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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

Chapter 35

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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).
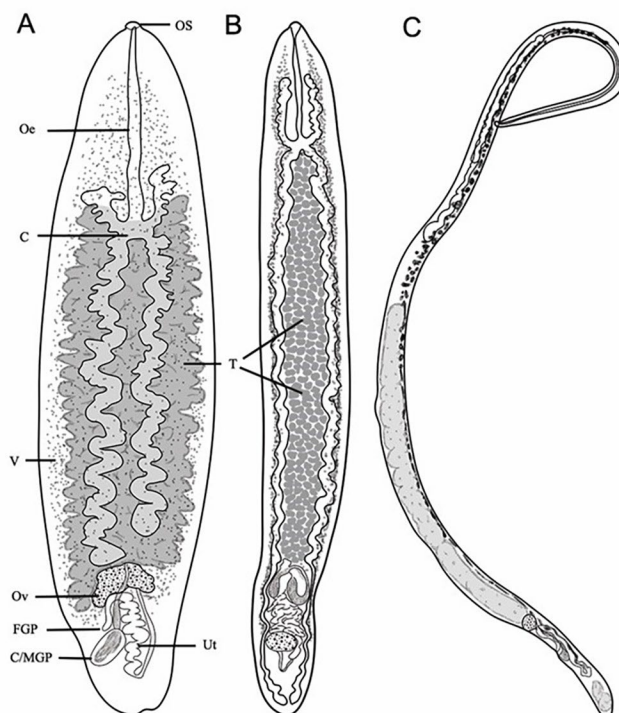

Figure 1: Selected aporocotylid species demonstrating their general body plans. A) *Psettarium pandora* Yong et al., 2018 (after Yong et al., 2018), a good example of a typical aporocotylid; B) *Aporocotyle simplex* Odhner, 1900 (after Thulin, 1980), showing a similar body plan but possessing multiple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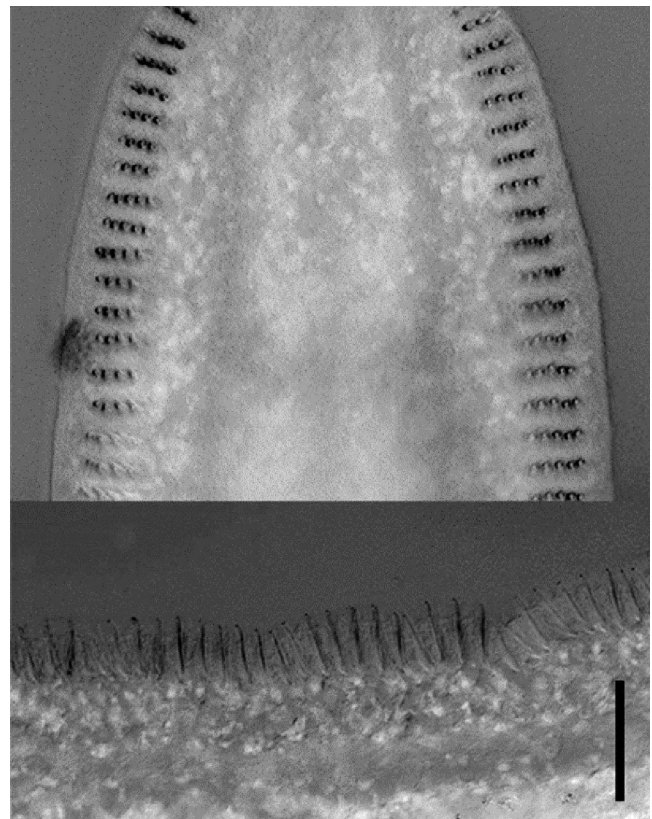

Figure 2: Ventral (top) and lateral (bottom) views of the tegumental spines of aporocotylids, as exemplified by *Cardicola suni* Yong et al., 2016. Note the hooked ends of the spines (seen most clearly in lateral view), and their arrangement in regular rows along the ventrolateral body margins. Scale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

flukes: The **Spirorchiidae**, which infect reptiles, and the **Schistosomatidae**, which infect endothermic tetrapods (that is, mammals and birds) (Smith, 2002). The overwhelming majority of species occur in actinopterygian fishes, with only 9 known from chondrichthyans (Cutmore et al., 2018).

