Systematic Review of Cestodes Infecting Freshwater Stingrays (Chondrichthyes: Potamotrygonidae) Including Four New Species from Venezuela

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ABSTRACT: Cestode parasites were collected in freshwater stingrays from localities in Colombia, Venezuela, Brazil, and Paraguay. Four new species are described from Venezuela. Potamotrygonocestus orinocoensis sp. n. from Potamotrygon reticulatus in the delta of the Orinoco River resembles P. magdalenensis by having compact rather than follicular vitellaria, but differs by having larger and differently shaped bothridial hooks. Acanthobothrium regoi sp. n. from P. hystrix in the delta of the Orinoco River most closely resembles A. quinonesi, but differs by having larger bothridial hooks. Rhinebothroides glandularis sp. n. from Potamotrygon hystrix in the delta of the Orinoco River most closely resembles R. scorzai by having a coiled vagina and poral ovarian lobes which extend anterior to the posterior margin of the cirrus sac, but differs in numbers of bothridial loculi and testes, and by exhibiting prominent parenchymal gland cells surrounding the terminal male genitalia. Rhinebothroides venezuelensis sp. n. from Potamotrygon hystrix in the delta of the Orinoco River and P. yepezi from the Rio Cachiri, Zulia, Venezuela, resembles R. moralarai and R. circularis by having a straight vagina and poral ovarian lobes not extending anteriorly beyond the posterior margin of the cirrus sac, but differs in testes number and number of bothridial loculi.

Eutetrarhynchus araya was collected in the new hosts Potamotrygon reticulatus and P. falkneri from the delta of the Orinoco River and the new locality of the Paraná River near Hohenau, Paraguay, respectively. Eutetrarhynchus baeri is a junior synonym of E. araya. Phylogenetic analysis suggests that E. araya is a member of a monophyletic group of species, including E. thalassius, E. caribbensis, E. schmidtii, and E. geraschmidti.

Potamotrygonocestus represents a monophyletic group of three known species of uncertain affinities with other tetraphyllidean cestodes. Potamotrygonocestus amazonensis was collected in the new host Potamotrygon yepezi from the Rio Cachiri, Zulia, Venezuela, a new locality. Phylogenetic analysis of the three species of Potamotrygonocestus suggests that P. amazonensis represents the plesiomorphic sister-species of the sister-species P. magdalenensis and P. orinocoensis. Potamotrygonocestus travassosi is considered a species inquirenda.

The four species of Acanthobothrium infecting freshwater stingrays form a monophyletic group most closely related to A. holarhini, A. cartagenensis, and A. urolophi. Acanthobothrium quinonesi is reported in the new host Potamotrygon yepezi from the new locality Rio Cachiri, Zulia, Venezuela.

Rhinebothrium paratrygoni was collected in the new hosts Potamotrygon hystrix and P. reticulatus from the new locality of the delta of the Orinoco River and in P. falkneri, another new host, from the Paraná River, near Hohenau, Paraguay, a new locality. Rhinebothrium paratrygoni belongs to a monophyletic group containing R. urobutidium, R. diesteticum, R. spinicephalum, R. tetralobatum, and a new species being described elsewhere.

Rhinebothroides represents a monophyletic group of six known species most closely related to Phyllobothrium kingae and its relatives. Rhinebothroides moralarai, R. circularis, and R. venezuelensis form one monophyletic group within the genus and R. glandularis, R. freitasi, and R. scorzai another. Rhinebothroides scorzai is reported from the new hosts Potamotrygon reticulatus and Elipesurus spicicaua from various localities in the delta of the Orinoco River.

The cestode fauna of potamotrygonids comprises elements with closest relationships to species infecting marine elasmobranchs and not freshwater fishes.

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Figure 1. Map showing localities from which helminth parasites infecting potamotrygonid stingrays have been reported. Closed circles represent localities reported by the present authors, open circles localities reported by other authors.

Prior to 1976, two reports listed a total of three cestode species infecting freshwater stingrays (family Potamotrygonidae) (Woodland, 1934; López-Neyra and Díaz-Ungría, 1958). Subsequently, four reports (Brooks and Thorson, 1976; Rego and Dias, 1976; Mayes et al., 1978; Rego, 1979; Mayes et al., 1981) have added an additional 10 species. This study presents descriptions of four new species collected in Venezuela and reviews the systematic status of the 14 presently known cestode species infecting potamotrygonids. Figure 1 shows localities from which potamotrygonid parasites have been reported.

Hosts were collected by seine, gig, or dipnet and dissected within 2 hr if taken alive, or immediately if collected dead. Worms were removed from host spiral valves, examined alive, then fixed with warm AFA and stored in 70% ethanol. Spiral valves were fixed with 10% formalin to preserve any overlooked helminths. Worms were stained with Delafield’s or Mayer’s hematoxylin and mounted in Canada balsam for study as whole mounts; serial cross sections of some speci-
mens cut at 8 \( \mu \text{m} \) and stained with hematoxylin-eosin were used to confirm some aspects of proglottid morphology. Measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube. The abbreviation USNM Helm. Coll. refers to the U.S. National Museum Helminthological Collection, Beltsville, Maryland; UNSM refers to the University of Nebraska State Museum, Lincoln, Nebraska.

