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Monogenoidean parasites of freshwater stingrays (Rajiformes: Potamotrygonidae) from the Negro River, Amazon, Brazil: species of *Potamotrygonocotyle* (Monocotylidae) and *Paraheteronchocotyle* (Hexabothriidae)

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Abstract. Five new species of *Potamotrygonocotyle* (Monocotylidae) are described and *Paraheteronchocotyle amazonense* Mayes, Brooks et Thorson, 1981 (Hexabothriidae) is redescribed from monogenoideans collected on the gills of species of Potamotrygonidae from the Negro River, Amazon, Brazil. *Potamotrygonocotyle quadracotyle* sp. n. and *P. umbella* sp. n. were found parasitizing an undescribed species of *Potamotrygon*; *Potamotrygonocotyle rarum* sp. n. is described from *Potamotrygon schroederi*; *Potamotrygonocotyle rionegrense* inhabits *Potamotrygon* cf. *motoro*; *Potamotrygonocotyle aramasae* sp. n. is a parasite of *Paratrygon aiereba*; and *Paraheteronchocotyle amazonense* is reported from *Potamotrygon orbignyi*. The diagnosis of *Paraheteronchocotyle* is emended, and *P. amazonense* is redescribed.

Recent efforts to uncover the diversity of monogenoidean parasites of potamotrygonids have revealed the existence of a considerable number of derived lineages that have been overlooked for more than two decades, since the first descriptions of two species restricted to Neotropical freshwater stingrays by Mayes et al. (1981) (see Domingues and Marques 2007). As expected, the fauna of monogenoideans inhabiting potamotrygonids is represented by monocotylids and hexabothriids, groups commonly found on marine elasmobranchs, tracing the origin of the hosts, which are presumed to have been derived from a marine ancestor during marine incursions through the Miocene in the northern part of South America (Lovejoy et al. 1998, 2006, Marques 2000).

Although it is evident that the fauna of monogenoideans is somehow related to its marine counterparts, it seems early to address their relationships while we still know very little about the lineages that are now confined to the rivers of South America. Thus far, only six species of monogenoideans have been reported from potamotrygonids, five belonging to *Potamotrygonocotyle* Mayes, Brooks et Thorson, 1981 (Monocotylidae) and one to the monotypic *Paraheteronchocotyle* Mayes, Brooks et Thorson, 1981 (Hexabothriidae) (Mayes et al. 1981, Domingues and Marques 2007). Most of the diversity reported to date is found in the southern hydrographic basins of South America (i.e., Paraná / Paraguay / Uruguay), probably because that area has been targeted for monogenoideans from potamotrygonids (Domingues and Marques 2007). However, as we surveyed other river systems in South America we have found that some regions host a parallel diversity of monogenoideans inhabiting potamotrygonids.

Here, we address the fauna of monogenoidean parasites of potamotrygonids from the Negro River by describing five new species of *Potamotrygonocotyle* (Monocotylidae) and redescribing *Paraheteronchocotyle amazonense* found in this river. At least 450 species of fishes representing 41 to 46 families have been reported from the Negro River, a river known for its black and acid water and highly endemic fauna of fishes and parasites (Suriano 1985, Boeger and Kritsky 1988, Goulding et al. 1988, Van Every and Kritsky 1992, Kritsky et al. 1996, 1997, 1998, Kritsky and Boeger 2002). Among the large number of fishes reported from the Negro River, we found four nominal species of Potamotrygonidae: *Paratrygon aiereba* (Müller et Henle, 1841), *Potamotrygon motoro* (Müller et Henle, 1841), *P. schroederi* Fernández-Yépez, 1957, *P. orbignyi* (Castelnau, 1855), and at least one new species, which is under description (de Carvalho et al. 2003). Most of these species are also found in the adjacent Orinoco River Basin to which the Negro River is connected through the Cassiquiari (Goulding et al. 1988, Hubert and Renno 2006). Thus, we would not be surprised if the species reported from the Negro River would also be found in the Orinoco Basin.