The phylogeny of the Aporocotylidae is of great interest, because there is evidence of broad cospeciation with their host fishes, that is, the more phylogenetically basal parasite species infect phylogenetically basal fish taxa (Figure 3). Only 1 group of 3 species which infect the milkfish *Chanos chanos*, a basal teleost, bucks this trend, with all 3 species grouping in a monophyletic clade formed otherwise by species infecting more derived Teleostei. This is interpreted as an example of secondary host-switching into ancient or less derived fish taxa (Yong et al., 2016). Interestingly, the fact that the chondrichthyan-infecting blood flukes form a monophyletic clade with those of actinopterygian fishes puts the understanding of aporocotylid evolutionary history at odds with the current understanding of craniate evolution, which
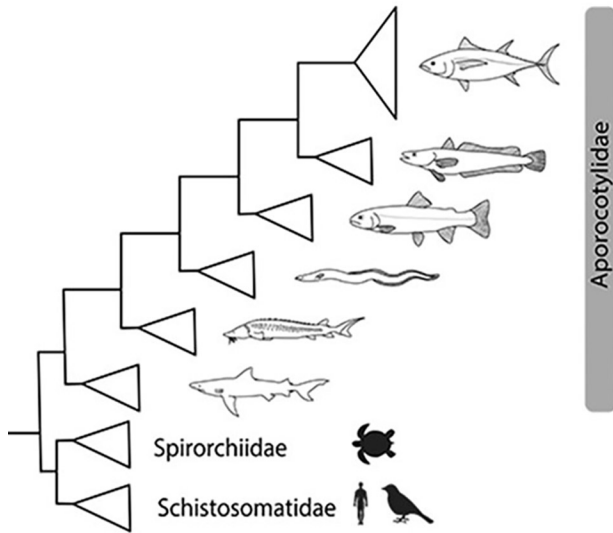
Figure 3: A simplified phylogenetic tree of the Aporocotylidae showing its relationships to the 2 other schistosomatoid families, the Schistosomatidae (blood flukes of mammals and birds) and the Spirorchiidae (blood flukes of reptiles), and cophyly with progressively evolutionarily-advanced groups of fishes from chondrichthyans, to chondrosteans (for example, sturgeon), to basal teleosts (such as, elopomorphs, like eels, and basal euteleosts, like salmoniforms and gadiforms), to advanced euteleosts (perciform fishes). Source: R. Q.-Y. Yong. License: CC BY-NC-SA 4.0.
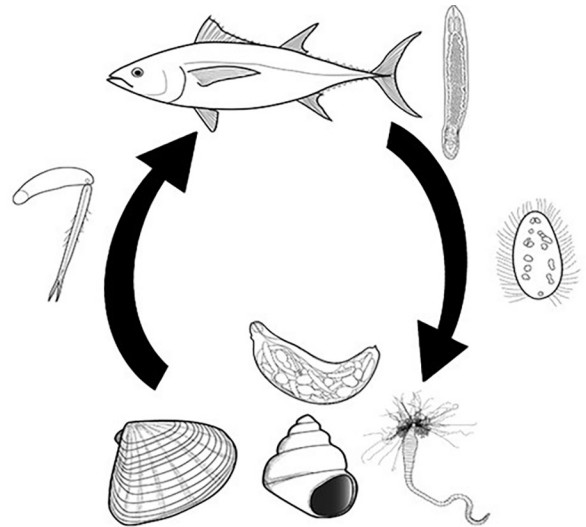
Figure 4: A generalized life cycle for aporocotylids. Adult worms live in the fish host and produce eggs (top); miracidial larvae (right) hatch, leave the fish and infect either a bivalve (bottom left; chondrichthyan-infecting species), gastropod (bottom middle; freshwater teleost-infecting species), or polychaete worm (bottom right; marine teleost-infecting species) intermediate host. The miracidium develops into a sporocyst, which in turn clonally produces daughter sporocysts or rediae (bottom center), both of which asexually produce cercariae (left) that leave the intermediate host, infect the definitive fish host, and develop into sexual adults. Source: R. Q.-Y. Yong. License: CC BY-NC-SA 4.0.

holds that tetrapods (that is, terrestrial craniates) and sarcopterygian fishes (such as lungfishes and coelacanths) diverged from the common ancestor of actinopterygian fishes (Orélis-Ribeiro et al., 2014).