**Trypanorhynchus Diesing, 1863**  
**Eutetrarhynchidae Guiart, 1927**  
**Eutetrarhynchus** Pintner, 1913

*Eutetrarhynchus araya* (Woodland, 1934) Rego and Dias, 1976


**HOSTS:** *Trygon* sp. (=*Potamotrygon* sp. or *Elipesurus* sp.); *Potamotrygon hystrix* (Müller and Troschel);\(^2\) *P. motoro* (Müller and Henle); *P. reticulatus* (Gunter), new host;\(^2\) *P. falkneri*, new host.

**LOCALITIES:** Itacuai River, Brazil; delta of Orinoco River, Venezuela; Rio Salobra, Mato Grosso, Brazil; delta of Orinoco River, Venezuela, near El Toro and at km 82 of main channel, new localities; Paraná River, Paraguay, near Hohenau, new locality.

**SITE OF INFECTION:** Middle ½ of spiral valve.

**SPECIMENS EXAMINED:** UNSM No. 21011.

Rego and Dias (1976) described trypanorhynch specimens collected in *Potamotrygon motoro* from the Rio Salobra, Mato Grosso, Brazil. They concluded that their specimens, those described by Woodland (1934) from “*Trygon* sp.” (“brown specimen speckled with black” 45 cm in disc length) in the Amazon River, and *Eutetrarhynchus baeri* López-Neyra and Diaz-Ungriá, 1958, from *P. hystrix* in the delta of the Orinoco River were conspecific. Based on our collections from the Orinoco and from the Paraná, we concur with Rego and Dias (1976). The tentacular armature of our specimens is identical to that reported by Rego and Dias and different from that reported by Woodland or López-Neyra and Diaz-Ungriá, who gave only cursory sketches. Further, our specimens possess 300–400 testes per proglottid but they lie in two layers in the parenchyma; Woodland (1934) noted that the “over 200” testes in his specimens also lay in two layers. López-Neyra and Diaz-Ungriá reported 150–200 testes per proglottid for *E. baeri*, but they may have interpreted their specimens as having cylindrical

\(^2\) Our identifications of *P. hystrix* and *P. reticulatus* must be considered tentative pending a taxonomic revision of the potamotrygonids. According to Dr. Reeve Bailey, University of Michigan (personal communication), those specimens we identify as *P. hystrix* may actually be *P. humboldti*, if *P. hystrix* (s.s.) is found to be endemic to the Río de la Plata. Further, our specimens identified as *P. reticulatus* may represent a single species which has been called *P. reticulatus*, *P. bruni*, or *P. brachyurus*. Rego and Dias (1976) listed *Elipesurus* sp. as one host. *Elipesurus* has been used to refer to members of *Potamotrygon* as well as to *Elipesurus spinicauda*. The latter species, one specimen of which we examined in Venezuela, is apparently identical with *Disceus thayeri*. Specimens of hosts we examined have been deposited in the U.S. National Museum of Natural History, Washington, D.C., for future reference.
tetrotyrphynchus araya most closely resembles E. thalassius Kovacs and Schmidt, 1980, and E. caribbensis Kovacs and Schmidt, 1980, which infect Urolophus jamaicensis Cuvier in Jamaica. The above species possess rosethorn-shaped hooks in the metabasal armature, a trait no other species in Eutetra rhynchus exhibit. Those of E. araya are positioned in an overlapping longitudinal row of eight hooks, whereas in the other species the rosethorn-shaped hooks are either nonoverlapping (E. caribbensis) or scattered (E. thalassius).

Only two other members of Eutetra rhynchus exhibit overlapping hook rows and similar holdfast proportions—E. schmidtii Heinz and Dailey, 1974, and E. geraschmidtii Dollfus, 1974. The former species exhibits a glandular apical organ found in E. araya, E. thalassius, and E. caribbensis but lacking in E. geraschmidtii or other members of the genus. Figure 2 depicts the distribution of the above special traits among the five species by means of a cladogram (cf. Hennig, 1966).

Tetraphyllidea Carus, 1863
Onchobothriidae Braun, 1900
Potamotrygonocestus Brooks and Thorson, 1976
Potamotrygonocestus orinocoensis sp. n.
(Figs. 3–4)

DESCRIPTION (based on 11 specimens): Strobila hyperapolytic, acraspedote, 1.3–1.6 mm long, composed of 5–10 proglottids. Scolex 153–230 long by 204–220 wide, composed of 4 simple bothridia each armed with pair of simple hooks and
surmounted by apical portion of bothridium modified as accessory suckerlike structure 51–61 in diameter. Bothridia 240–265 long by 71–107 wide. Hooks dissimilar in shape (Fig. 3); outer hook base 30–50 (\( \bar{x} = 42, n = 17 \)) long, prong 73–108 (\( \bar{x} = 92, n = 17 \)) long; inner hook base 25–48 (\( \bar{x} = 37, n = 17 \)) long, prong 80–125 (\( \bar{x} = 104, n = 17 \)) long. Scolex and extremely short neck spinose; spines up to 8 long. Detached mature proglottids 714–1,020 long by 235–296 wide. Testes in 2 single file lines in anterior 3/4 of proglottid, 8–14 (\( \bar{x} = 10, n = 15 \)) porally, 9–14 (\( \bar{x} = 11, n = 15 \)) aporally, 17–25 (\( \bar{x} = 21, n = 15 \)) in number; 41–66 in diameter. Cirrus sac lateral to ovary, 77–153 long by 31–46 wide, containing spined eversible cirrus up to 107 long. Ovary in posterior 1/4 of proglottid, theta (\( \theta \)) shaped, 214–311 long by 112–143 wide at isthmus. Vagina anterior to cirrus sac, vaginal sphincter lacking. Genital pore 68–77% of proglottid length from anterior end. Vitellaria composed of 2 lateral compact lines extending from 20–28% of proglottid length from anterior end to anterior margin of ovary, 32–41% of proglottid length from posterior end. Gravid proglottids not collected.