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MATERIALS AND METHODS

Specimens of potamotrygonids were collected using spears, gill nets, or long lines from the Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), during January and February 2004. The gills were removed and placed in plastic bags containing heated (~65°C) 4% formaldehyde solution. Unstained helminths were mounted in Hoyer's or Gray & Wess medium to study sclerotized structures (Humason 1979). Whole mounts of monogenoideans were stained with Gomory's trichrome to determine internal features (Humason 1979). Measurements, all in micrometres, were performed following Mizelle and Klucka (1953), except for the measurements of the hexabothriid sucker sclerite that are represented by (1) length and width of the shaft and (2) length of the point (Fig. 41). The dimensions of organs and other structures represent the greatest measurement in dorsoventral view; lengths of curved or bent structures (anchors, male copulatory organ) represent straight line distances between extreme ends. The average measurements are followed by ranges and number of specimens measured (n) in parentheses. Illustrations were prepared with aid of a drawing tube on an Olympus BX-51 microscope with differential interference contrast optics. Specimens prepared for scanning electron microscopy were post fixed in 1% osmium tetroxide for 1–2 hours at room temperature, dehydrated in a graded ethanol series, critical point dried and sputter-coated with gold. Type specimens and vouchers were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil (CHIOC); Coleção Helmintológica do Museu de Zoologia da Universidade de São Paulo, São Paulo, SP, Brazil (MZUSP); Instituto de Pesquisas da Amazônia (INPA), Manaus, AM, Brazil; the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, NE, USA; the United States National Parasite Collection (USNPC), Beltsville, MD, USA; and the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (IPCR). The following museum specimens were examined: holotype, *Paraheteronchocotyle amazonense* (USNPC 77159); 2 paratypes and 1 paratype, *Paraheteronchocotyle amazonense* (USNPC 77160 and HWML 21391, respectively). Morphological terminology of monocotylids and hexabothriids follows Chisholm et al. (1995) and Boeger and Kritsky (1989), respectively.

DESCRIPTIONS

Polyonchoinea Bychowsky, 1937
Monocotylidae Taschenberg, 1879
Heterocotylinae Chisholm, Wheeler et Beverley-Burton, 1995
Potamotrygonocotyle Mayes, Brooks et Thorson, 1981

Potamotrygonocotyle quadracotyle sp. n.

