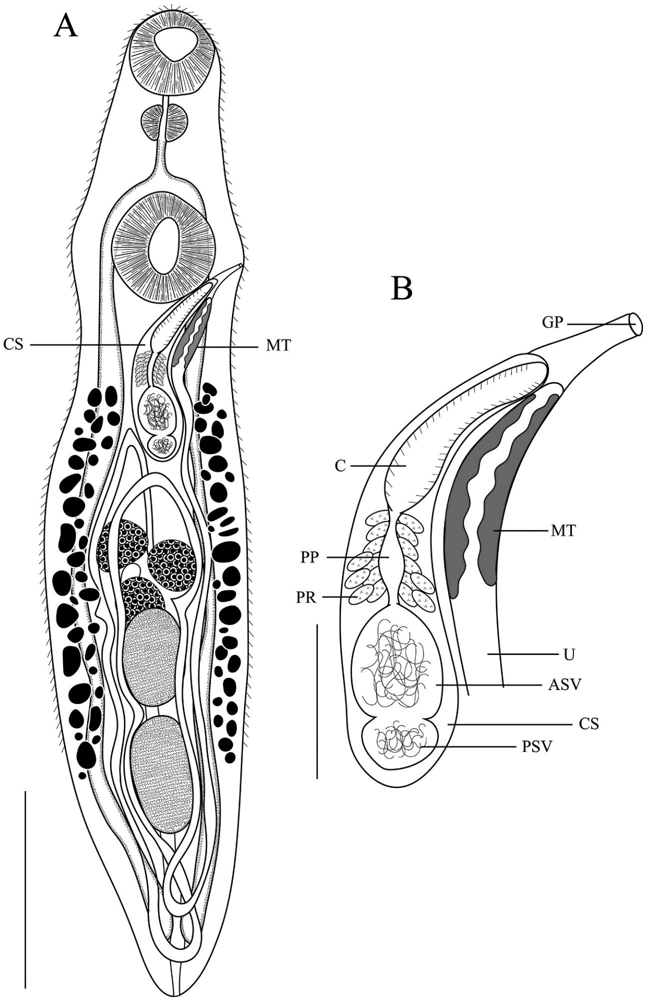

Figure 4. A typical lissorchiid, *Lissorchis hypentelii* (Fischthal, 1942). A) Whole worm, ventral view; B) Terminal genitalia, ventral view. Abbreviations: ASV: Anterior seminal vesicle; C: Cirrus; CS: Cirrus sac; GP: Genital pore; MT: Metraterm; PP: Pars prostatica; PR: Prostatic cells; PSV: Posterior seminal vesicle; U: Uterus. Scale bars: A) 400 µm; B) 200 µm. Source: Adapted from Fischthal, 1942. License: CC BY-NC-SA 4.0.

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

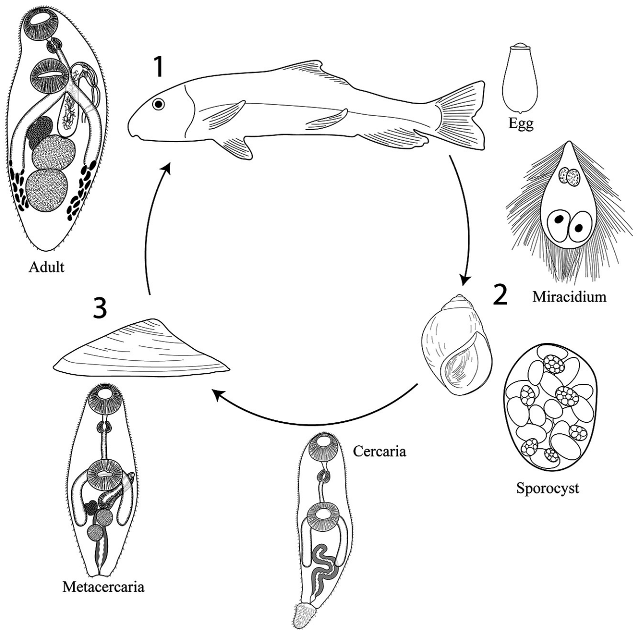

Figure 5. Life cycle of *Neopaleorchis catostomi* Schell, 1973 (Lissorchiidae). Source: Adapted from Schell, 1973. License: CC BY-NC-SA 4.0.

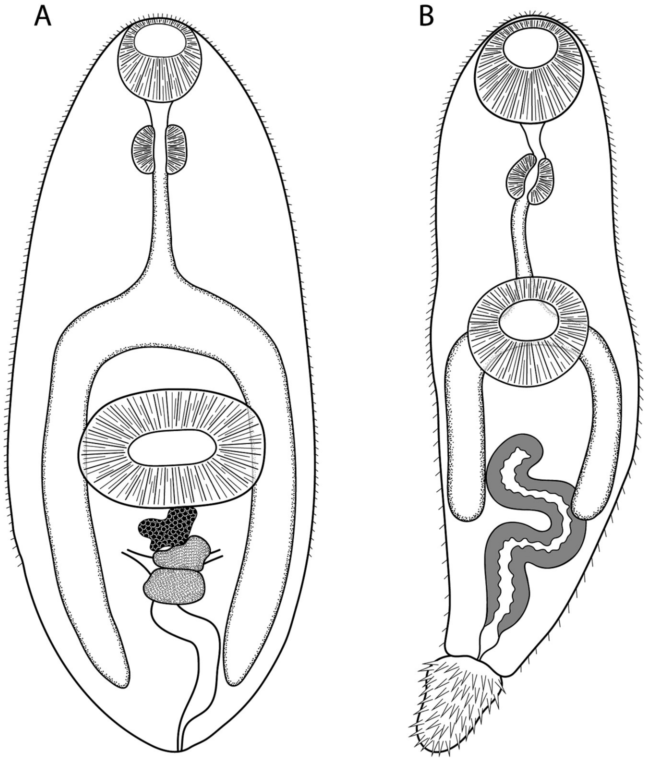

Figure 6. Lissorchiid cercariae showing different body shapes. A) *Lissorchis mutabile* (Cort, 1918); B) *Neopaleorchis catostomi* Schell, 1973. Source: Adapted from Wallace, 1941; Schell, 1973. License: CC BY-NC-SA 4.0.

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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42

DIGENEA, PLAGIORCHIIDA

Opisthorchis (Genus)

Sue Ann Gardner, compiler

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)

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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

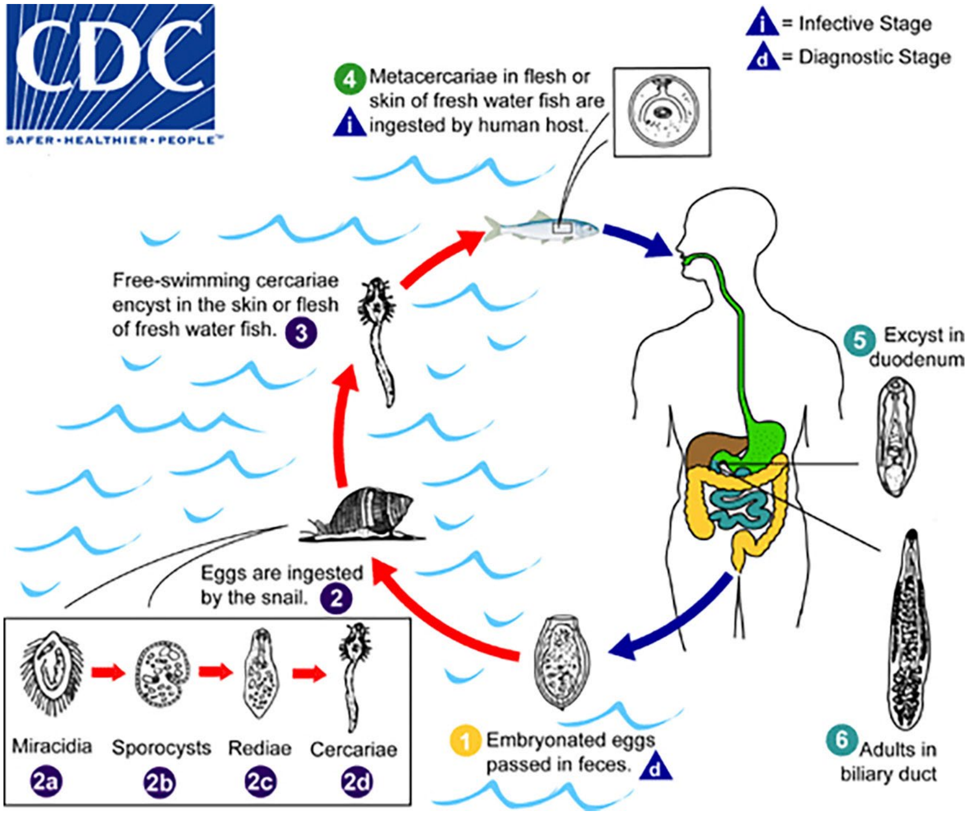

Figure 1. The adult flukes deposit fully developed eggs that are passed in the feces (1). After ingestion by a suitable snail (first intermediate host) (2), the eggs release miracidia (2a), which undergo in the snail several developmental stages (sporocysts (2b), rediae (2c), cercariae (2d)). Cercariae 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.

Use of material from United States federal agencies does not constitute its endorsement or recommendation by the US Government, Department of Health and Human Services, or Centers for Disease Control and Prevention (CDC). The material from the CDC is otherwise available on the agency website for no charge.

