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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

doi:10.32873/unl.dc.ciap034
2024. *In* S. L. Gardner and S. A. Gardner, eds. Concepts in Animal Parasitology. Zea Books, Lincoln, Nebraska, United States.

Chapter 34

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

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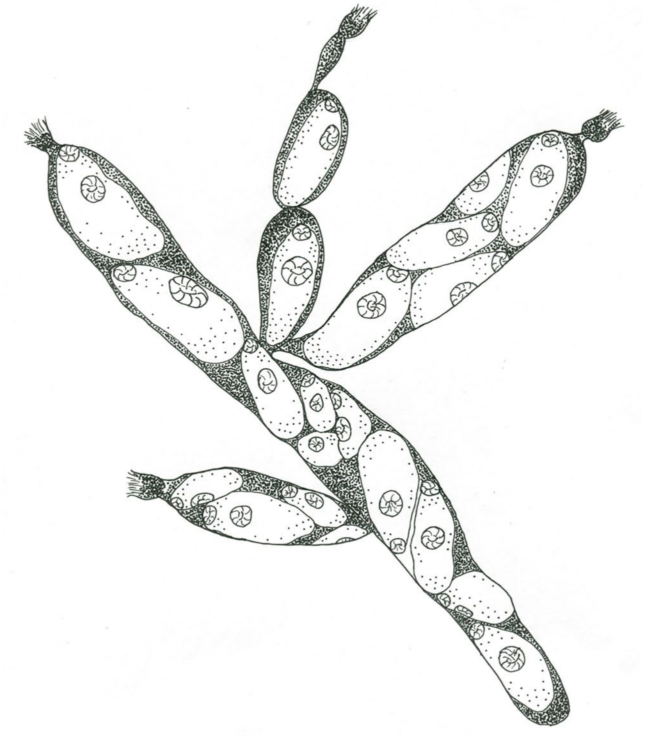

Figure 1. Branched sporocyst of Brachylaimoidea. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

Introduction

The order **Diplostomida** constitutes 1 of the 2 main lineages from which digeneans (subclass **Digenea**) have diversified (Olson et al., 2003). Using a Bayesian analysis, the order Diplostomida was established by Olson and others in 2003 and includes only 1 suborder, **Diplostomata** (Olson et al., 2003). Although some discrepancies have been found between the mt (meaning, mitochondrial) genomic phylogeny and the rDNA genomic phylogeny, a study by Locke et al. (2018) used a much larger genomic dataset which supported the validity of Diplostomida, arriving at a similar result as that of Olson and colleagues (2003).

Probably, the most relevant feature of the morphology of these digeneans is the position of the genital pore posterior to the ventral sucker in the adult stage.

Diplostomida Systematics

This order comprises 3 main lineages or superfamilies: **Brachylaimoidea**, including **Leucochloridiidae**; **Diplostomoidea**, in which members of **Diplostomidae** and **Strigeidae** are intermingled; and the blood flukes, or superfamily **Schistosomatoidea**, in which **Schistosomatidae**, **Sanguinicolidae**, **Spirorchidae**, and **Clinostomidae** are included (Olson et al., 2003). Following are descriptions of some Diplostomida groups.

Superfamily Brachylaimoidea

The Brachylaimoidea comprises a group of digeneans that are difficult to identify and that have poorly understood phylogeny. Larvae in the mollusc intermediate host have branched sporocysts (Figure 1) and cercariae with a poorly developed or absent tail are probably the most relevant morphological features. The main diagnostic features are the position of the genital pore, the extent of the vitellaria through the body, the arrangement of the gonads, the presence of a cirrus sac, and the pattern of the life cycle. Adult worms are characterized by a very short or absent esophagus, which may be more patent in the cercariae. The body may be of variable shape and armed or unarmed with fine spines. The pharynx is stout and muscular and, commonly, preceded by a prepharynx. Usually, the 2 blind ceca reach close to the posterior extremity. Gonads in tandem or a triangle are posterior to the ventral sucker. For more detailed data on the morphology of Brachylaimoidea, see Pojmańska (2002h).