**HOST:** *Potamotrygon reticulatus.*

**SITE OF INFECTION:** Anteriormost chamber of spiral valve.

**LOCALITIES:** Delta of Orinoco River, Venezuela, near El Toro (type) and at km 82 of main channel.

**HOLOTYPE:** USNM Helm. Coll. No. 75713.

Etymology: The species is named for the Orinoco River, in which it was collected.

Potamotrygonocestus orinocoensis differs from the other two members of the genus by having hook prongs joining their bases at one end rather than in the middle. The new species' hooks are also larger than those of P. amazonensis Mayes, Brooks, and Thorson, 1981 (58–78 μm long) or P. magdalenensis Brooks and Thorson, 1976 (43–45 μm long). Its ovarian shape is unique. Potamotrygonocestus orinocoensis most closely resembles P. magdalenensis by having compact rather than follicular vitellaria and by lacking a genital atrium.

Potamotrygonocestus magdalenensis Brooks and Thorson, 1976

Host: Potamotrygon magdalenae Dumeril.

Site of infection: Anteriormost chamber of spiral valve.

Locality: Magdalena River and associated cienagas near San Cristóbal, Bolívar, Colombia.

Specimens examined: USNM Helm. Coll. No. 73542 (holotype) and 73543 (paratype); UNSM No. 20254 (paratypes).


Potamotrygonocestus amazonensis Mayes, Brooks, and Thorson, 1981

Hosts: Potamotrygon circularis Garman; P. yepezi, Castex and Castello, 1970, new host; P. reticulatus, new host.

Site of infection: Anteriormost chamber of spiral valve.

Localities: Itacuá River, Brazil, 5 km south Atalaia do Norte, Brazil (type); Represa de Tulé, Río Cachiri, Zulía, Venezuela, new locality; Orinoco River delta, Venezuela, near km 82 of main channel, new locality.

Specimens examined: USNM Helm. Coll. No. 76363 (holotype); UNSM No. 21019 (paratypes).


Potamotrygonocestus travassosi Rego, 1979, species inquirenda

Host: Potamotrygon hystrix.

Site of infection: Spiral valve.

Locality: Amazon River, Maicuru, Para, Brazil.

Specimens examined: None.

Rego (1979) described this species on the basis of “... dois proglotes livres e un plerocercide ... .” Examination of Rego’s Figure 6 suggests that the free proglottids belong to Eutetrarhynchus araya; Figures 4 and 5 clearly depict the scolex of a species of Potamotrygonocestus. However, Figure 4 illustrates bothridial hook morphology similar to that exhibited by P. amazonensis, whereas Figure 5 depicts a single hook appearing more similar to those of P. orinocoensis.
We, therefore, consider *P. travassosi* a *species inquirenda* pending further collections of material from Maicuru.

**Key to Species of *Potamotrygonocestus***

1a. Maximum bothridial hook length less than 80 \( \mu \)m, hook prongs join bases near middle .................................................. 2

1b. Maximum bothridial hook length more than 100 \( \mu \)m, prongs join bases at end ........................................................................................................... *orinocoensis*

2a. Vitellaria compact, hooks 43–55 \( \mu \)m long ........................................... *magdalenensis*

2b. Vitellaria follicular, hooks 58–78 \( \mu \)m long ........................................... *amazonensis*

**Phylogenetic Relationships**

*Potamotrygonocestus* species exhibit perhaps the most simplified scolex morphology of any onchobothriid tetraphyllideans, possessing simple hooks and non-septate bothridia. Members of *Potamotrygonocestus* are unusual because they exhibit posterolateral or postovarian genital pores and terminal genitalia. The three species of this monophyletic group may be related phylogenetically in one of four ways. The most parsimonious, based on the following four characters, is depicted in Figure 5.

1. Bothridial hook length. 0 = maximum bothridial hook length up to 78 \( \mu \)m; 1 = maximum hook length up to 125 \( \mu \)m; \(-1\) = maximum hook length less than 60 \( \mu \)m.

2. Ovarian shape. 0 = inverted “A”; 1 = theta-shaped.

3. Hook prong attachment. 0 = prongs join bases near middle; 1 = prongs join bases at one end.

4. Vitelline configuration. 0 = vitellaria follicular; 1 = vitellaria compact.

The above characters support the phylogenetic hypothesis that *P. orinocoensis*
and *P. magdalenensis* are more closely related to each other than either is to any other species on the basis of relative recency of common ancestry.