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43

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

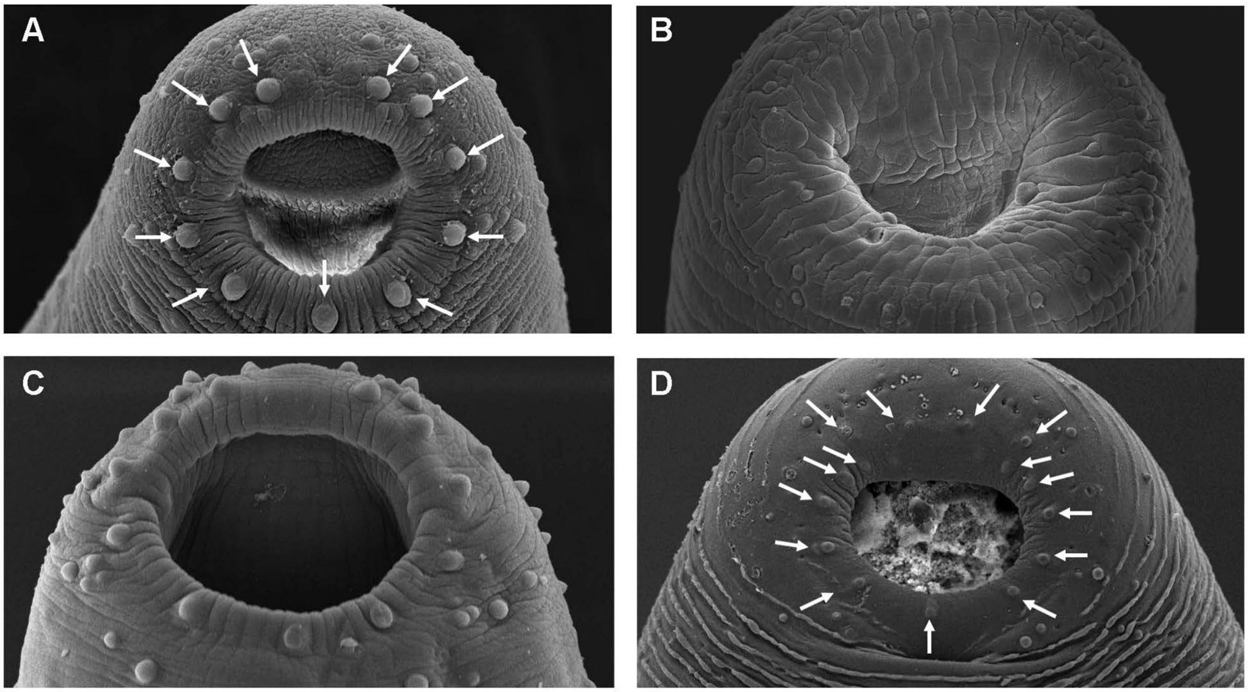


Figure 1. Scanning electron microscopy microphotographs of 4 species of allocreadiids lacking muscular oral lobes on the oral sucker. A) *Margotrema resolanae* from *Xenotaenia resolanae*. Note the arrows pointing to 15 dome-like papillae; B) *Wallinia mexicana* from *Astyanax mexicanus*; C) *Pseudoparacreptotrema 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.

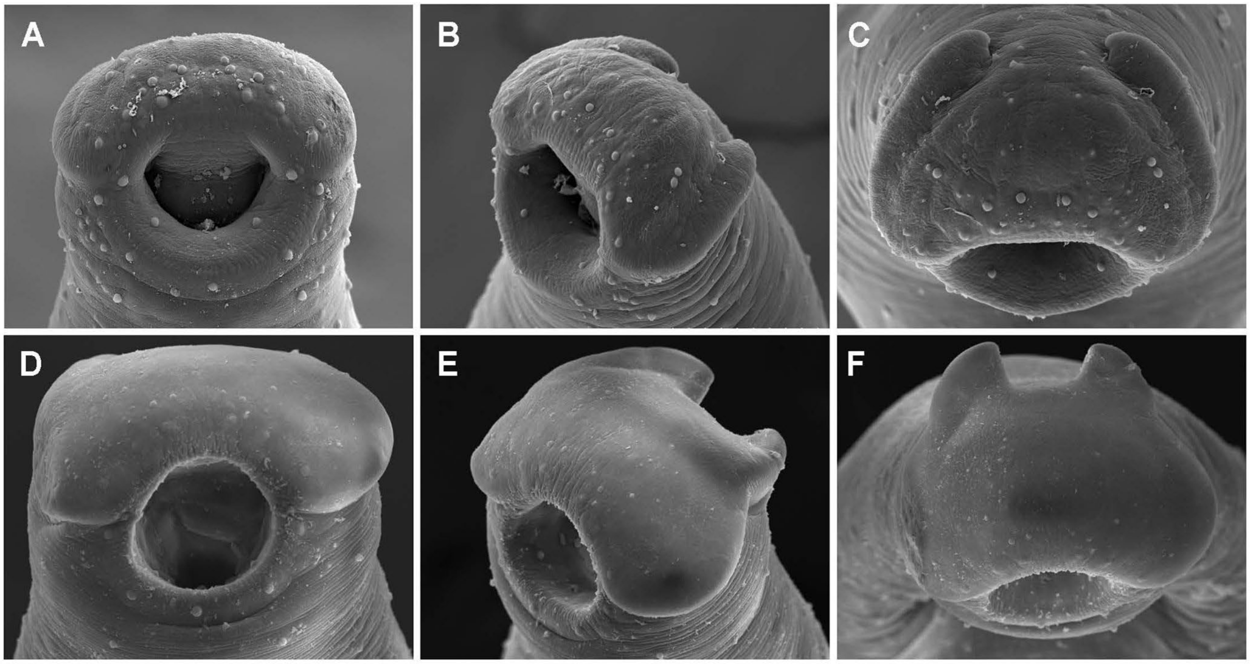


Figure 2. Scanning electron microscopy microphotographs of 2 species of allocreadiids possessing muscular oral lobes on the oral sucker (frontal, lateral, and anterior views); A–C) *Auriculostoma totonacapenensis* from *Astyanax mexicanus*; D–F) *A. lobata* from *Brycon guatemalensis*. Sources: A–C) 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.

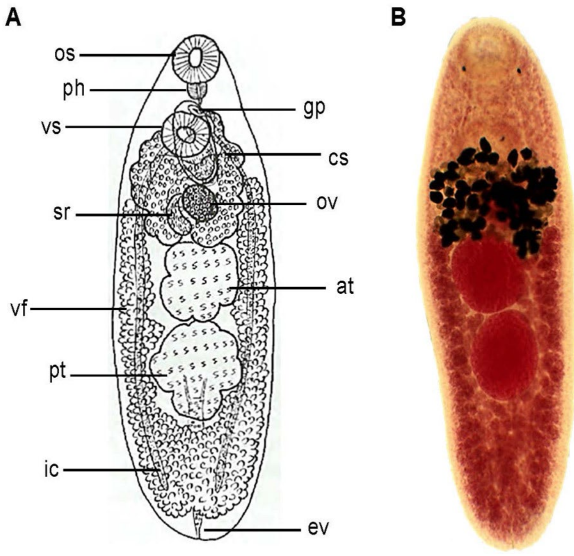


Figure 3. *Allocreadium lobatum*, ventral view. A). Line drawing; B) Microphotograph of a stained specimen from *Semotilus atromaculatus*. Note the pretesticular position of the uterus (eggs hydrated), and the eye spots on the posterior border of the oral sucker. Sources: A) Adapted from Hoffman, 1999; B) A. Choudhury. License: CC BY-NC-SA 4.0.

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.

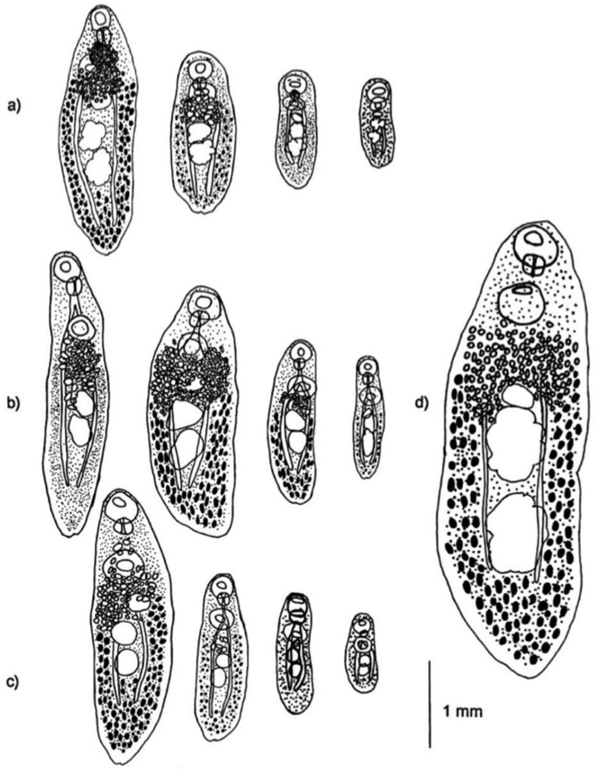


Figure 4. Morphological types in descending size classes of *Allocreadium lobatum*: a) lobate; b) asymmetrical; c) round; and d) syntype. Sources: Willis, 2002: a) Left to right, HWML slides 35119 (37-5), 35119 (37-4), 35117 (24-3), 35120 (53-8); b) Left to right, slides 35126 (79-2), 35114 (1-10), 35127 (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.

