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Eucestoda

Rhinebothriidea Healy et al., 2009 (Order)

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Phylum Platyhelminthes

Class Cestoda

Subclass Eucestoda

Order Rhinebothriidea

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Chapter 29

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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

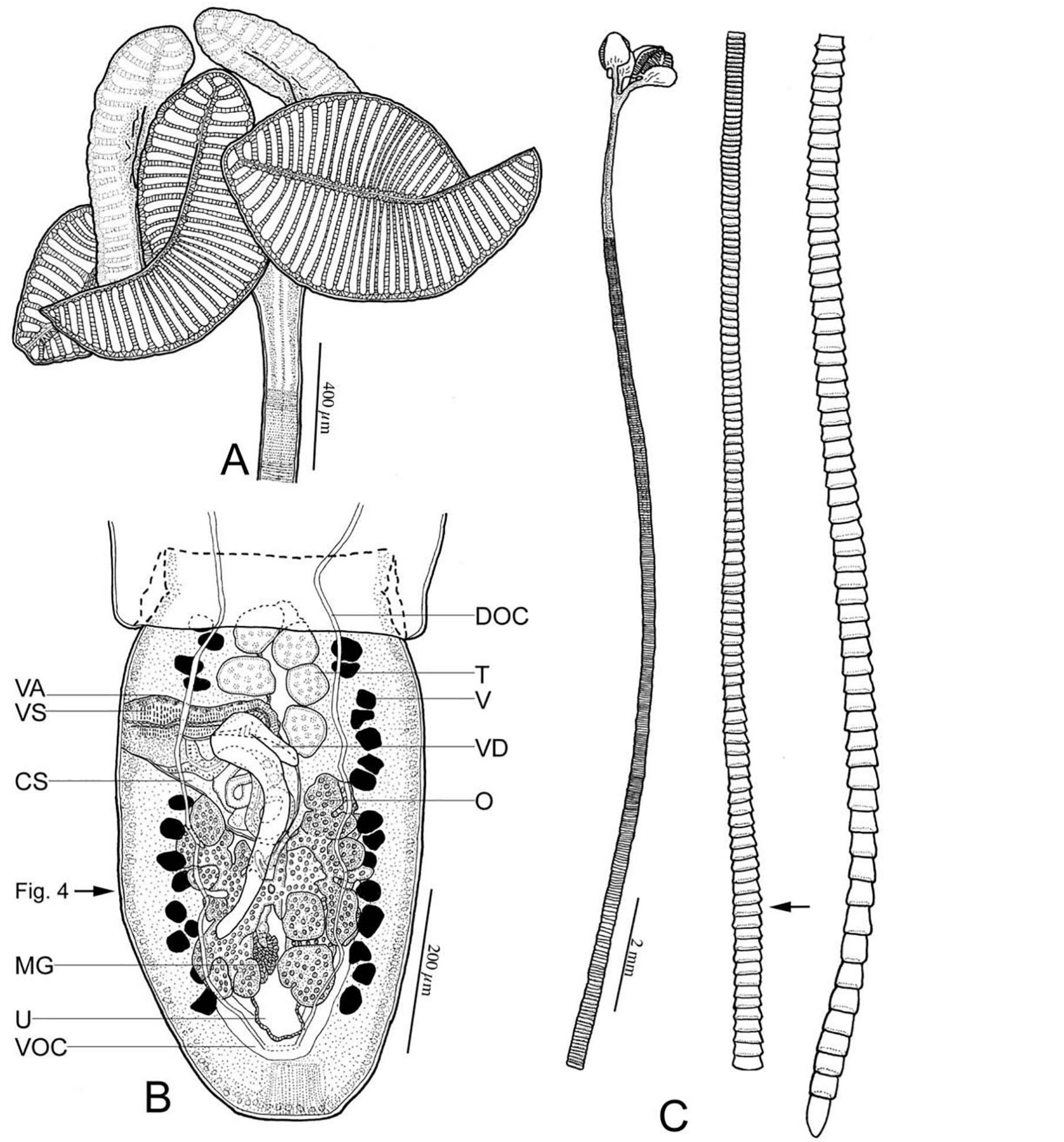

Figure 1. Line drawings of *Rhinebothrium paratrygoni* Rego & Dias, 1976 collected from the type locality. A) Scolex of voucher (MZUSP 6214); B) terminal mature proglottid of voucher (MZUSP 6214). The vas deferens is above the cirrus sac. The arrow indicates the location of the section shown in the portion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

