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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

Chapter 20

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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 tri-loculate) 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 Gangesii-nae 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
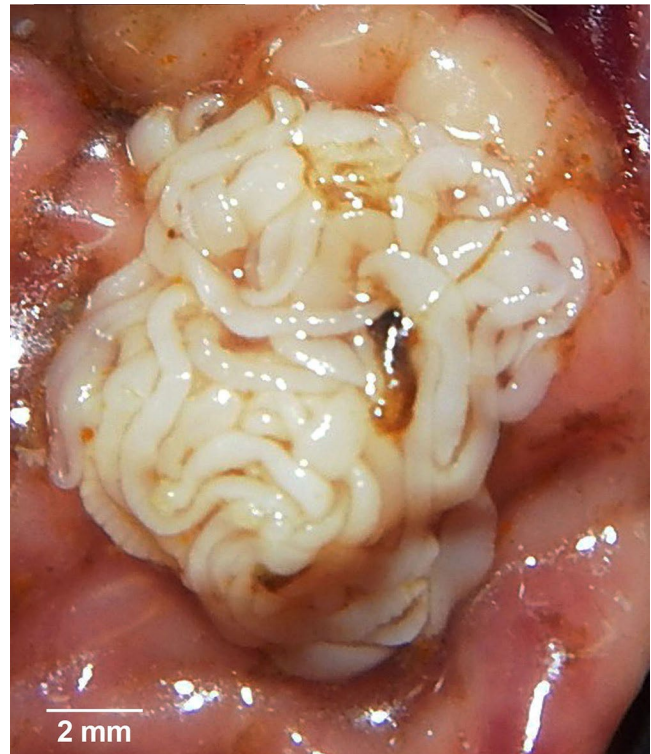

Figure 1. *Proteocephalus perplexus* in the intestine of *Amia calva*, United States. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

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 colleagues (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).

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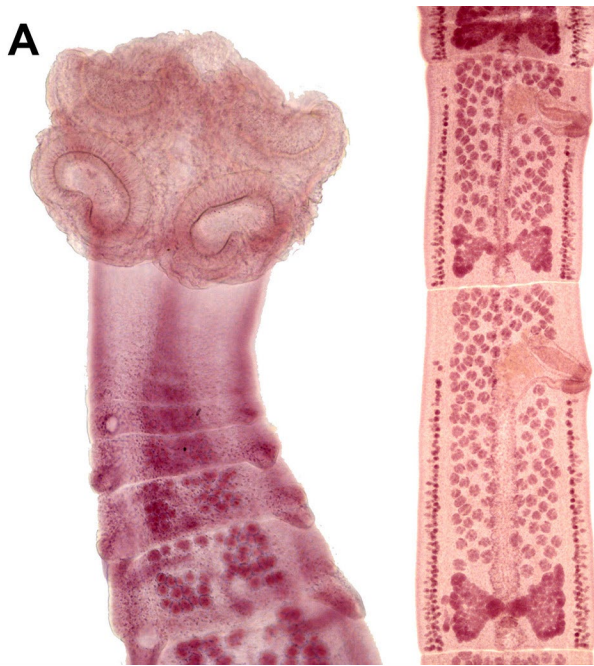
Figure 2. Adults from pimelodid catfishes in Peru (scolex and anterior proglottids of *Pseudocrepidobothrium eirasi* from *Phractocephalus hemioliopterus* and mature proglottids of *Proteocephalus sophiae* from *Paulicea luetkeni*. Source: R. Kuchta and T. Scholz. License: CC BY-NC-SA 4.0.

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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