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

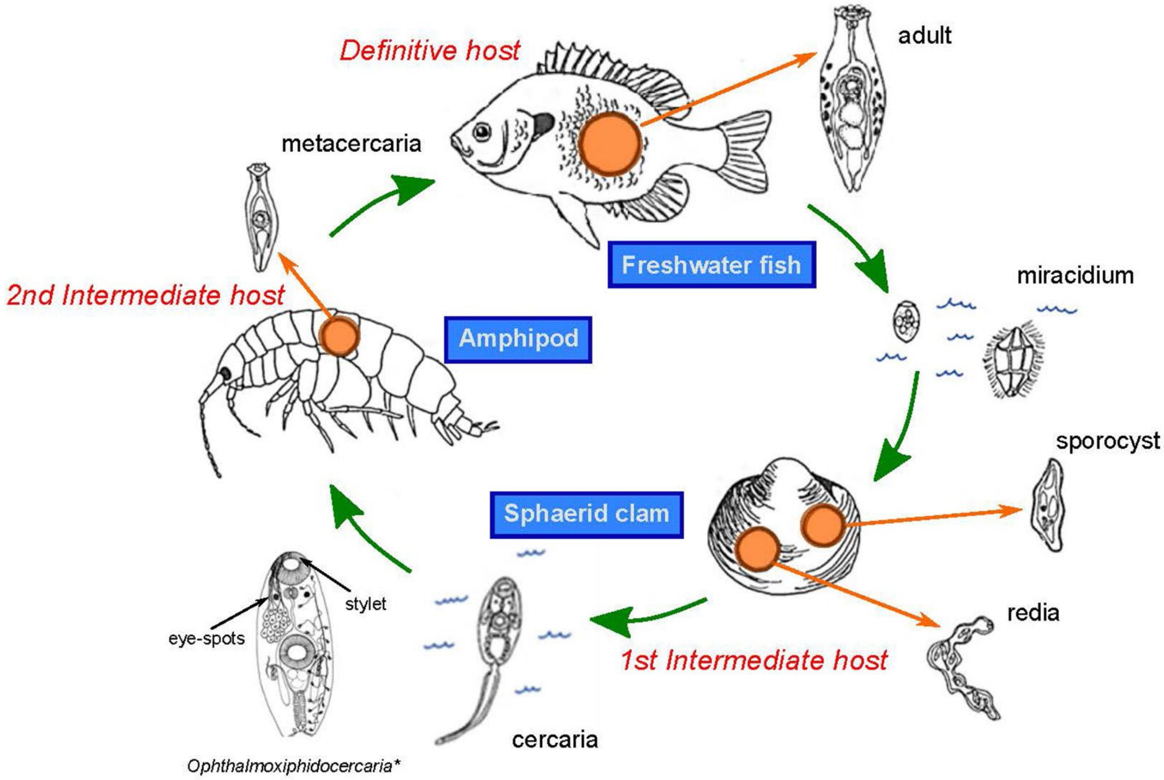


Figure 5. Generalized 3-host life cycle of an allocreadiid trematode. Sources: Adapted from Caira, 1989; Niewiadomska and Valtonen, 2007. License: CC BY-NC-SA 4.0.

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

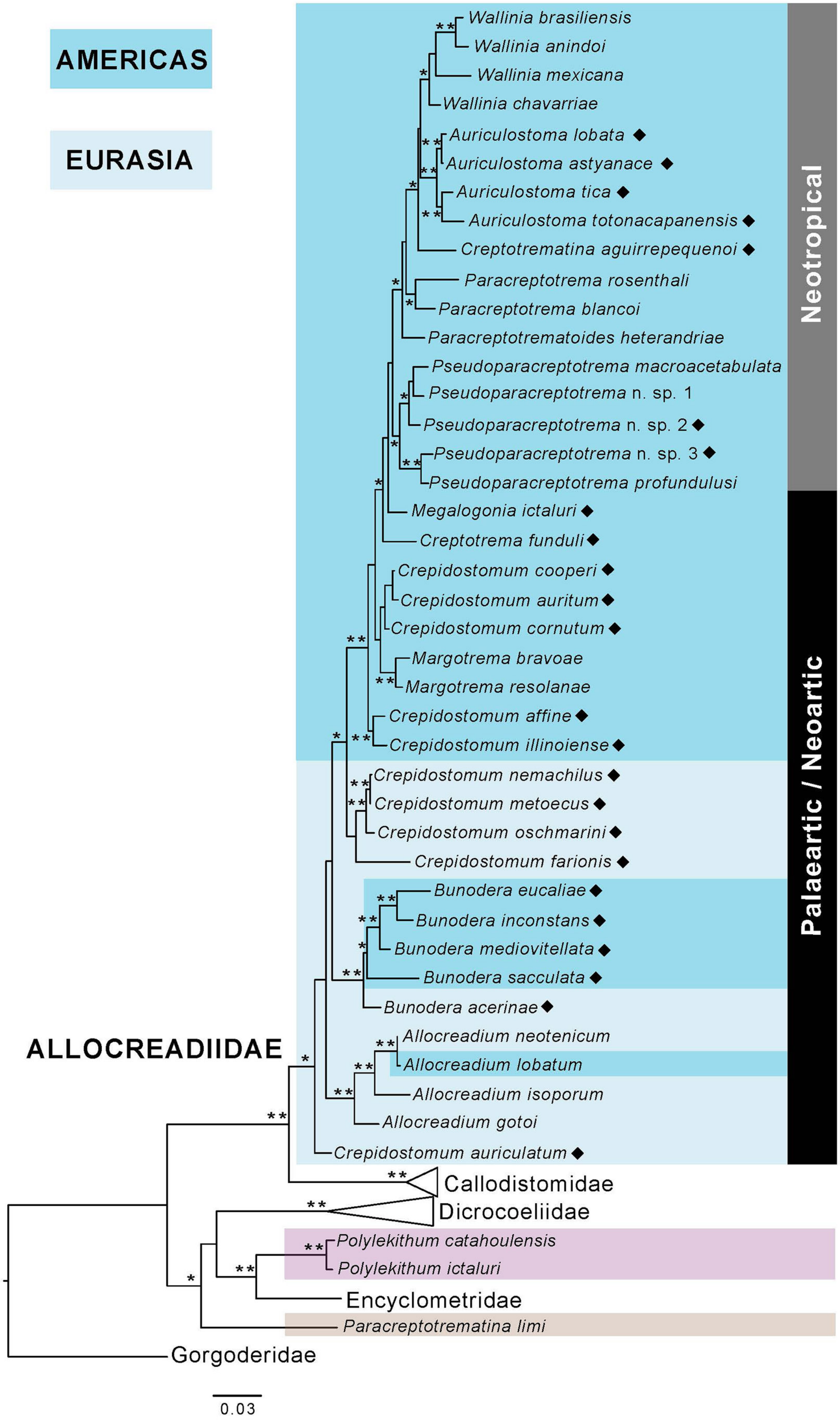
A taxonomic assessment of the family Allocreadiidae was conducted by Caira and Bogéa (2005). A cladistics analysis using morphological characters was conducted earlier by Caira (1989) and Caira and Bogéa (2005). Morphology was not very useful to assess the interrelationships among members of the family, 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

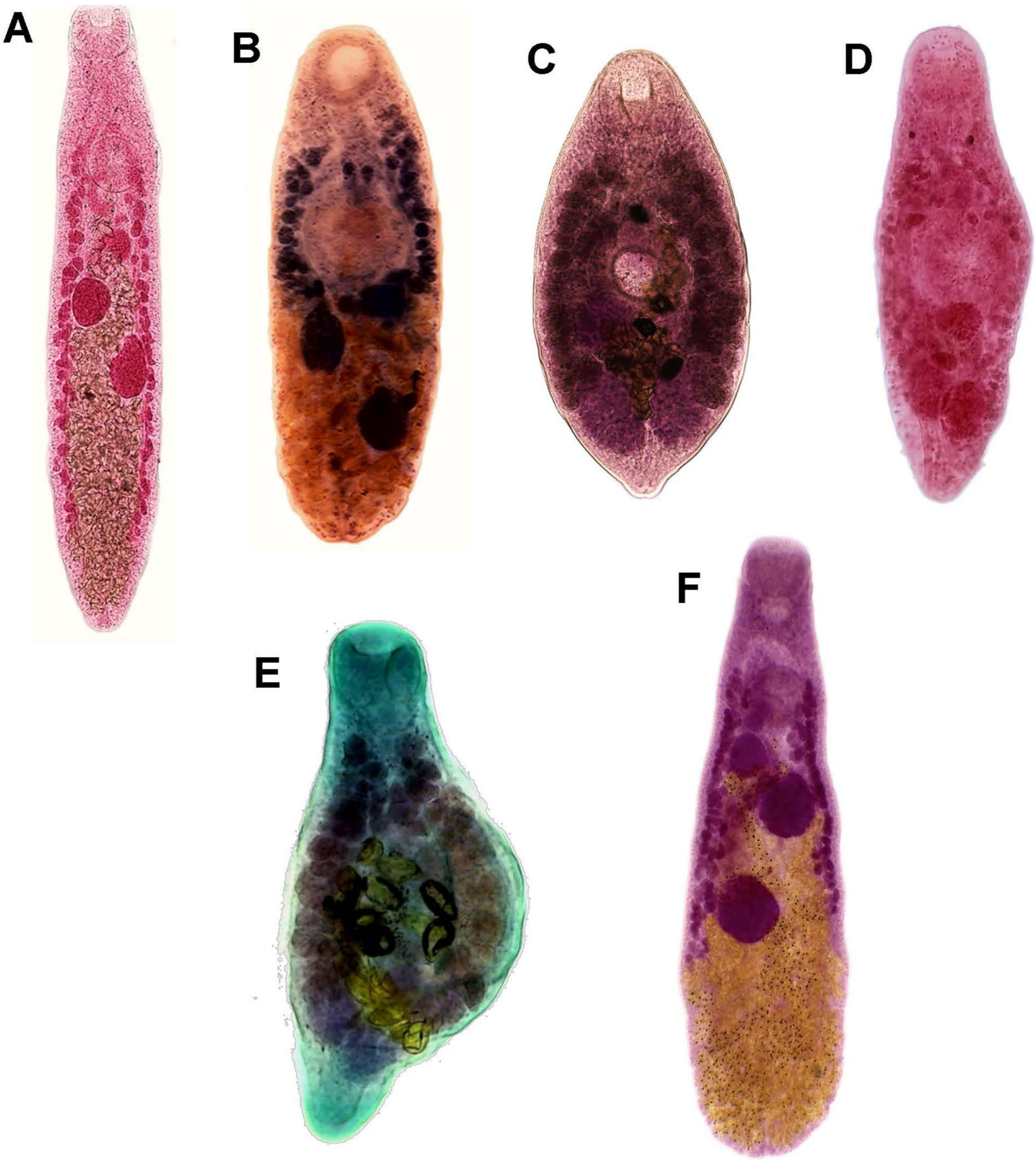


Figure 7. Microphotographs of stained specimens of allocreadiids representing some of the genera in the family. A) *Creptotrematina aguirrepequenoi,* B) *Margotrema resolanae,* C) *Paracreptotrema blancoi,* D) *Paracreptotrematoides heterandriae,* E) *Pseudoparacreptotrema magacetabulata,* F) *Wallinia 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 three-spine 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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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