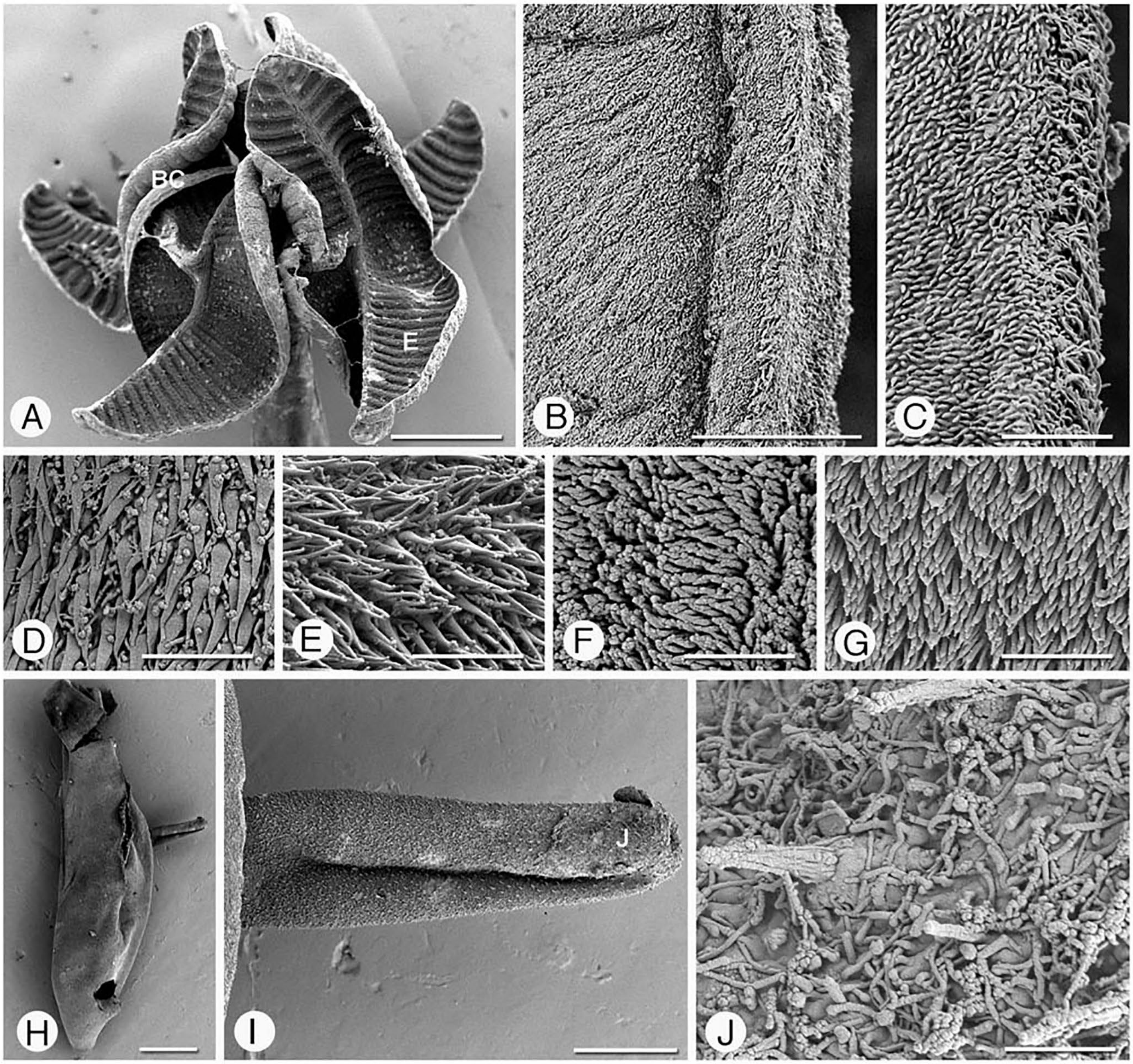

Figure 2. Scanning electron micrographs of *Rhinebothrium paratrygoni*. Scolex, Figures A–G. A) Scolex; B) small letter indicates the locations of details shown in B–C and E. Proximal surface of the rim of the bothridium; C) proximal bothridial surface adjacent to the bothridial rim; D) proximal bothridial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