Figs. 1–11

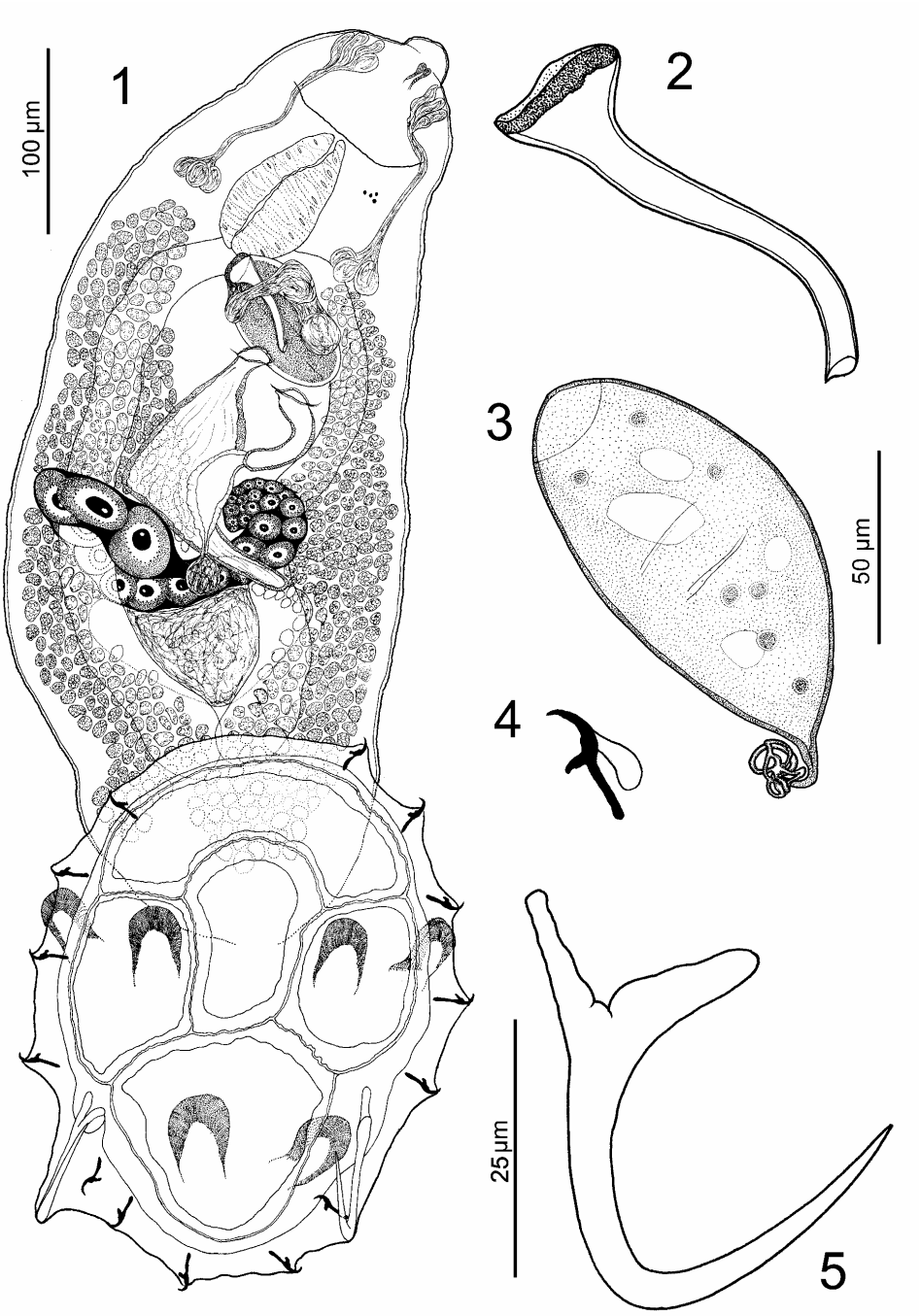
Description (based on 37 specimens): Body fusiform, total length excluding haptor 273 (220–380; n = 11); 130 (100–180; n = 12) wide at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs converging to unique pore (Fig. 8); cephalic glands unicellular, posterolateral to pharynx, with rod-shaped secretion. Anteromedial gland not observed. Dispersed pigment granules laterodorsally to pharynx, infrequently absent. Mouth surrounded by sclerotized ridges (Fig. 9). Pharynx elongate ovate, 37 (28–48; n = 12) long, 25 (n = 12) wide; oesophagus short; two intestinal caeca, nonconfluent, partially overlapping, lacking diverticula. Haptor subhexagonal, 134 (113–160; n = 13) long, 110 (95–125; n = 12) wide, with one central and four peripheral loculi (one anterior, two lateral, one posterior) (Fig. 10); septa ventrally surrounded by slightly sinuous sclerotized ridge (Fig. 11). Dorsal surface of haptor with 2 pairs of haptoral accessory structures associated with lateral and posterior peripheral loculi; each dorsal haptoral accessory structure with sclerotized margins. Anterior pair of dorsal haptoral accessory structures bilobate, with well separated lobes; each lobe elongate, posterior pair of dorsal haptoral accessory structures elongate (Fig. 7). Anchors 42 (36–47; n = 21) long, base 23 (20–25; n = 10) wide, with heavy diverging roots, evenly curved shaft and point. Hooks similar, 11 (10–14; n = 162) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ sclerotized, long straight slightly tapered tube, 36 (30–44; n = 6) long, distal portion sinuous, aperture terminal; base with small proximal flap. Accessory piece absent. Testis transversally ovate, 32 (30–35; n = 8) long, 57 (43–73; n = 8) wide; vas deferens not observed; seminal vesicle C-shaped, entering dorsal surface of posterior region of ejaculatory bulb. Ejaculatory bulb muscular, ovate without distinct internal chambers; glands associated with ejaculatory bulb not observed. Germarium tubular, unbranched, looping right intestinal caecum; distal end ascendant; Mehlis' glands not observed. Vagina muscular. Vaginal pore sinistroventral at level of common genital pore; seminal receptacle spherical. Oötype well developed. Vitellaria coextensive with gut, absent in regions of reproductive organs. Egg ovate, 126 (120–133; n = 4) long, 59 (43–65; n = 4) wide, with short filament; distal end of filament reticulate. For comparative measurements see Table 1.

Holotype, type host, type locality: MZUSP 6367; *Potamotrygon* sp., Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), 23 January 2005.