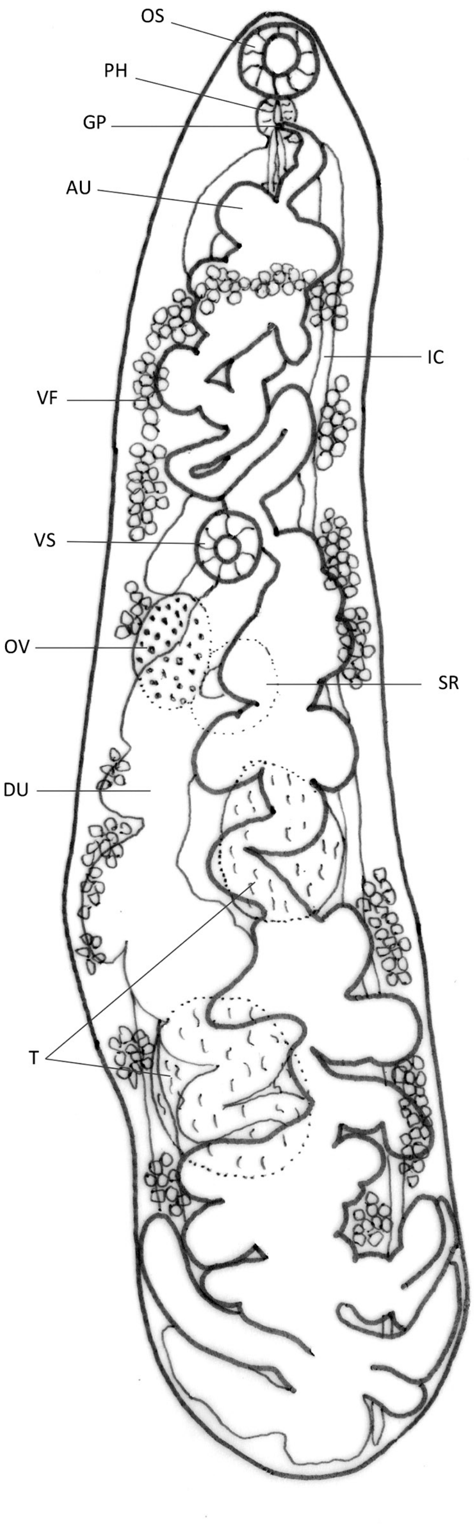

Figure 1. *Haematoloechus caballeroi*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

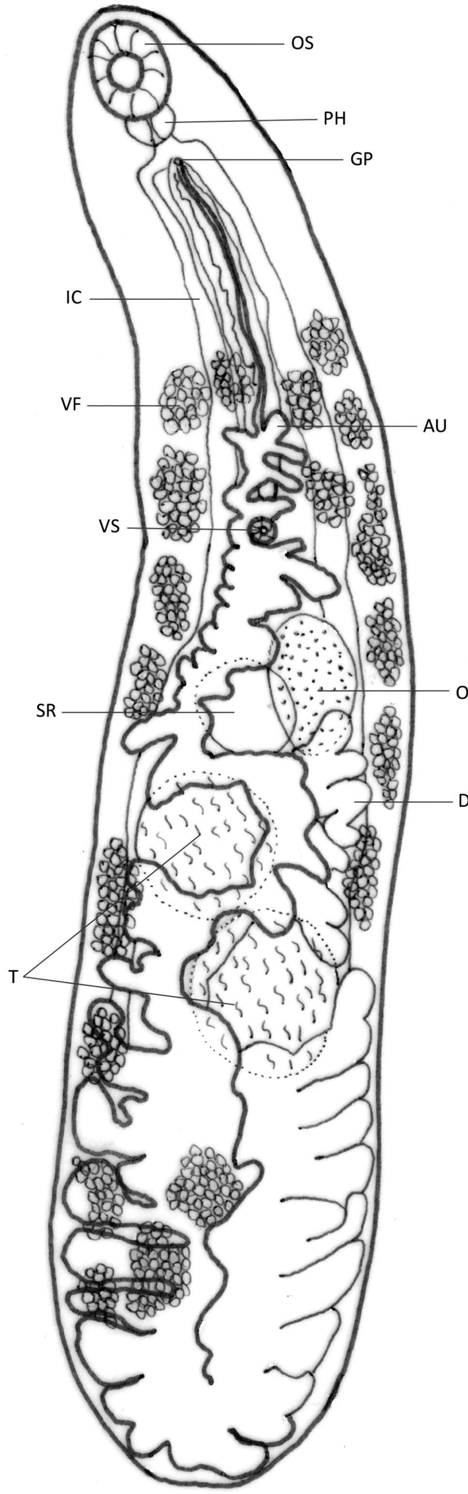

Figure 2. *Haematoloechus meridionalis*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

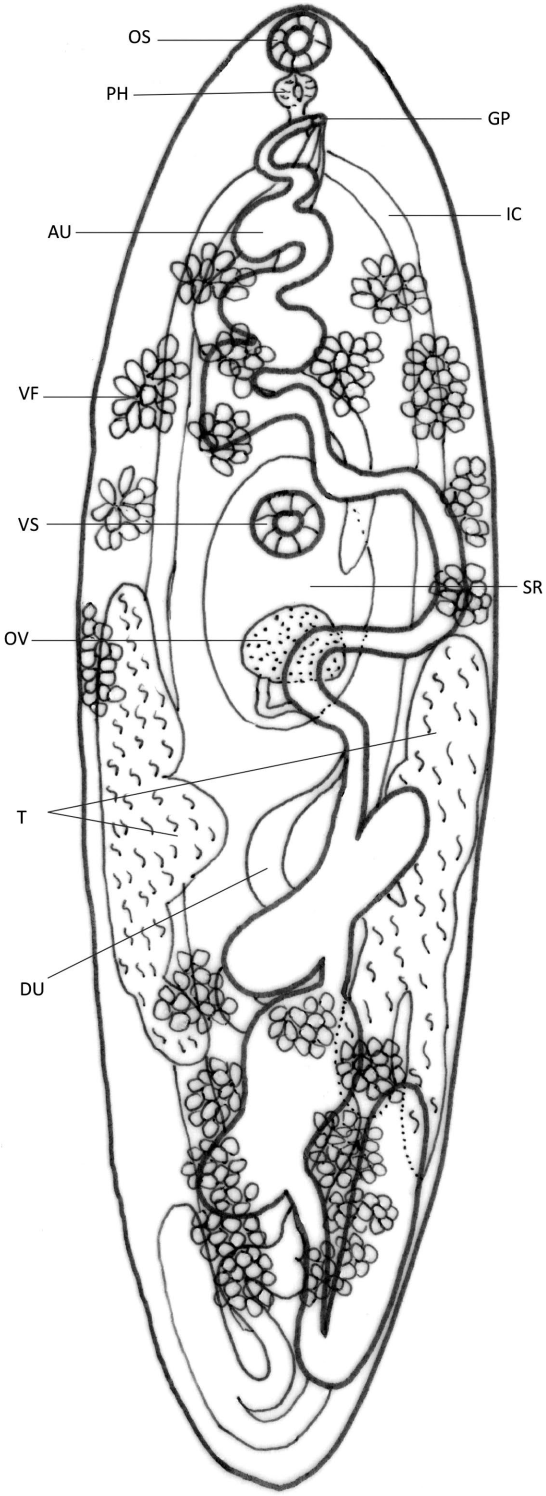

Figure 3. *Haematoloechus exoterorchis*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

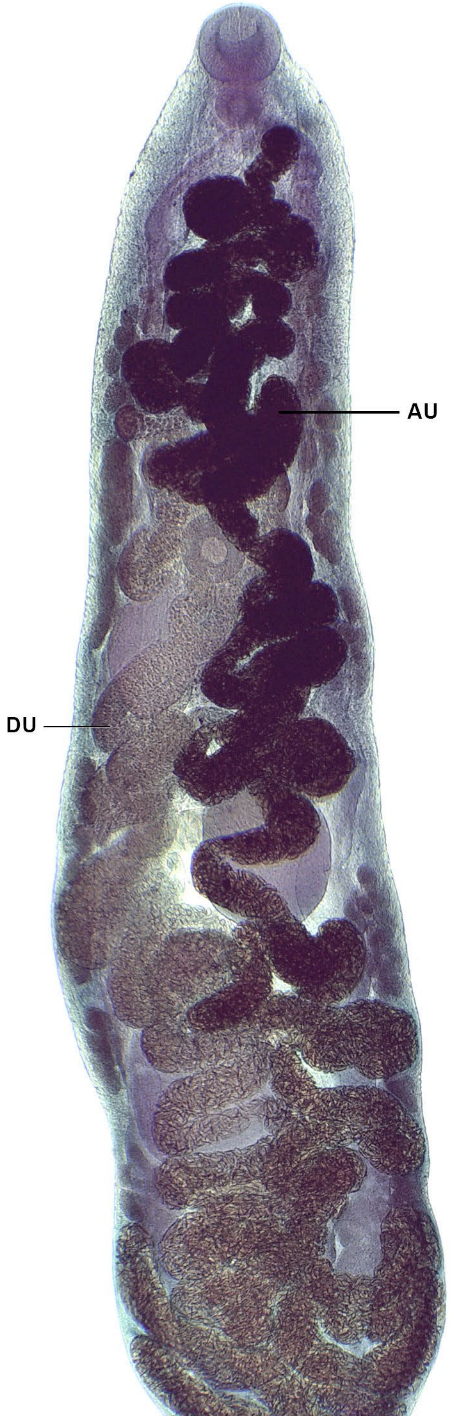

Figure 4. *Haematoloechus caballeroi*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