*Acanthobothrium Van Beneden, 1849
*Acanthobothrium regoi* sp. n.
(Figs. 6–8)

**Description** (based on 5 complete and 6 fragmented specimens): Strobila acraspedote, apolytic, up to 45 mm long, composed of 87–120 proglottids. Scolex 700–900 long by 800–1,100 wide, composed of 3 triloculate bothridia each armed with pair of bifid hooks and surmounted by apical sucker and pad. Posterior margins of bothridia attached to scolex; velum lacking. Bothridia 500–600 long by 300–350 wide; ratio of length to width 1:0.6–0.7 (\(\bar{x} = 1:0.64, n = 20\)). Anterior loculus 200–220 long, middle loculus 80–100 long, posterior loculus 100–130 long; average ratio of locular lengths 1:0.45:0.55. Apical sucker 61–102 in diameter, pads 126–179 in diameter. Hook formula (modified from that of Euzet, 1956) for 30 hooks:

\[
\begin{array}{cccc}
31–41 (35) & 87–128 (105) & 66–82 (75) \\
122–163 (142)
\end{array}
\]


**Host:** *Potamotrygon hystrix.*

**Site of infection:** Middle \(\frac{1}{2}\) of spiral valve.

**Localities:** Orinoco River delta, Venezuela, near Curiapo (type); Orinoco River near Los Castillos, Venezuela.

**Holotype:** USNM Helm. Coll. No. 75709.

**Paratypes:** USNM Helm. Coll. No. 75710; UNSM No. 21012, 21013.

**Etymology:** This species is named in honor of Dr. A. Arandas Rego, Instituto Oswaldo Cruz, Rio de Janeiro, in recognition of his contributions to our knowledge of Neotropical cestodes.

By possessing recurved cirrus sacs, *Acanthobothrium regoi* most closely resembles *A. quinonesi* Mayes, Brooks, and Thorson, 1978, infecting *Potamotrygon magdalenae* in the Magdalena River of Colombia. The new species differs by having a larger scolex (800–1,100 \(\mu\)m wide vs. 460–620 \(\mu\)m wide), more pro-
Acanthobothrium regoi.


The four species of Acanthobothrium parasitizing freshwater stingrays form a monophyletic group characterized by a special (uniquely derived) bothridial hook dimorphism (Fig. 7), H-shaped ovaries, postequatorial genital pores, and scoleces with sessile bothridia and expanded necks. Acanthobothrium terezae Rego and Dias, 1976, differs greatly from the other species in testes number, scolex size, and bothridial hook size. Characters distinguishing the other three species are slight but consistent. We consider them indicative of specific differences but if they are not, because of their consistency, the variations delimit morphotypes relating either to host-induced variation or to geographic (subspecific) variation. However, cestodes collected in three localities (Magdalena River, Lake Maracaibo area, and lower Orinoco River) from three different hosts (Potamotrygon...
Table 1. Comparison of selected morphological characters among four species of *Acanthobothrium* infecting South American freshwater stingrays. The superscript ¹ refers to specimens of *A. quinonesi* collected in the Magdalena River of Colombia whereas² refers to those collected in the Maracaibo area of Venezuela.

<table>
<thead>
<tr>
<th>Character</th>
<th>terezae</th>
<th>quinonesi¹</th>
<th>quinonesi²</th>
<th>amazonensis</th>
<th>regoi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scolex width</td>
<td>2,000–3,000</td>
<td>508–620</td>
<td>460–620</td>
<td>612–790</td>
<td>800–1,100</td>
</tr>
<tr>
<td>Number of proglottids</td>
<td>200–260</td>
<td>55–75</td>
<td>50–60</td>
<td>75–100</td>
<td>87–120</td>
</tr>
<tr>
<td>Strobilar length (mm)</td>
<td>88–110</td>
<td>up to 25</td>
<td>up to 25</td>
<td>up to 35</td>
<td>up to 45</td>
</tr>
<tr>
<td>Total bothridial hook length</td>
<td>range</td>
<td>180–326</td>
<td>100–142</td>
<td>100–140</td>
<td>145–184</td>
</tr>
<tr>
<td></td>
<td>mean (x)</td>
<td>253</td>
<td>118</td>
<td>120</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>mean (x)</td>
<td>120–140</td>
<td>43–60</td>
<td>50–55</td>
<td>50–72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130</td>
<td>52</td>
<td>52</td>
<td>62</td>
</tr>
<tr>
<td>Position of genital pores</td>
<td>post-eq.</td>
<td>post-eq.</td>
<td>post-eq.</td>
<td>post-eq.</td>
<td>post-eq.</td>
</tr>
<tr>
<td>Ovarian shape</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Presence or absence of spines</td>
<td>absent</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
</tr>
</tbody>
</table>

*magdalenae, P. yepezi, and P. hystrix* all uniformly possess recurved cirrus sacs. Therefore, we consider *A. amazonensis* Mayes, Brooks, and Thorson, 1978, from *Potamotrygon circularis* in the upper Amazon River drainage, with straight cirrus sacs, distinct. Likewise, because specimens from *P. magdalenae* in Colombia and *P. yepezi* in the Lake Maracaibo area exhibit very similar scolex sizes, apical sucker diameters, bothridial hook sizes, and proglottid numbers, we consider them distinct from specimens parasitizing *P. hystrix* in the Orinoco. Table 1 presents a comparison for all species of *Acanthobothrium* infecting potamotrygonids showing pertinent characters.

**Acanthobothrium terezae** Rego and Dias, 1976

HOSTS: *Potamotrygon motoro* (as *Paratrygon m.*); *Elipesurus* sp.

SITE OF INFECTION: Spiral valve.

LOCALITY: Rio Salobra, Mato Grosso, Brazil.

SPECIMENS EXAMINED: None.

DIAGNOSTIC FEATURES: See Table 1.

**Acanthobothrium quinonesi** Mayes, Brooks and Thorson, 1978

HOSTS: *Potamotrygon magdalenae* (type); *P. yepezi*, new host.

SITE OF INFECTION: Middle ⅔ of spiral valve.