Other specimens: 36 paratypes; 18 MZUSP 6368a–r, 4 CHIOC 36883a–d, 4 HWML 48537–48540, 5 INPA 508a–e, 4 USNPC 99793, 1 IPCR M-451; Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), January and February 2005.

Site of infection: Gills.

Etymology: The specific name refers to the presence of four peripheral loculi in the haptor.

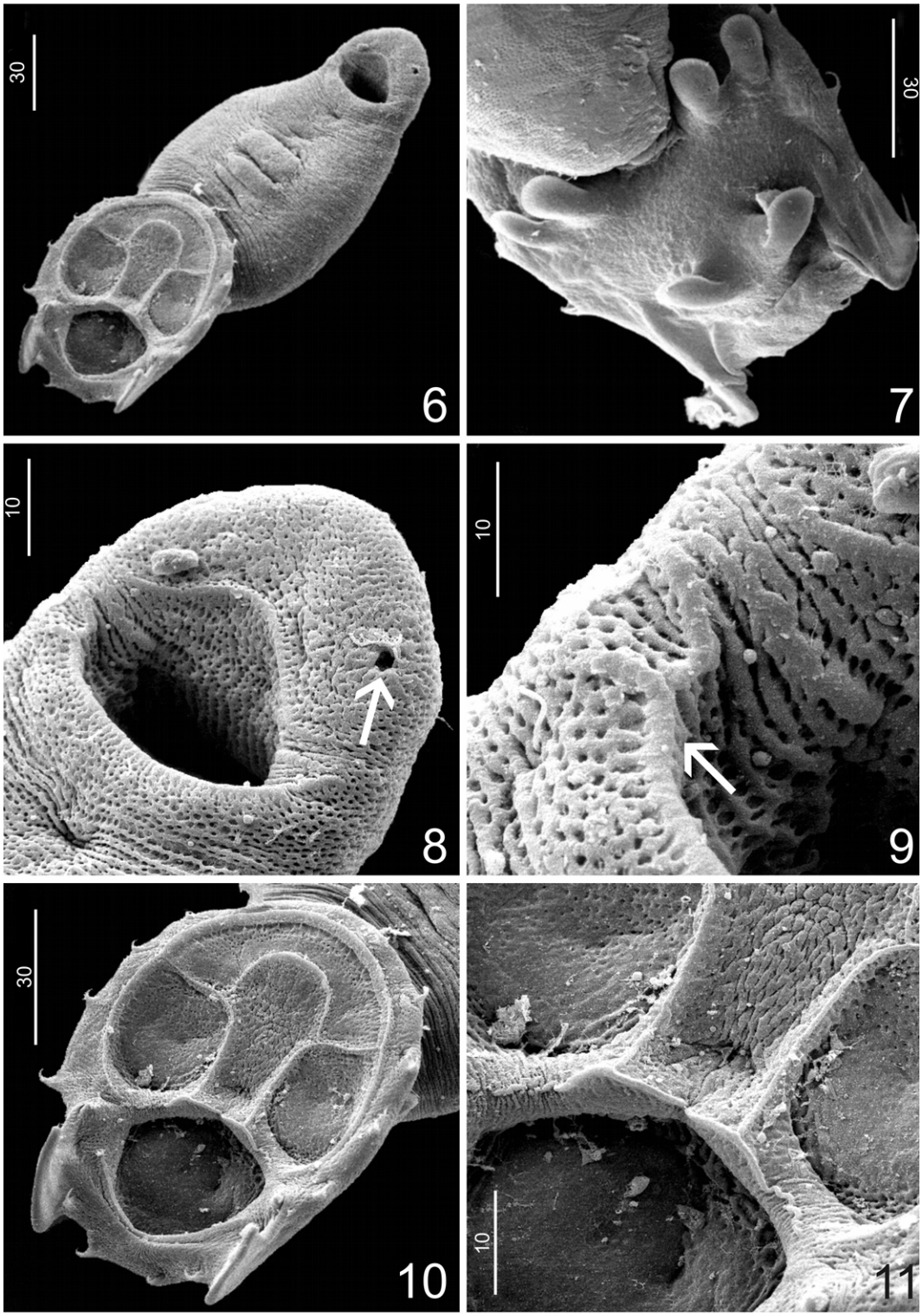


Figs. 1–5. *Potamotrygonocotyle quadracotyle* sp. n. **Fig. 1.** Whole mount (holotype). **Fig. 2.** Male copulatory organ. **Fig. 3.** Egg. **Fig. 4.** Hook. **Fig. 5.** Anchor.