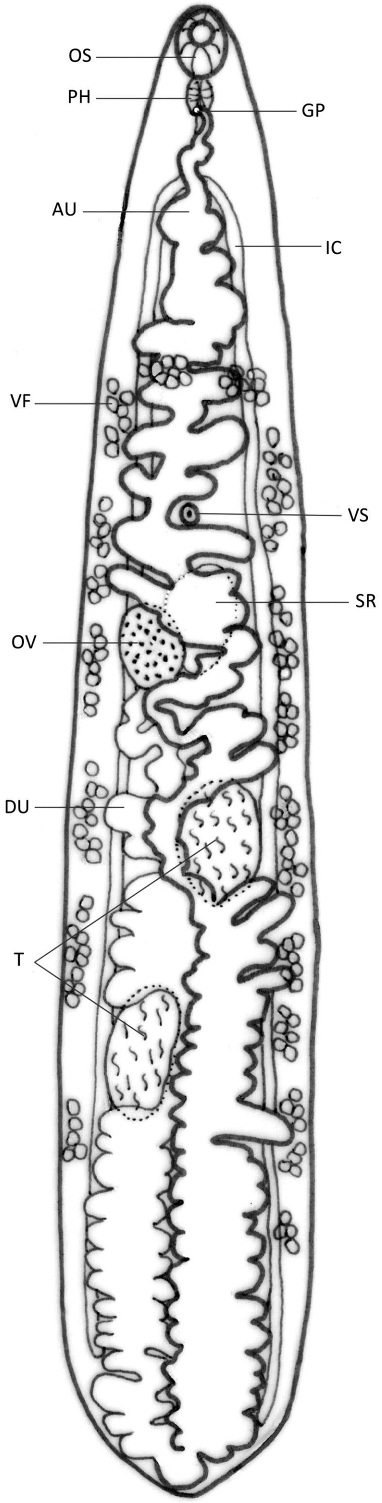

Figure 5. *Haematoloechus medioplexus*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

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.

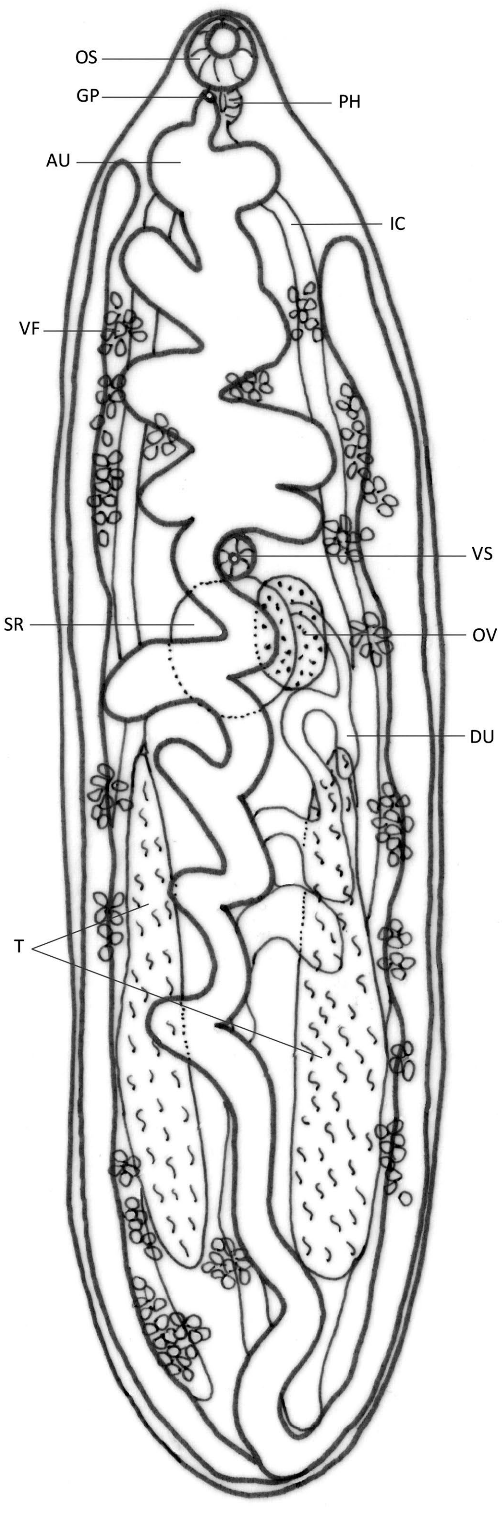

Figure 6. *Haematoloechus longiplexus*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

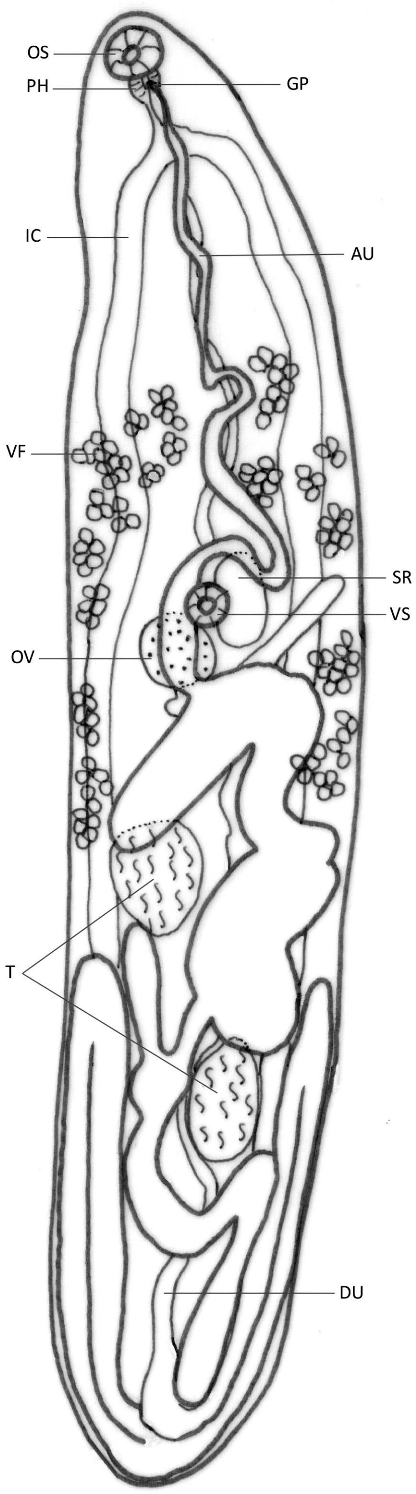

Figure 7. *Haematoloechus similis*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

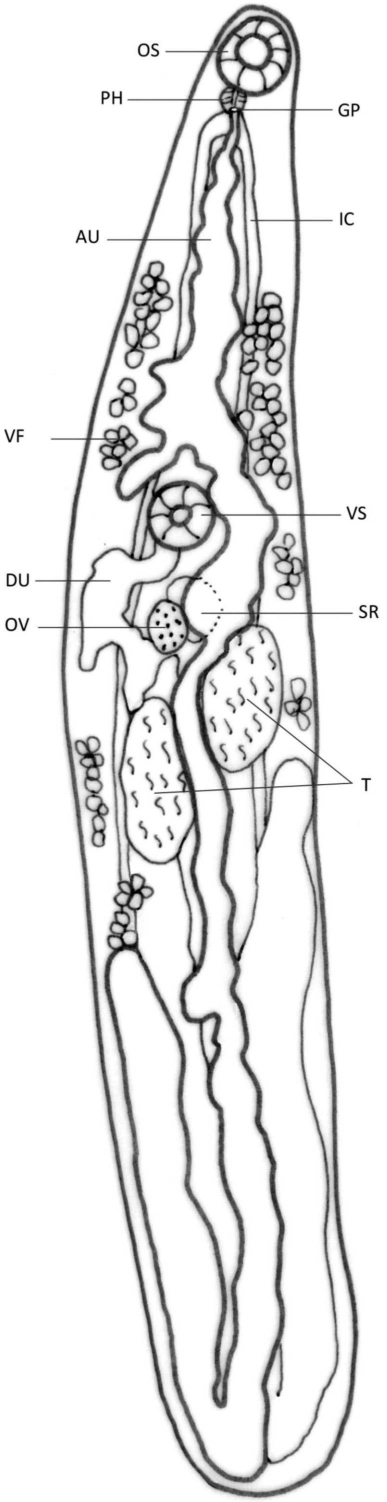

Figure 8. *Haematoloechus asper*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles; VS: Ventral sucker. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

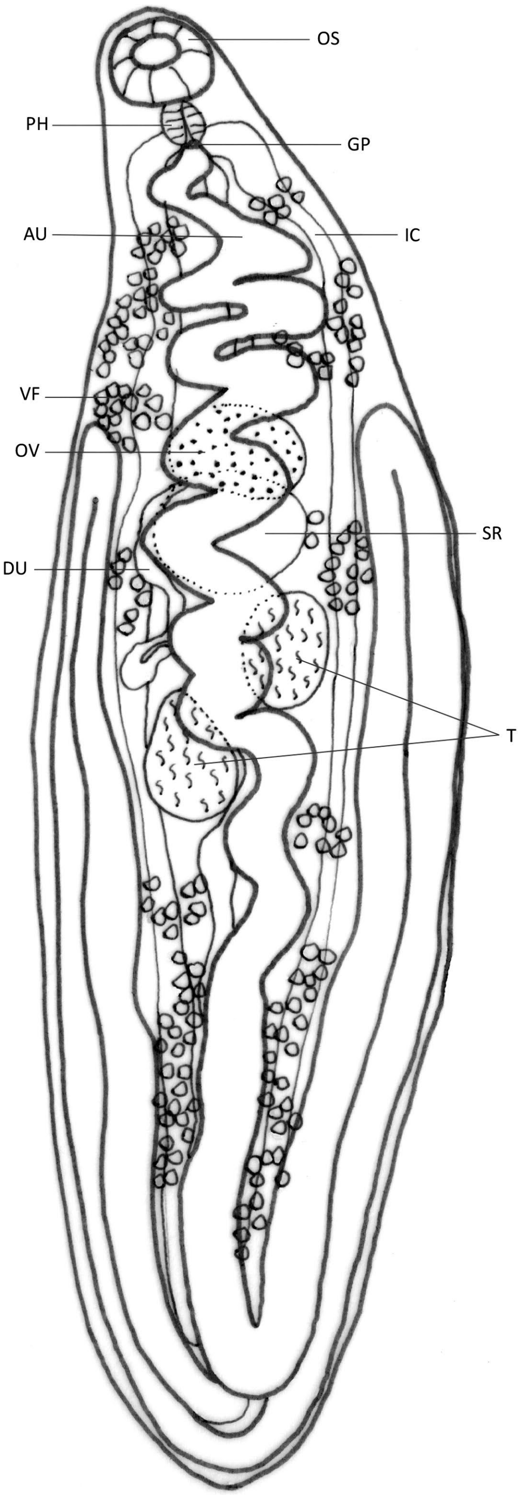

Figure 9. *Haematoloechus neivai*. AU: Ascending uterus; DU: Descending uterus; GP: Genital pore; IC: Intestinal ceca; OS: Oral sucker; OV: Ovary; PH: Pharynx; SR: Seminal receptacle; T: Testes; VF: Vitelline follicles. Source: V. León-Règagnon. License: CC BY-NC-SA 4.0.