Members of Brachylaimoidea are mainly parasites of birds and mammals, affecting domestic animals, poultry, wild game birds and, rarely, amphibians or reptiles (Joyeux et al., 1934; Harkema, 1939; Heneberg et al., 2016). Some members of Brachylaimoidea have been found to parasitize humans (Butcher et al., 1996; 1998; Butcher and Grove, 2001).

The history of the superfamily Brachylaimoidea shows a rather controversial classification. The superfamily was first recognized by Allison (1943), with Brachylaimidae as the type family (Joyeux and Foley, 1930), and retained in the classifications published in the 1960s and 1970s. A phylogenetic analysis by Olson and colleagues (2003) included this superfamily within the order Diplostomida and the suborder Diplostomata.

Although several phylogenetic and molecular studies have been performed to help differentiate the members of Brachylaimoidea (Machalska, 1978; Casey et al., 2003; Olson et al., 2003; Iwaki et al, 2009; Locke et al., 2012; Zhukova et al., 2012; Heneberg et al., 2016), the phylogenetic relationships are still mainly based on morphology and life cycles. However, conflicting opinions exist and there are several genera, such as *Michajlovia, Urorygma*, or *Zeylamurotrema*, that are currently considered as incertae sedis (Pojmańska, 2002h).

The life cycles of Brachylaimoidea differ markedly from one another. Together with Dicrocoeliidae, they are the only trematodes that are able to complete their life cycle outside of wetlands, sometimes even in more xeric, arid habitats. Only species of the Leucochloriodiomorphidae complete their life cycle in an aquatic environment (Sirgel et al., 2012). According to Pojmańska (2002h), 3 main patterns of life cycle can be distinguished within Brachylaimoidea:

- Life cycle with 2 intermediate hosts completed in an aquatic environment (as that of the Leucochloriodiomorphidae). This pattern includes a mother sporocyst, a daughter sporocyst, furcocercous cercariae with a poorly developed tail, unencysted metacercariae in the second intermediate host, and water birds as the definitive host.
- Life cycle with 2 intermediate hosts completed in a terrestrial environment (for example, Brachylaimidae and Panopistidae). These life cycles include a single generation of sporocysts, cercariae without a tail leaving the first intermediate host, encysted or unencysted metacercariae within the second intermediate host, and adults in birds and mammals, though sometimes (rarely) in amphibians or reptiles.
- Life cycle with 1 intermediate host completed in a terrestrial environment (for example, Hasstilesiidae and Leucochloridiiade). This example includes a single generation of sporocysts, cercariae with an absent or rudimentary tail not leaving the sporocyst, encysted or unencysted metacercariae within the sporocyst, and adults in birds and mammals.

Following is the taxonomy of Brachylaimoidea proposed by Pojmańska and colleagues (2002h), including a total of 8 families and 4 genera incertae sedis.

Family Brachylaimidae

The family Brachylaimidae contains numerous species of terrestrial trematodes that infect mammals, birds, reptiles, and, rarely, amphibians (Gibson and Bray, 1994). *Brachylaima* is the most representative genus within this family and the type genus. Adult worms of Brachylaimidae are characterized by an elongated, or occasionally oval or subglobular, body, sometimes with fine spines. The suckers are well-developed and usually at the anterior end. There is usually a prepharynx, the pharynx is muscular, the esophagus, if present, is short and the ceca long, terminating near the posterior end. The gonads are posterior to the ventral sucker and the genital pore opens on the ventral surface.

Regarding the larval stages, the main characteristic features are branched daughter sporocyts, and cercariae with rudimentary, stumpy tails. Metacercariae, encysted or not, inhabit the kidney or pericardium of the second intermediate host.

Pojmańska (2002a) recognizes subfamilies within Brachylaimidae. The subfamily Brachylaiminae, including 5 genera (*Brachylaima, Ectosiphomus, Glaphyrostomum, Parabrachylaima*, and *Postharmostomum*) and the subfamily Ityogoniminae with 2 genera (*Ityogonimus* and *Scaphiostomum*). Both subfamilies are differentiated based on the adult morphology. Brachylaiminae is characterized by adults with a plump or elongate body, well developed suckers, and an esophagus that is practically absent. In contrast, adult worms of Ityogoniminae are filiform, with small suckers and a short esophagus.