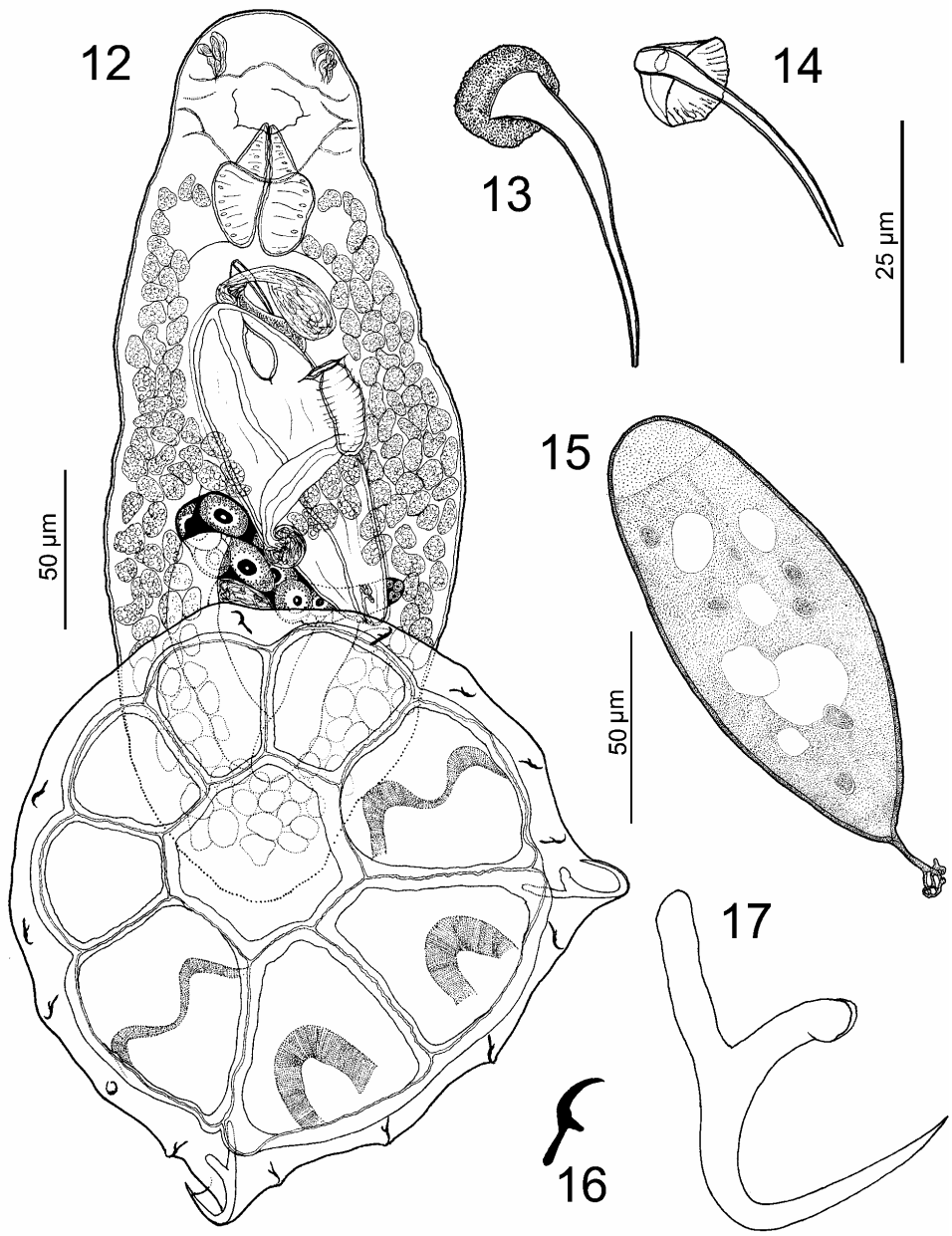
Remarks. *Potamotrygonocotyle quadracotyle* differs from its congeneric species by having a haptor with one central and four peripheral loculi instead of one central and eight peripheral loculi (Figs. 1, 6, 10). This character is apparently derived within the genus and represents a putative autapomorphy for *P. quadracotyle*. Future discoveries of more species of this genus may justify erection of a separate genus for *P. quadracotyle* based in part on the presence of a haptor with one central and four peripheral loculi.