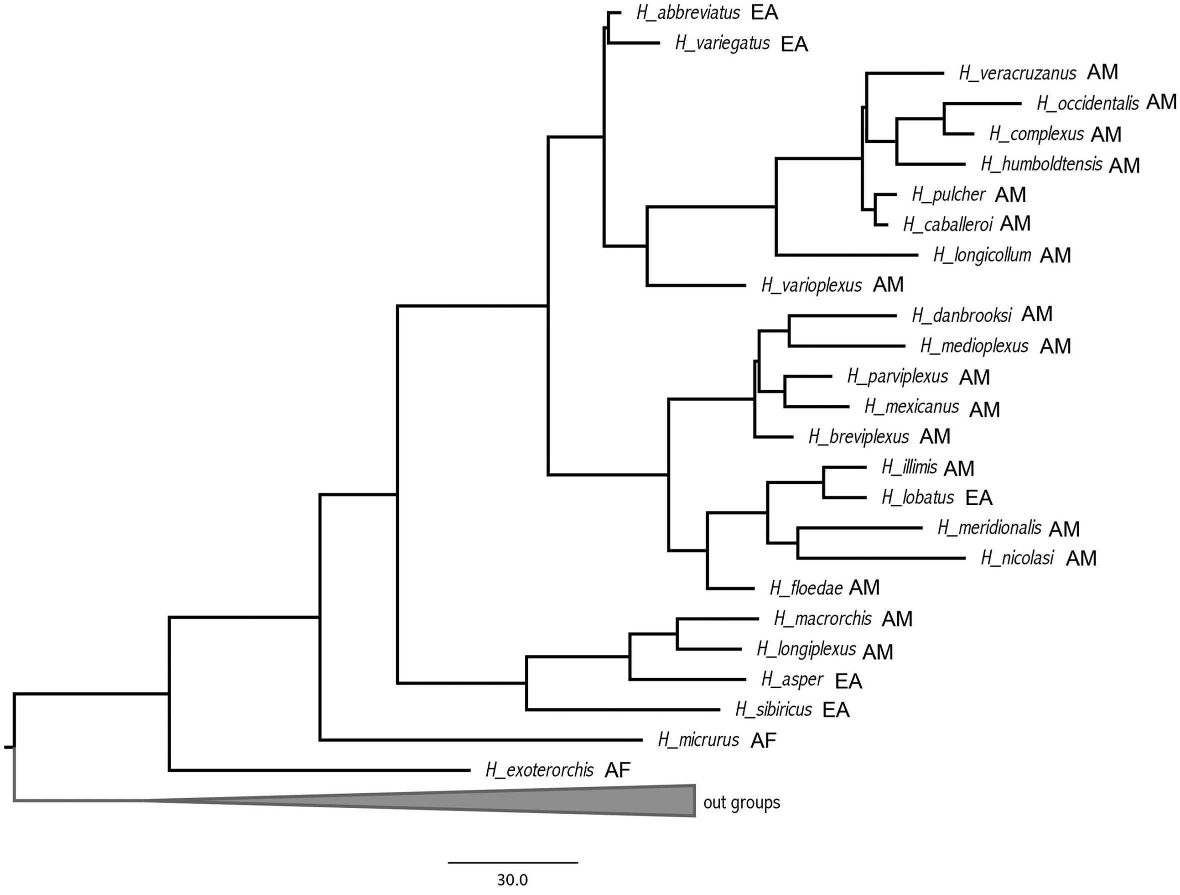

Figure 10. Phylogenetic hypothesis of *Haematoloechus* spp. based on mitochondrial and ribosomal DNA. AF: Africa, AM: America, EA: Eurasia, Out groups: *Brachycoelium salamandrae*, *Glypthelmins brownorumae*, *Opisthioglyphe ranae*, and *Plagiorchis koreanus*. Source: Adapted from León-Règagnon and Topan, 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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DIGENEA, PLAGIORCHIIDA

XIPHIDIATA

Lecithodendriidae Lühe, 1901 (Family)

Jeffrey M. Lotz

Phylum Platyhelminthes
  Class Trematoda
    Subclass Digenea
      Order Plagiorchiida
        Family Lecithodendriidae

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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

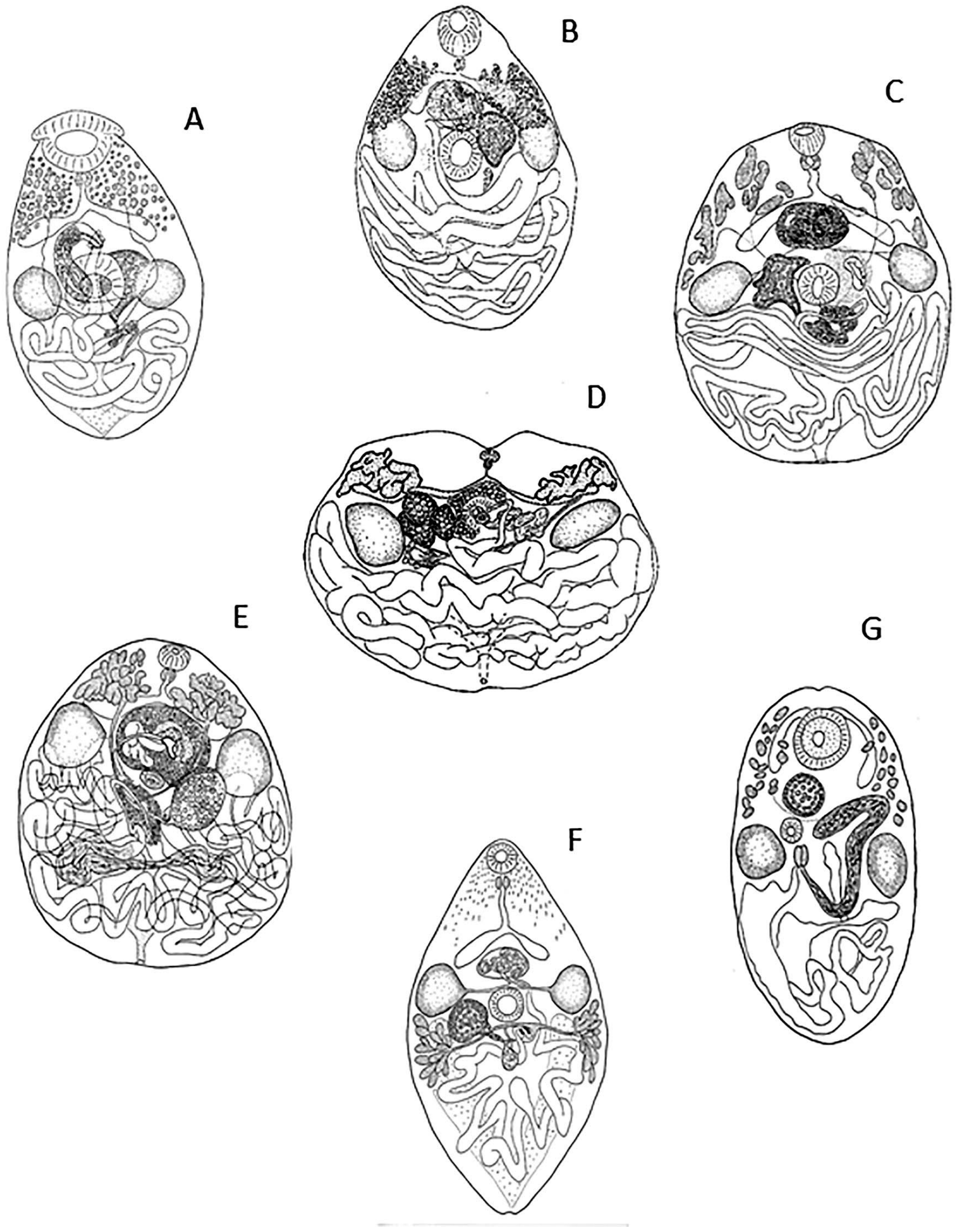

Figure 1. A) *Glyptoporus noctophilus*. B) *Paralecithodendrium swansoni* Macy, 1936. C) *Ochoterenatrema labda*. D) *Castroia silvai* Travassos, 1928. E) *Acanthatrium nycteridis* Faust, 1919. F) *Lecithodendrium linstowi*, Dollfus, 1931. G) *Ophiosacculus mehelyi* (Modlinger, 1930) Macy, 1935. Source: 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