Adult flukes in this family are found in mammals and birds, and occasionally amphibians, and have a complex 3-stage life cycle. There are 2 intermediate hosts, both terrestrial molluscs. The cercariae leave the first intermediate host with easily shed, rudimentary tails, and the metacercariae in the second intermediate host may or may not be encysted.

As mentioned above, *Brachylaima* is the type genus of this family. This genus has had many synonyms with no fewer than 4 spellings having been used in the literature (Yamaguti, 1971; Kamiya and Machida, 1977). Many of the species have been poorly described, with incomplete life cycles and a lack of detailed information for accurate identification. This problem is compounded by the morphological similarity of many of the adult worms. The first and second intermediate hosts of brachylaimids are either the same or 2 different species of terrestrial snail species. The definitive host can be either a mammal or a bird. Humans have also been reported as an incidental definitive host for 1 species of the genus *Brachylaima*, namely, *B. cribbi* (Butcher et al., 1996; 1998). These infections were reported from South Australia (Butcher et al., 1996; 1998; Butcher and Grove, 2001) where the life cycle is maintained between mice, *Mus musculus*, and helicid and hygromiid snails (Butcher et al., 1996).

Humans often accidentally ingest these snails with vegetables from house gardens or local markets (Butcher et al., 1996; 1998). Infections in humans usually become chronic and can persist as long as 18 months (Butcher et al., 1996). Clinical symptoms depend on the parasite load and heavy infections are associated with diarrhea, abdominal pain, low-grade fever, and fatigue (Butcher et al., 1996; Toledo et al., 2006).

Family Hasstilesiidae

The family Hasstilesiidae has been often recognized as a subfamily of Brachylaimidae. However, its status at the family level has been recognized with *Hasstilesia* as the type genus (Pojmańska et al., 2002b). Hasstilesiidae are similar to Brachylaimidae in the position of the genital pore between or anterior to the testes on the ventral side, but differ in the pattern of the life cycle. They are intestinal parasites of mammals such as rabbits, pikas, bats, goats, and sheep (Rowan, 1955; Nogueira et al., 2004), and the life cycle includes 1 intermediate host, a cercarium with a rudimentary tail, and metacercariae that remain unencysted within the ramified sporocysts.

Adult worms are characterized by their wide body and small, ovoid suckers, testes larger than the ovary in a triangle or in tandem, and an elongated cirrus sac in the median line of the body. There is an ovary located between the testes and short vitelline fields. This family includes 2 genera (*Hasstilesia* and *Strzeleckia*) that can be differentiated by body shape, extent of the ceca, position of the genital pore, and the arrangement of the gonads (Pojmańska et al., 2002b).

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).