Aporocotylids show varying patterns of radiation among different groups. Species of some genera, such as those of *Psettarium* Goto & Ozaki, 1930, and *Paradeontacylix* McIntosh, 1934, have radiated only among particular fish taxa. In the case of the former, in tetraodontiform fishes (Yong et al., 2018b) and, in the latter, perciform fishes of the family Carangidae (Repullés-Albelda et al., 2008). Others, like those of *Aporocotyle* and *Cardicola* Short, 1953, infect much broader ranges of hosts; 5 orders and 10 families of fishes in the case of the former, and 5 orders and 17 families in the latter. Some radiations, such as those of *Ankistromeces*, *Phthinomita*, and *Cardicola* Short, 1953 that infect rabbitfishes (Perciformes: Siganidae), show highly-conserved morphologies and relatively little molecular divergence, indicating cryptic or incipient speciation (Nolan and Cribb, 2006; Brooks et al., 2017). Other lineages of aporocotylids have shown limited capacity for radiation in other fish groups; for instance, only 1 species is known to infect damselfishes (Pomacentridae) and only 2

are known from wrasses (Labridae), 2 of the most speciose fish families in the world, with over 400 and 500 species, respectively, despite both these families being surveyed extensively for aporocotylids (Nolan and Cribb, 2005; 2006; Yong et al., 2018a).

Aporocotylids in Relation to Other Organisms

All known aporocotylid life cycles involve 2 hosts, an invertebrate intermediate host and a fish final host (Figure 4). As for all blood flukes, sexual reproduction occurs within the host circulatory system and the eggs are shed into the host bloodstream. The eggs travel either to the gills, where they cause erosive pathology which eventually brings them into contact with seawater, or to the gut via the mesenteric vessels, where they exit with the feces. In both routes, egg escape is probably mediated by significant antigenic reactions on the part of the host and, in cases of heavy infection, can be compounded by environmental stress and cause host mortality.

Once free of the fish, the eggs hatch and a ciliated miracidium infects an intermediate host. Among freshwater species, the intermediate host is a gastropod mollusc or snail, whereas those of marine bony fishes infect polychaete worms

(Cribb et al., 2017). No full life cycles are known for chondrichthyan-infecting aporocotylids, but Cribb and colleagues (2017) found intermediate stages in a bivalve mollusc which formed a molecular phylogenetic clade with sequence data for elasmobranch-infecting species. Having penetrated the host, the miracidia infect the digestive and reproductive organs and develop into mother sporocysts, which in turn asexually produce daughter sporocysts. These asexually produce cercariae, which emerge from the intermediate host and enter the final hosts by direct penetration (Kirk and Lewis, 1993). Aporocotylid cercariae have varying capacities to seek out their final hosts; some species have well-developed tails and actively swim to find their final hosts. Others, such as those of tuna-infecting species of *Cardicola*, have rudimentary tails and are evidently poor swimmers. It is not known how they come into contact with their final hosts (Cribb et al., 2011; Shirakashi et al., 2015). The act of penetration by cercariae can be traumatic for the hosts, particularly if the larvae are densely concentrated (Wales, 1958). Infection by aporocotylids causes pathological reactions which, if sufficiently severe, can permanently impair or kill the host. This is discussed in further detail below.

Most Important Groups

Aporocotylids are that rare aquatic trematode family which receives human attention due to the propensity for high infection rates to cause mass mortalities in the fish aquaculture industry. Several species in both freshwater and marine systems cause diseases that reduce production in aquaculture production facilities (reviewed in Ogawa, 2014). Rainbow trout (family Salmonidae: *Oncorhynchus mykiss*) farms in the western United States have reported losses of up to a million fish in a short amount of time due to infection with these trematodes (Wales, 1958), while significant losses have also been reported by farmers of brook trout (family Salmonidae: *Salvelinus fontinalis*) in the United States (Hoffman et al., 1985), tiger pufferfish (family Tetraodontidae: *Takifugu rubripes*) in China and Japan (Ogawa et al., 2007), amberjack (family Carangidae: *Seriola* spp.) in Japan and Spain (Crespo et al., 1994; Ogawa and Fukudome, 1994), and bluefin tuna (family Scombridae: *Thunnus* spp.) in Japan (Ogawa et al., 2010).