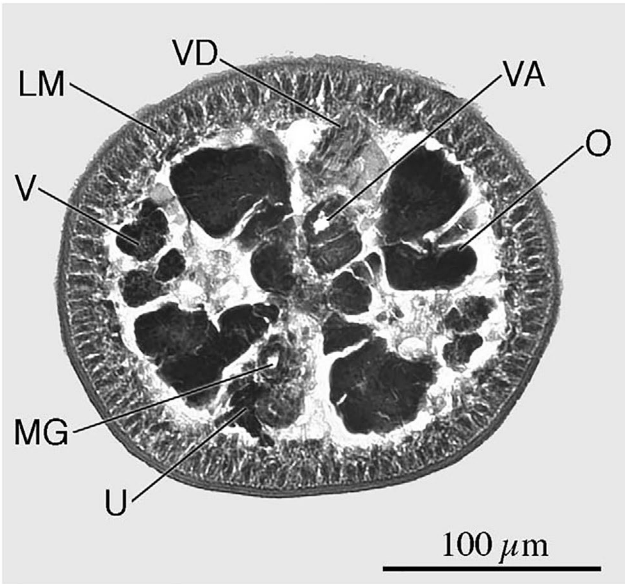

Figure 3. Cross section through a mature proglottid of *Rhinebothrium paratrygoni* at the level of the ovarian isthmus. Abbreviations: LM) Longitudinal muscles; MG) Mehlis' gland; O) ovary; U) uterus; V) vitellaria; VA) vagina; VD) vas deferens. Source: Reyda and Marques, 2011. License: CC BY.

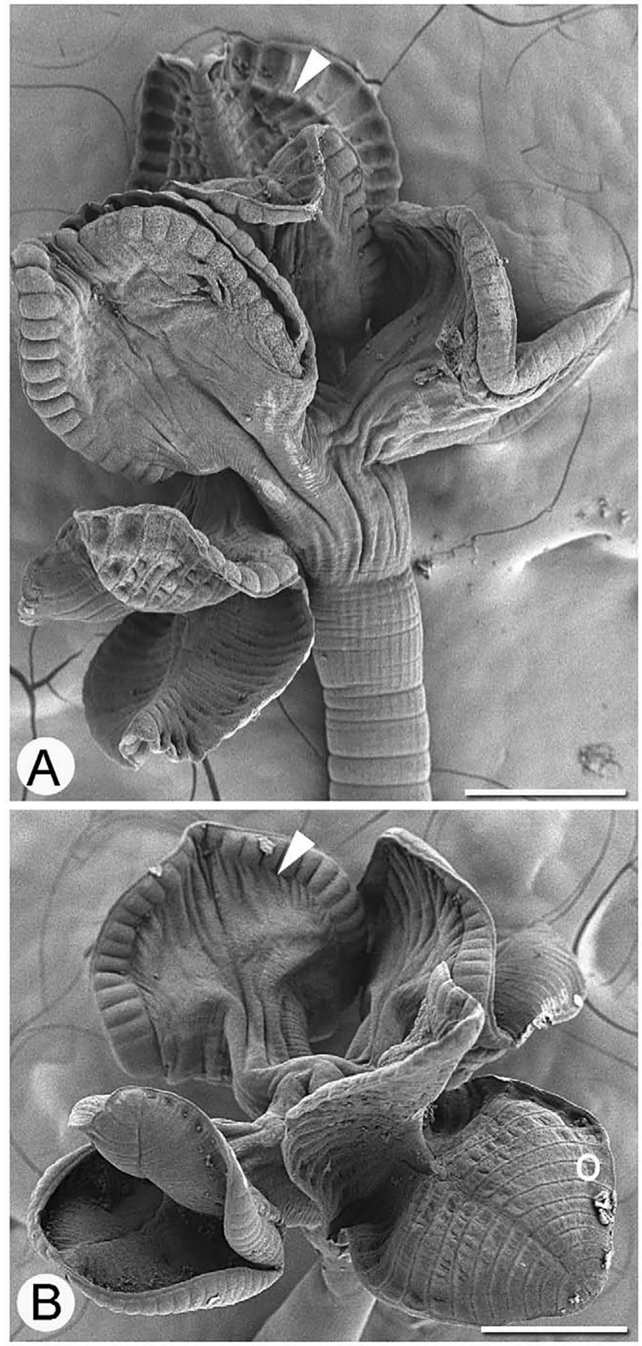

Figure 4. Scoleces of *Rhinebothrium copianullum*. A) Scolex in which marginal longitudinal septa are visible, indicated by the white arrow; B) scolex in which marginal longitudinal septa are visible on the proximal bothridial surface, indicated by the white arrow. The white circle indicates the position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

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 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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