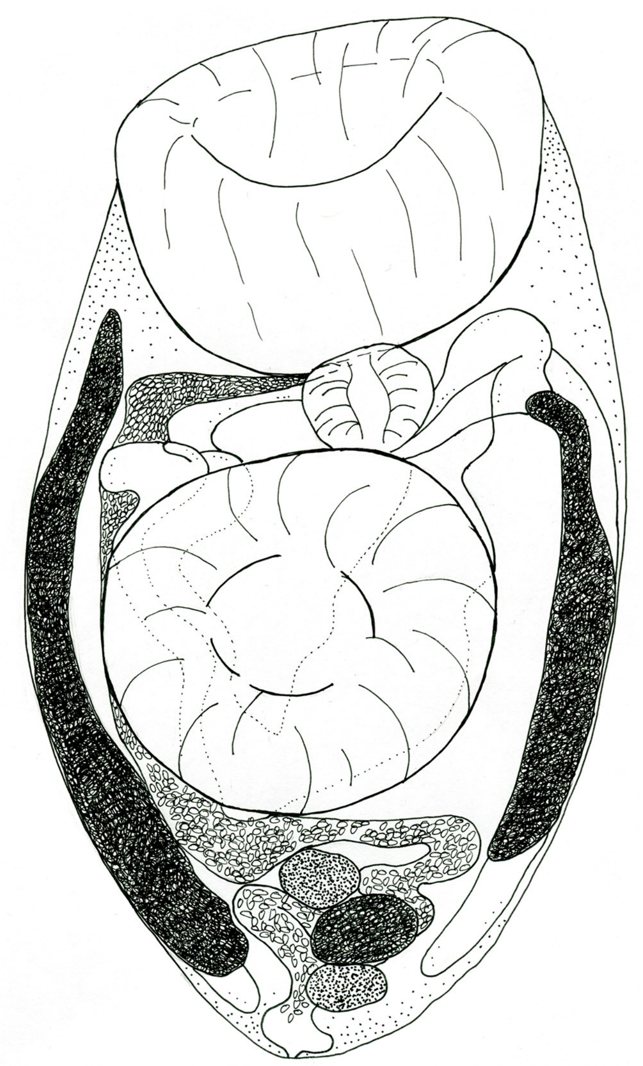

Figure 2. General scheme of an adult specimen of *Urogonimus* sp. (Leucochloridiidae). Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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,

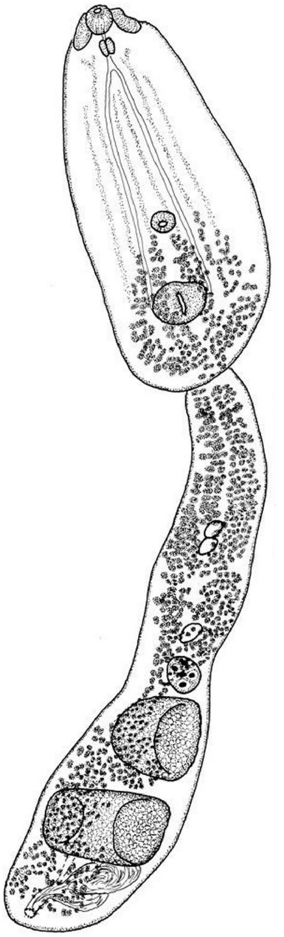

Figure 3. Adult specimen of *Diplostomum pseudospathaceum* (family Diplostomidae). Source: Pérez del Olmo et al., 2014. License: CC BY 4.0.

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

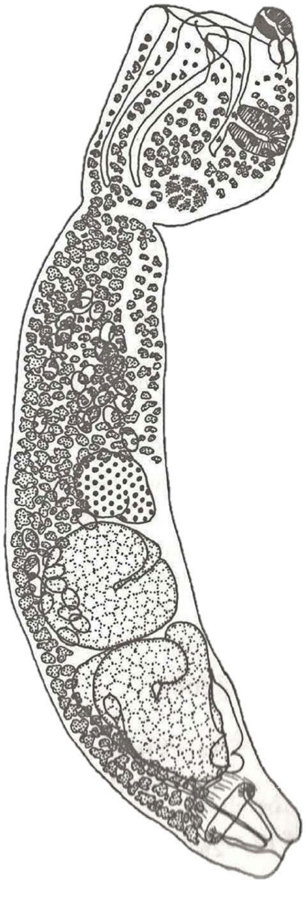

Figure 4. Adult specimen of *Strigea falconis* (family Strigeidae). Source: Dubois, 1968. License: Public domain.

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.

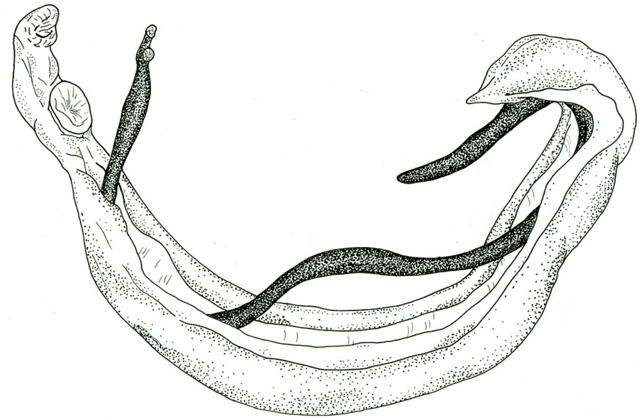

Figure 5. Adults of *Schistosoma* spp. Source: L. Acosta Soto, B. Fried, and R. Toledo. License: CC BY-NC-SA 4.0.