The cumulative pathogenic effects of aporocotylid infection are collectively known as sanguinicoliasis. The symptoms of sanguinicoliasis fall into 2 phases: Those which result from initial infection of fish by cercariae, and those related to mature infections. In the first phase, the physical trauma resulting from penetration by large numbers of cercariae can rapidly and severely compromise, and even kill, a fish. In the second phase, the consumption of host blood and the release of eggs into the organs lead to severe pathological effects such as inflammation, ulceration of the gut wall (Yong

et al., 2018a), and erosion of gill tissue (Bullard and Overstreet, 2002). One species, *Cardallagium anthicum* (Bullard & Overstreet, 2006), which infects cobia (family Rachycentridae: *Rachycentron canadum*), even induces dramatic alterations to its attachment site. By lacing itself into spaces in the heart tissue, it induces a fibromatous tissue response, leading to the formation of a sort of fibrotic collar which surrounds the worm (Bullard and Overstreet, 2006; Warren et al., 2017). Although some fish are able to survive these effects, they may still incur significant loss of body condition and may have increased susceptibility to infection by other pathogens (Iqbal and Sommerville, 1986; Kumon et al., 2002). More typically, however, particularly in aquaculture settings, these effects lead to severe trauma and, ultimately, death. Through all these phases, the severity of disease suffered is directly proportionate to the number of worms to which fishes are exposed. Since most aquacultured fish are kept in stationary facilities, often adjacent to large populations of intermediate hosts, they are unable to escape high concentrations of cercariae. Treatment of sanguinicoliasis hence depends not just on treatment of the infection using drugs (anthelminthics such as praziquantel), but on interrupting the fluke life cycle, either by removing intermediate hosts from the immediate area (Malevitskaya, 1950) or moving the fish away from infection sites (Kirchhoff et al., 2011). To cite an example, bluefin tuna ranchers in South Australia combat infection of their stock by *Cardicola* spp. by shifting their sea cages further offshore to minimize encounters between the tuna and the aporocotylid intermediate hosts, which are polychaete worms of the family Terebellidae (Kirchhoff et al., 2011).

Other Relevant Related Topics

The inherent difficulties associated with working on aporocotylids mean that, for most of the family's history, the rate of discovery lagged behind those of many other trematode groups. The fragility of individuals upon collection of most species poses problems with collecting specimens for preservation and study, with specimens readily fragmenting, even during targeted dissections. Once removed from their infection sites, they often rapidly degrade, losing their tegumental spines and quickly dying (Bullard et al., 2008). Compounding the issue is the fact that their chief infection sites—blood vessels, gills, and cardiac tissue—are difficult to dissect, requiring great care and precision. As a result, aporocotylids have often been discoveries of chance, appearing in washings of guts and other dissections, their real sites of infection unknown until systematic searching was performed. This is a pervasive and characteristic problem of collecting small helminth parasites from larger hosts with little time, and usually with inadequate training of the persons doing the necropsies.

However, since the turn of the 21st century, the number of described aporocotylid species has increased rapidly because of concerted efforts to characterize the fauna of this family, to try for a better understanding of both the nature of parasites affecting aquaculture and how to optimize targeted searching. Seventeen of the 36 known genera and 86 of the 164 known species were described or proposed post-2000, including many of the species which infect fish of aquacultural significance. The pace of discovery for this family continues unabated, with new taxa continuing to be proposed and described year after year. Nevertheless, many fish groups are still under-surveyed or unstudied for the presence of blood flukes, many regions of the world have been little-studied for parasites in general, and a high proportion of the genera within the Aporocotylidae are monotypic. The fish blood flukes, therefore, can still be regarded as a highly exciting potential trove of discovery.

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