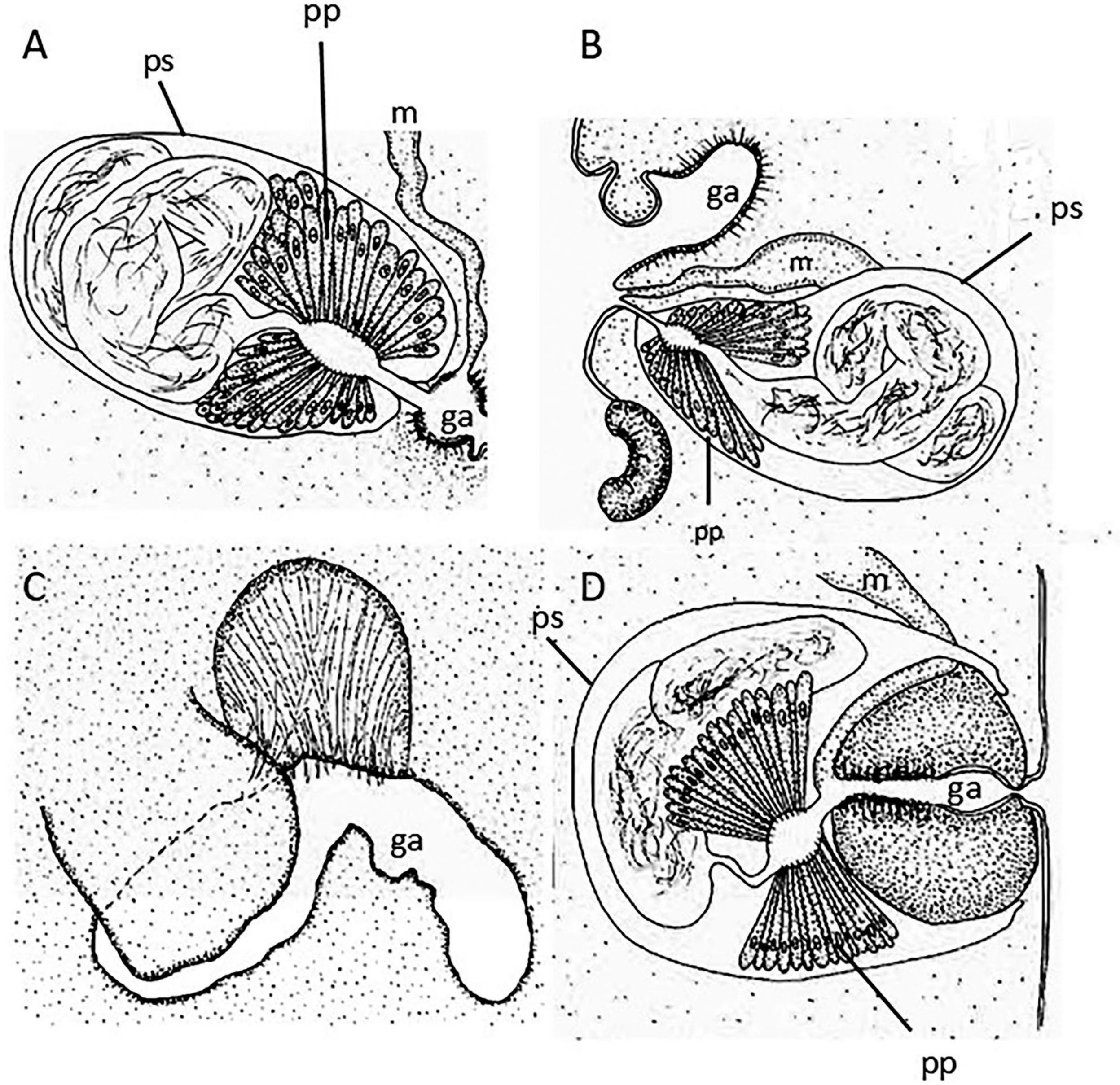

Figure 2. A) *Paralecithodendrium ovimagnosum* (Bhalero, 1926); B) *Papillatrium parvouterus* (Bhalero, 1926); C) *Acanthatrium eptesici* Alicata, 1932; D) *Caprimulgorchis molenkampi* (Lie Kian Joe, 1951). A, B, and D are sagittal sections. C is a ventral view of terminal genitalia of whole mount. Ps: 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*,

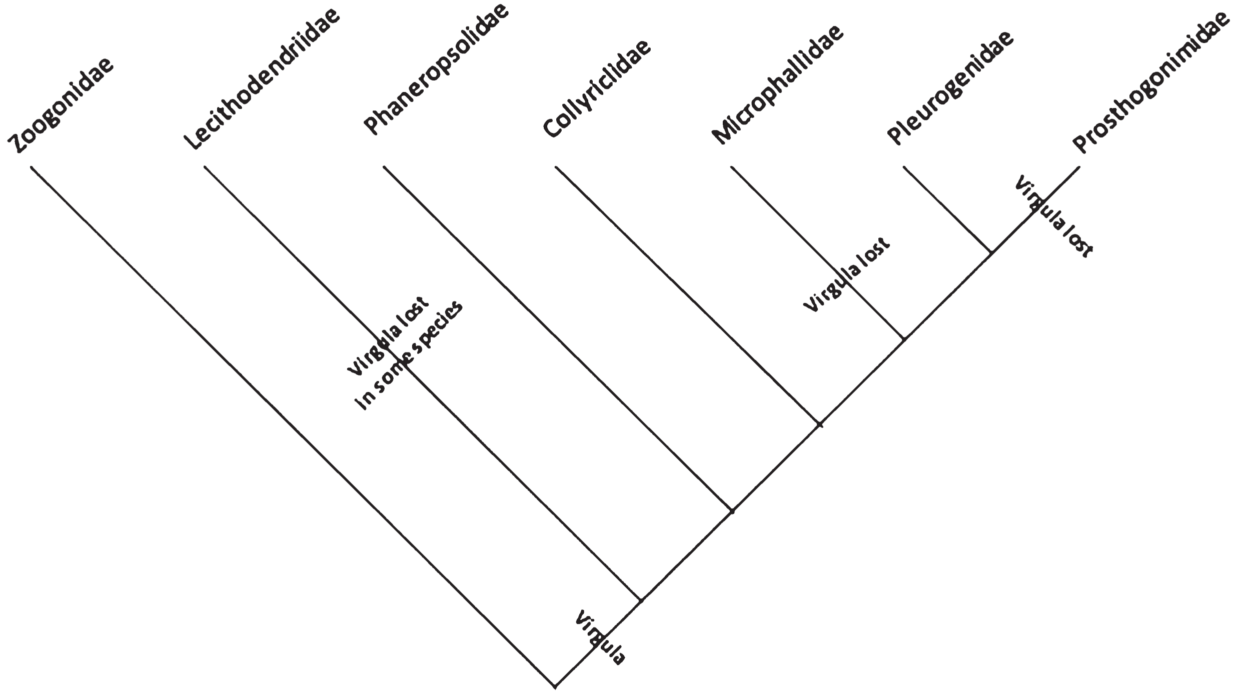

Figure 3. Distribution of virgulate cercariae among families of the Microphalloidea for which cercariae are known. Cladogram of the Microphalloidea. Sources: Adapted from Olson et al., 2013; Kanarek et al., 2014. License: CC BY-NC-SA 4.0.

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).

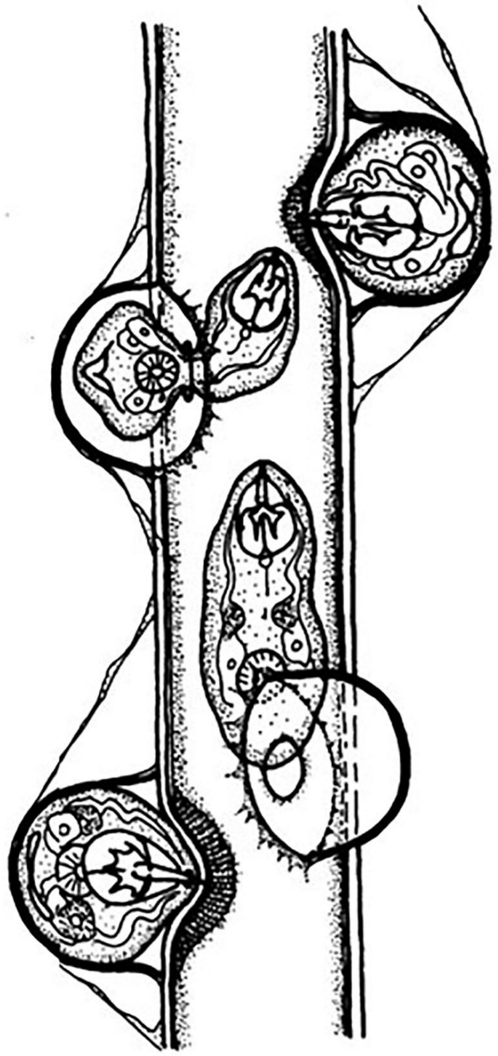

Figure 4. Cercaria of *Acanthatrium oregonense* encysting on and penetrating the gill of a larval caddisfly Source: Adapted from Burns, 1961b. License: CC BY-NC-SA 4.0.