LOCALITIES: Magdalen River and associated cienagas near San Cristóbal, Bolívar, Colombia; Lake Maracaibo area near El Congo and Represa de Tulé, Rio Cachiri, Zulia, Venezuela, new localities.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 74804 (holotype) and 74805 (paratypes); UNSM No. 20563 (originally listed as 74806) (paratypes) and 21021, 21022, 21023 (voucher specimen from *P. yepezi*).
Diagnostic features: See Table 1. Mayes et al. (1978) reported that this species lacked tegumental spines, but small spines were discovered upon reexamination of the type material. Venezuelan specimens tend to have follicular ovaries, whereas Magdalenean ones have compact ovaries.

**Acanthobothrium amazonensis** Mayes, Brooks, and Thorson, 1978

**Host:** *Potamotrygon circularis* Garman.

**Site of infection:** Middle 1/3 of spiral valve.

**Locality:** Itacuai River, 5 km south of Atalaia do Norte, Brazil.

**Specimens examined:** USNM Helm. Coll. No. 74806 (holotype) and 74807 (paratype); UNSM No. 20562 (paratype).

**Diagnostic features:** See Table 1.

**Key to Species of Acanthobothrium Infecting Freshwater Stingrays**

1a. Posterior end of cirrus sac recurved ........................................ 2
1b. Posterior end of cirrus sac straight ........................................ 3
2a. Bothridial hooks averaging 142 μm in total length, scolices 800-1,100 μm wide ........................................ regoi
2b. Bothridial hooks averaging 118-120 μm in total length, scolices 460-620 μm wide ........................................ quinonesi
3a. Testes averaging 62 in number, bothridial hooks averaging 168 μm in total length ........................................ amazonensis
3b. Testes averaging 130 in number, bothridial hooks averaging 253 μm in total length ........................................ terezae

**Phylogenetic Relationships**

We noted four possible cladograms representing the phylogenetic relationships of the three species of *Potamotrygonocestus*; for the four species of *Acanthobothrium* infecting potamotrygonids the number of possible cladograms is 26. However, we prefer the cladogram depicted in Figure 9, which is most consistent with the characters listed below.

1. Shape of cirrus sac. 0 = straight; 1 = recurved.
2. Strobilar spination. 0 = lacking; 1 = present.
3. Bothridial hook size. 0 = total hook length averaging 140–170 μm; 1 = total hook length averaging 118–120 μm; −1 = total hook length averaging 253 μm.
4. Number of proglottids. 0 = 75–120; 1 = 50–75; −1 = 200–260.

**Phyllobothriidae Braun, 1900**

**Rhinebothrium Linton, 1889**

**Rhinebothrium paratrygoni** Rego and Dias, 1976

**Hosts:** *Elipesurus* sp. (type); *Potamotrygon hystrix, P. reticulatus; P. falkneri,* new hosts.

**Site of infection:** Middle 1/3 of spiral valve.

**Localities:** Rio Salobra, Mato Grosso, Brazil (type); Orinoco River delta, near Curiaçó and near km 82 of main channel, Venezuela, new localities; Paraná River, Paraguay, near Hohenau, new locality.

**Specimens examined:** UNSM No. 21010 (voucher specimens from Orinoco) and 21016 (voucher specimens from Paraná).
Our specimens agree closely with those described by Rego and Dias (1976). They reported that proglottids generally contained five and occasionally contained six testes; we found four to eight testes with a mean of five (n = 50). Rego and Dias were not able to provide an accurate count of bothridial loculi, but our specimens exhibited 72–76 loculi arranged as follows: bothridia comprising two lobes separated by a horizontal hingelike constriction; bothridia with 36–38 horizontal septa divided by a median longitudinal septum forming 72–76 total loculi. Rego and Dias compared *R. paratrygoni* with *R. maccallumi* Campbell, 1970, *R. lintoni* Campbell, 1970, *R. walga* Euzet, 1956, and *R. minimum* Euzet, 1956. We do not believe that any of those species are closely related to *R. paratrygoni*. Rather, five other species and *R. paratrygoni* form a monophyletic group characterized by being small worms with relatively to markedly long cephalic peduncles, more than 25 proglottids per strobila, craspedote proglottids which are wider than long (except terminal proglottids), bilobed bothridia with a single median longitudinal septum and at least 32 loculi, and an average of fewer than 10 testes per proglottid. Those species include *R. urobatidium* (Young, 1955) Appy and Dailey, 1978, *R. spinicephalum* Campbell, 1969, *R. tetralobatum* Brooks, 1977, *R. diesticulum* Appy and Dailey, 1978, and a new species being described elsewhere, in addition to *R. paratrygoni*. The phylogenetic relationships of those five species, depicted in Figure 10, were estimated using the following characters and their coded states.

1. Testes number. 0 = 6–12 (\(\bar{x} = 8\)); 1 = 4–8 (\(\bar{x} = 5\)); 2 = 3–6 (\(\bar{x} = 4\)); 3 = 2.
3. Relative length of cephalic peduncle. 0 = short; 1 = long.
4. Ovarian morphology. 0 = compact; 1 = fragmented.
Figure 10. Cladogram depicting phylogenetic relationships of *Rhinebothrium paratrygoni* and its closest relatives.

The most parsimonious arrangement of those six species based on the above characters indicates a parallel reduction in testes number to a total of two (*R. ditesticulum* and *R. spinicephalum/R. tetalobatum*) and a reduction in bothridial loculi number following a trend of increasing loculi numbers, a form of evolutionary reversal (*R. spinicephalum*).