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

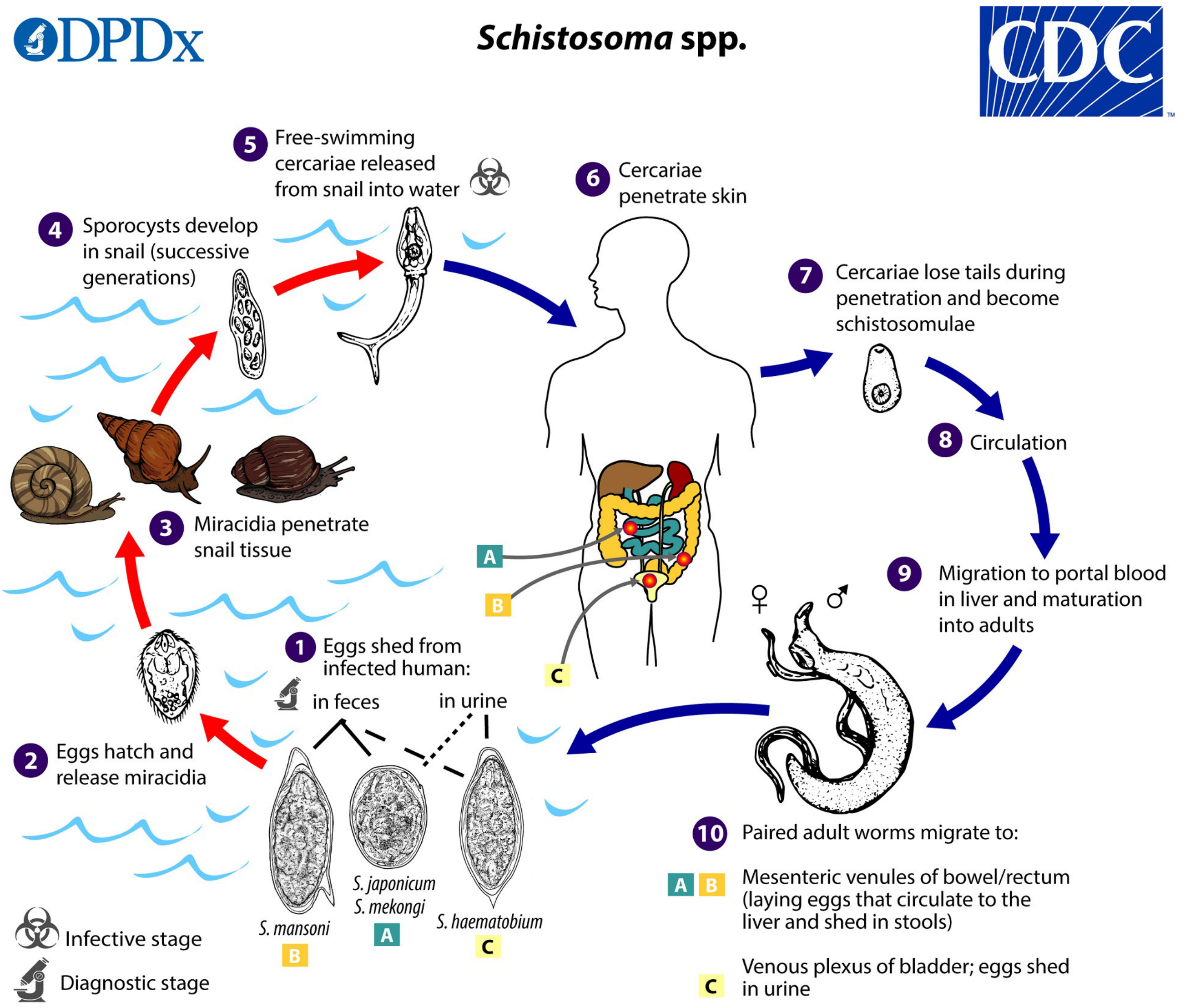

Figure 6. *Schistosoma* eggs are eliminated with feces or urine, depending on species (1). Under appropriate conditions the eggs hatch and release miracidia (2), which swim and penetrate specific snail intermediate hosts (3). The stages in the snail include 2 generations of sporocysts (4) and the production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