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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46

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

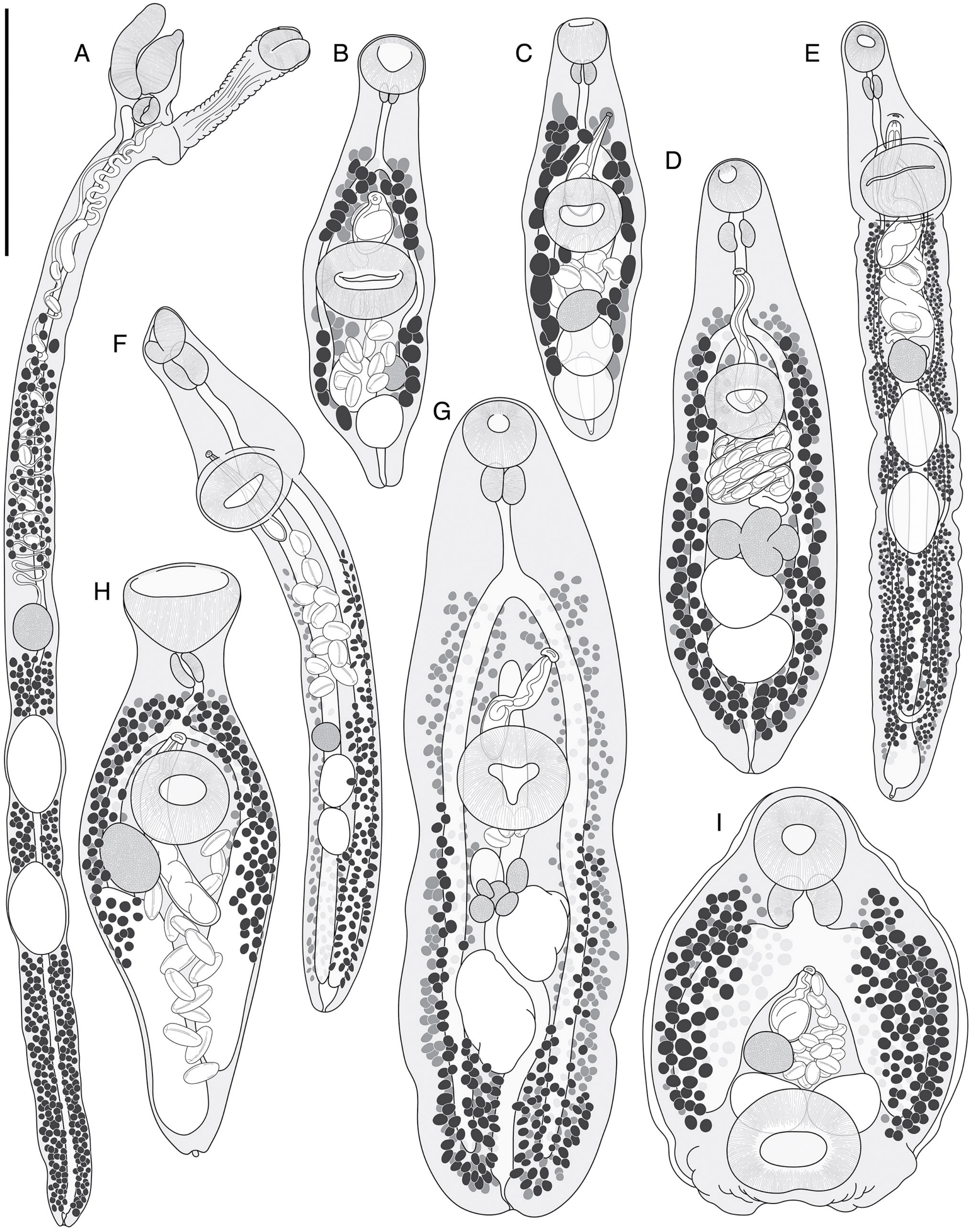
The Opecoelidae Ozaki, 1925 is the richest of all trematode families. It comprises over 1,000 described species presently arranged into about 100 genera. Adult opecoelids are benign endoparasites, typically residing in the intestines, pyloric ceca, or rectum of phylogenetically and ecologically diverse 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. License: CC BY-NC-SA 4.0.

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),

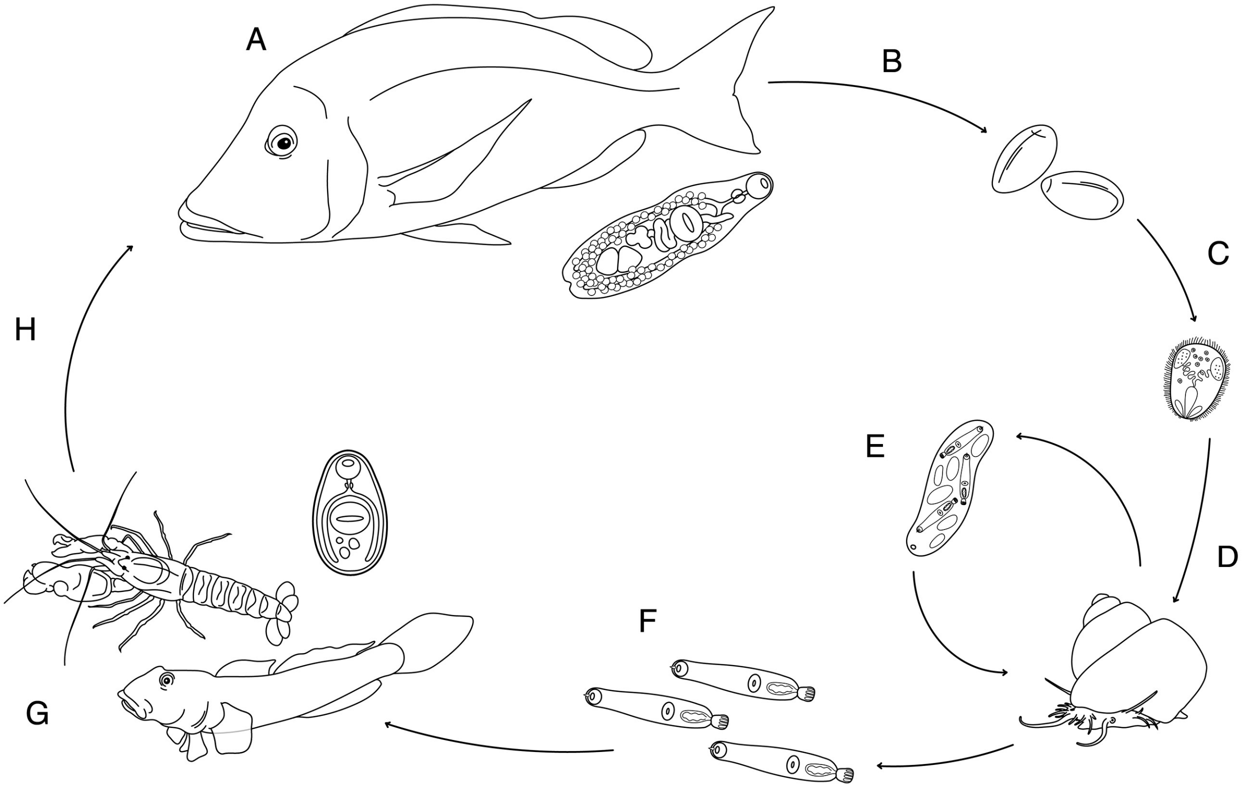

Figure 2. Generalized opecoelid life cycle. A) Adult opecoelids reside within the intestines of teleost fishes; B) Opecoelid eggs are passed to the environment in definitive host feces; C) Eggs hatch in the environment, giving rise to the ciliated miracidia larvae; D) Miracidia seek and penetrate the 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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47

DIGENEA

Summary of the Digenea (Subclass): Insights and Lessons from a Prominent Parasitologist

Robin M. Overstreet

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 *Deropristis inflata* (Deropristidae) 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

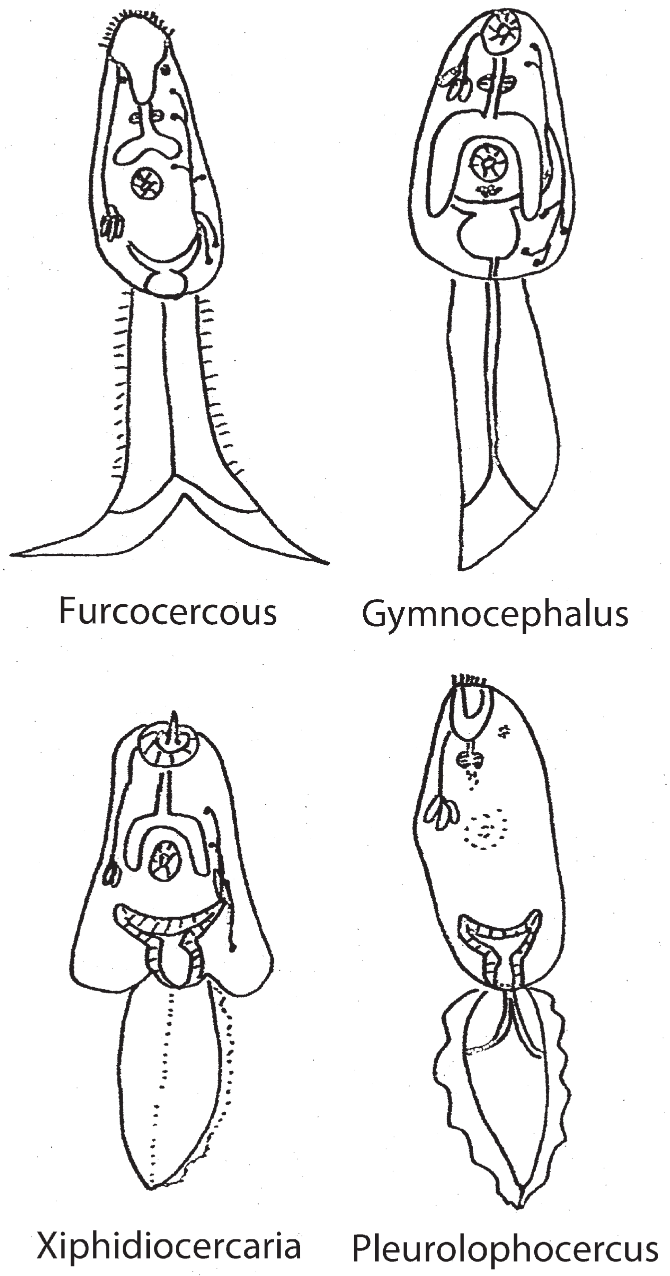
Sporocyst Stage

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).



Furcocercous

Gymnocephalus

Xiphidiocercaria

Pleurolophocercus

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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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.

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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 edi-tors capitalized on the availability of visiting scholars in the Harold W. Manter Laboratory of Parasitology (Lincoln, Ne-braska, 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 to-gether 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 au-thors 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 ad-vancement of knowledge of animal parasitology have the po-tential 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.

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