Rego and Dias (1976) noted the presence of a very long cephalic peduncle in *Rhinebothrium paratrygoni* and suggested that the trait invalidated the generic concept of *Caulobothrium* Baer, 1948. The critical characteristic separating *Caulobothrium* species from *Rhinebothrium* species is the presence of postvaginal testes in *Caulobothrium* and their absence in *Rhinebothrium*. In fact, three species of *Caulobothrium*—*C. anacolum* Brooks, 1977, from *Himantura schmardae* in Colombia, *C. myliobatidis* Carvajal, 1977, from *Myliobatis chilensis* in Chile, and *C. multorchidum* (Young, 1955) Appy and Dailey, 1978, from *Urolophus halleri* in California—all possess short cephalic peduncles. Appy and Dailey (1978) treated the generic status of *Rhinebothrium, Caulobothrium,* and *Rhabdobotrium* Baer, 1948. They observed that *Rhinebothrium* and *Caulobothrium* appeared valid because they retained their membership intact without regard for relative peduncle length, but that *Rhabdobotrium*, which differs from *Caulobothrium* by lacking any cephalic peduncles, might not be justifiably distinct. We concur with Appy and Dailey, recognizing *Rhinebothrium* for those species lacking postvaginal testes and *Caulobothrium* for those species possessing postvaginal testes. We consider the relative length of the cephalic peduncle a plastic trait exhibiting wide variation among the representatives of both genera.

**Rhinebothroides Mayes, Brooks, and Thorson, 1981**

**Rhinebothroides glandularis** sp. n.

(Figs. 11–14)

**Description** (based on 20 specimens): Strobila craspedote, apolytic, up to 50 mm long, composed of 25–30 proglottids. Scolex with 4 pedicellated, bilobed, squared bothridia; rostellum lacking. Pedicels contractile, up to 50 long. Bothridia 610–714 long by 460–714 wide; indistinct hingelike constriction between lobes;
divided into marginal and medial portions by marginal septum; divided horizontally by 25–29 septa; medial loculi 51–59 in number; marginal loculi 51–59 in number. Immature proglottids wider than long; mature ones 1.6–1.9 mm long by 460–510 wide. Testes in 2 broad fields in anterior ½ of proglottid, 41–51 in number ($\bar{x} = 45, n = 50$); 40–61 in diameter. Cirrus sac in posterior ½ of proglottid, 331–408 long by 57–102 wide, surrounded by darkly staining cells lying free in the parenchyma, containing spined eversible cirrus and internal seminal vesicle. External seminal vesicle extending length of cirrus sac, joining cirrus sac near poral end and vas deferens near posterior end of proglottid. Genital atrium shallow; genital pores alternating irregularly in posterior ¼ of proglottid. Vagina anterior to cirrus sac, coiled; vaginal sphincter and seminal receptacle present. Ovary bilobed in frontal view, X-shaped in cross section; aporal lobe 638–868 long, extending anteriorly to middle of proglottid; poral lobe extending anteriorly to middle of cirrus sac; 348–468 wide at isthmus. Vitelline follicles lateral, extending entire length of proglottid, not interrupted near genital pore; 20–32 in diameter. Gravid proglottids 1.90–2.35 mm long by 460–510 wide, devoid of gonads. Uterus saccate with 45–60 total lateral diverticula. Eggs 29–37 in diameter; oncospheres 27–31 in diameter, unembryonated in utero.

**Host:** *Potamotrygon hystrix.*

**Site of Infection:** Middle ½ of spiral valve.

**Locality:** Orinoco River delta near Curiape, Venezuela.

**Holotype:** USNM Helm. Coll. No. 75707.

**Paratypes:** USNM Helm. Coll. No. 75708; UNSM No. 21007.

**Etymology:** This species is named after its diagnostic feature, the parenchymal gland cells surrounding the terminal genitalia.

*Rhinebothroides glandularis* differs from all other members of the genus by possessing prominent gland cells surrounding the terminal genitalia. It resembles *R. scorzaii* more closely than any other species by having a coiled vagina, vitelline follicles uninterrupted near the genital pore, and poral ovarian lobes extending anterior to the posterior margin of the cirrus sac.

**Rhinebothroides venezuelensis** sp. n.

(Figs. 15–17)

**Description** (based on 25 specimens): Strobila craspedote, apolytic, up to 60 mm long, composed of 20–30 proglottids. Scolex with four pedicellated, bilobed, squared bothridia; rostellum lacking. Pedicels contractile, up to 300 long. Bothridia 360–720 long by 260–690 wide; indistinct hingelike constriction between lobes; divided into marginal and medial portions by marginal septum; divided longitudinally by indistinct median septum; divided horizontally by 25–26 septa; medial loculi 51–53 in number; marginal loculi 51–53 in number. Immature proglottids wider than long; mature ones 714–1,160 long by 210–357 wide. Testes in 2 broad fields in anterior ½ of proglottid, 45–64 ($\bar{x} = 53, n = 50$) in number, 40–80 in diameter. Cirrus sac in posterior ¼ of proglottid, 153–194 long by 31–51 wide, containing spined eversible cirrus and internal seminal vesicle. External seminal vesicle extending length of cirrus sac, joining cirrus sac near poral end, joining vas deferens near posterior end of proglottid. Genital atrium shallow; genital pores alternating irregularly in posterior ¼ of proglottid. Vagina anterior to cirrus sac, straight; vaginal sphincter and seminal receptacle present. Ovary
bilobed in frontal view, X-shaped in cross section. Aporal lobe 510–560 long, not extending into anterior ½ of proglottid; poral lobe extending anteriorly to posterior margin of cirrus sac; 123–160 wide at isthmus. Vitelline follicles lateral, extending entire length of proglottid, interrupted near genital pore; 20–40 in diameter. Gravid proglottids 1.53–3.05 mm long, devoid of gonads. Uterus saccate, with 80–100 total lateral diverticula. Eggs 19–24 in diameter; oncospheres 17–21 in diameter, unembryonated in utero.

**HOSTS:** *Potamotrygon hystrix* (type); *P. yepezi*.

**SITE OF INFECTION:** Middle ⅓ of spiral valve.

**LOCALITIES:** Orinoco River delta near Curiaipo, Venezuela (type); Orinoco River near Los Castillos, Venezuela; Lake Maracaibo area, near El Congo and Reserva de Tulé, Río Cachiri, Zulia, Venezuela.

**HOLOTYPE:** USNM Helm. Coll. No. 75705.

**PARATYPES:** USNM Helm. Coll. No. 75706; UNSM No. 21005, 21006.

**ETYMOLOGY:** This species is name for the Republic of Venezuela, the country in which it has been collected.

*Rhinebothroides venezuelensis* most closely resembles *R. moralarai* (Brooks and Thorson, 1976) Mayes, Brooks, and Thorson, 1981, and *R. circularisi* Mayes, Brooks, and Thorson, 1981, by having straight vaginae, vitelline follicles interrupted near the genital pore, and poral ovarian lobes extending anteriorly only to the posterior margin of the cirrus sac. The new species differs by having fewer testes (45–64) than *R. moralarai* (54–71) or *R. circularisi* (66–88) and by being craspedote rather than acraspedote. Specimens of *R. venezuelensis* from *Potamotrygon yepezi* in the Lake Maracaibo area differ from those collected in the Orinoco by having 45–64 (x = 54) rather than 45–55 (x = 51) testes per proglottid.

**Rhinebothroides scorzai** (López-Neyra and Diaz-Ungría, 1958)


**HOSTS:** *Potamotrygon hystrix* (type); *P. motoro*; *P. reticulatus*, new host; *Elipesurus* sp.; *Elipesurus spinicauda*, new host.

**SITE OF INFECTION:** Middle of ⅓ of spiral valve.

**LOCALITIES:** Orinoco River delta, Venezuela (type); Río Salobra, Mato Grosso, Brazil; Orinoco River delta, near El Toro, near km 82 of the main channel, and near Tucupita, Venezuela, new localities.

**SPECIMENS EXAMINED:** USNM Helm. Coll. No. 75704 (voucher specimen from *P. hystrix*); UNSM No. 21014 (voucher specimen from *E. spinicauda*), 21015 (voucher specimen from *P. reticulatus*).


Three reports (López-Neyra and Diaz-Ungría, 1958; Rego and Dias, 1976; present report), listing five hosts in five localities, exist for *R. scorzai*. Table 2 lists variability in testes number for *R. scorzai* based on those reports.
Rhinebothroides moralarai (Brooks and Thorson, 1976)
Mayes, Brooks, and Thorson, 1981

Rhinebothroides moralarai: Mayes, Brooks, and Thorson, 1981.

HOST: Potamotrygon magdalenae.
SITE OF INFECTION: Middle 1/3 of spiral valve.
LOCALITIES: Magdalena River, near San Cristóbal, Bolívar, and near La Dorada, Caldas, Colombia.
SPECIMENS EXAMINED: USNM Helm. Coll. No. 73544 (holotype) and 73545 (paratype); UNSM No. 20255 (incorrectly listed as 73546 in original description) (paratypes).

Rhinebothroides circularisi Mayes, Brooks, and Thorson, 1981

HOST: Potamotrygon circularis.
SITE OF INFECTION: Middle 1/3 of spiral valve.
LOCALITY: Itacuá River, Brazil, near Leticia, Colombia.
SPECIMENS EXAMINED: USNM Helm. Coll. No. 76361 (holotype) and 76362 (paratype); UNSM No. 21020 (paratype).

Rhinebothroides freitasi (Rego, 1979) comb. n.


HOST: Potamotrygon hystrix.
SITE OF INFECTION: Spiral valve.
LOCALITY: Amazon River, Maicuru, Para, Brazil.
SPECIMENS EXAMINED: None.

Key to Species of Rhinebothroides

1a. Vagina coiled, vitelline follicles not interrupted near genital pore, poral ovarian lobes extending anterior to posterior margin of cirrus sac 2
1b. Vagina straight, vitelline follicles interrupted near genital pore, poral ovarian lobes reaching anteriorly only to posterior margin of cirrus sac 4
2a. Strobila composed of 60–100 proglottids, testes 60–99 in number

    scorzai
Table 2. Comparison of range and mean testes numbers for specimens of *Rhinebothroides scorzai* collected from various freshwater stingrays in Venezuela and Brazil. Superscript ¹ refers to specimens reported by Lopez-Neyra and Díaz-Ungria (1958), ² to specimens reported by Rego and Dias (1977), ³ to specimens collected in the present study.

<table>
<thead>
<tr>
<th>Host</th>
<th>Range</th>
<th>Mean (±)</th>
<th>Sample size (n)</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potamotrygon hystrix¹</td>
<td>86–98</td>
<td>92 (est.)</td>
<td>—</td>
<td>Venezuela</td>
</tr>
<tr>
<td>Potamotrygon hystrix³</td>
<td>84–99</td>
<td>92</td>
<td>10</td>
<td>Venezuela</td>
</tr>
<tr>
<td>Potamotrygon reticulatus³</td>
<td>60–90</td>
<td>78</td>
<td>30</td>
<td>Venezuela</td>
</tr>
<tr>
<td>Potamotrygon motoro²</td>
<td>80–85</td>
<td>82.5 (est.)</td>
<td>—</td>
<td>Brazil</td>
</tr>
<tr>
<td>Elipesurus spinicauda³</td>
<td>78–90</td>
<td>82</td>
<td>10</td>
<td>Venezuela</td>
</tr>
<tr>
<td>Elipesurus sp.²</td>
<td>80–85</td>
<td>82.5 (est.)</td>
<td>—</td>
<td>Brazil</td>
</tr>
</tbody>
</table>

2b. Strobila composed of fewer than 35 proglottids, testes 41–64 in number .................................................. 3

3a. Prominent gland cells in parenchyma surrounding terminal genitalia, strobila craspedote .................................................. *glandularis*

3b. Vitellaria terminating at level of anteriormost extent of ovary, strobila acraspedote .................................................. *freitasi*

4a. Strobila craspedote, bothridial loculi 51–53 in number, testes 45–64 in number .................................................. *venezuelensis*

4b. Strobila acraspedote .................................................. 5

5a. Testes 54–71 in number, bothridial loculi 45–47 in number ............ *moralarai*

5b. Testes 66–68 in number, bothridial loculi 69–79 in number ............ *circularisi*

**Phylogenetic Relationships**

The six members of *Rhinebothroides* exhibit two traits possessed by no other tetraphyllideans presently known, their bothridial morphology and an internal seminal vesicle, which establishes them as a monophyletic group. The phylogenetic relationships of those six species (Fig. 18) were inferred using the following seven characters.

1. Vaginal morphology. 0 = vagina straight; 1 = vagina coiled.
2. Anterior extent of poral ovarian lobe. 0 = poral lobe extending anterior to posterior margin of cirrus sac; 1 = poral lobe reaching anteriorly only to posterior margin of cirrus sac.
3. Vitelline configuration. 0 = vitellaria not interrupted near genital pore; 1 = vitellaria interrupted near genital pore; −1 = vitellaria terminating preovarially.
4. Proglottid structure. 0 = acraspedote; 1 = craspedote.
5. Presence or absence of parenchymal gland cells surrounding terminal genitalia. 0 = lacking; 1 = present.
6. Testes number. 0 = 45–70; 1 = 60–100.
7. Bothridial loculi number. 0 = 69–79; 1 = 51–59; 2 = less than 50.

Study of numerous specimens of *Rhinebothroides*, including all five of the six known species, demonstrated that previous descriptions of bothridial morphology were incomplete. Bothridia are squared, bilobed, and hinged, with a median longitudinal septum and many horizontal septa forming numerous loculi as reported by Brooks and Thorson (1976) and Mayes et al. (1981). Two previously unreported
features include (1) the presence of an indistinct circular septum (best seen in immature specimens) inside the margin of the bothridium creating a ring of marginal loculi equal in number to the medial loculi and (2) the asymmetrical nature of the terminal loculi, there being two at one end and one at the other end of each bothridium (Fig. 19).

*Rhinebothroides* species resemble *Phyllobothrium kingae* Schmidt, 1978, and *Phyllobothrium cf. kingae* Brooks and Mayes, 1980, by having squared bothridia...
with indistinct horizontal septa and marginal loculi as well as proglottids with terminal genitalia situated near the ovary. The above-mentioned taxa differ from other phyllobothriid genera by virtue of those shared special traits. Members of *Rhinebothroides* have previously been classified with *Rhinebothrium* (López-Neyra and Diaz-Ungria, 1958; Brooks and Thorson, 1976; Mayes et al., 1981), with which they share many generalized features, including septate bothridia. However, at least 12 separate tetraphyllidean genera are characterized by septate bothridia; thus, the presence of septate bothridia in *Rhinebothroides* does not necessarily imply close relationship with *Rhinebothrium*. Indeed, special traits exhibited by *Rhinebothroides* suggest other affinities.

**Conclusions**

Potamotrygonid hosts a diverse cestode fauna which reflects their phylogenetic relationships with other elasmobranchs more than their ecological affinities with freshwater fishes. Both the Trypanorhynchida and Tetraphyllidea comprise species parasitic as adults in elasmobranchs, and are represented in the cestode fauna of potamotrygonids. On the other hand, no potamotrygonid examined thus far has hosted any members of the Proteocephalidea, a group of cestodes displaying its greatest diversity in South American freshwater fishes (Brooks, 1978). Each of the cestode taxa occurring in potamotrygonids comprises a monophyletic group, either a species-group (*Acanthobothrium*) or a distinct genus (*Potamotrygonocestus* or *Rhinebothroides*). These findings suggest that potamotrygonid cestodes are derived from a common ancestral helminth fauna and support the hypothesis that potamotrygonid themselves comprise a monophyletic group. A more thorough analysis of the evolutionary history of potamotrygonid parasites is being prepared by the authors.

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**Literature Cited**


Survey or Taxonomic Papers

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository such as the National Parasite Collection at Beltsville, Maryland and include the accession numbers in the manuscript.