Potamotrygonocotyle umbella sp. n. Figs. 12–17, 34

Description (based on 24 specimens): Body fusiform, total length excluding haptor 251 (200–300; n = 14); 107 (80–130; n = 14) wide; widest at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs converging to unique pore; cephalic glands posterolateral to pharynx. Anteromedial gland not observed. Pigment granules usually absent, infrequently present in cephalic, trunk



Figs. 6–11. General morphology of structures of *Potamotrygonocotyle quadracotyle* sp. n., scanning electron micrographs. **Fig. 6.** Whole specimen ventral view. **Fig. 7.** General morphology of dorsal haptoral accessory structures. **Fig. 8.** Detail of anterior region; arrow shows the single pore of head organs opening on left side of the head. **Fig. 9.** Detail of anterior region; arrow shows the sclerotisation around the mouth. **Fig. 10.** Ventral view of haptor showing loculi. **Fig. 11.** Detail of haptoral septa. Scale bars in μm.



Figs. 12–17. *Potamotrygonocotyle umbella* sp. n. **Fig. 12.** Whole mount (composite drawing). **Fig. 13.** Male copulatory organ. **Fig. 14.** Male copulatory organ (bent). **Fig. 15.** Egg. **Fig. 16.** Hook. **Fig. 17.** Anchor.

regions. Mouth surrounded by slight sclerotisations. Pharynx elongate ovate, 38 (35–40; n = 13) long, 28 (25–30; n = 13) wide; oesophagus short; two intestinal caeca, nonconfluent, partially overlapping, lacking diverticula. Haptor circular, haptoral disc approximately 2/3 of body length, 169 (155–180; n = 12) long, 169 (135–190; n = 12) wide, with one central and eight peripheral loculi (two anterior, four lateral, two posterior); septa ventrally surmounted by slightly sinuous sclerotized ridge. Dorsal pairs of haptoral accessory structures associated with the four posterior peripheral loculi; each pad with sclerotized margins. Anterior pair of dorsal haptoral accessory structures bilobate with divergent rounded lobes, each anterior pad with slightly concave separation between lobes, posterior pair of dorsal haptoral accessory structures elongate (Fig. 34). Anchors 37 (34–39; n = 12) long, base 15 (14–17; n = 11) wide, with heavily diverging roots, evenly curved shaft and point. Hooks similar, 10 (8–11; n = 36) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ sclerotized, arcuate, tapered tube, 36 (34–38; n = 5) long, distal portion acute, aperture terminal. Accessory piece absent. Testis transversal ovate, 41 (33–50; n = 14) long, 53 (48–68; n = 13) wide; vas deferens not observed; seminal vesicle sigmoid, entering dorsally at posterior region of ejaculatory bulb. Ejaculatory bulb muscular, ovate without

distinct internal chambers; glands associated with ejaculatory bulb not observed. Germarium tubular, unbranched, looping right intestinal caecum; distal end transversal to body; Mehlis' glands not observed. Vagina and vaginal canal muscular. Vaginal pore sinistroventral at level of common genital pore; seminal receptacle spherical. Oötype well developed. Vitellaria coextensive with gut, absent in regions of reproductive organs. Egg ovate, 121 (110–130; n = 9) long, 49 (43–58; n = 8) wide, with short filament; distal end reticulate. For comparative measurements see Table 1.

Holotype, type host, type locality: MZUSP 6369; *Potamotrygon* sp., Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), 23 January 2005.

Other specimens: 23 paratypes; 11 MZUSP 6370a–k, 2 CHIOC 36884a–b, 2 HWML 48541–48542, 5 INPA 509a–e, 2 USNPC 99794, 1 IPCR M-452; Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), January and February 2005.

Site of infection: Gills.

Etymology: The specific name is from Latin (*umbella* = an umbrella) and refers to the morphology of the haptoral disc.

Remarks. *Potamotrygonocotyle umbella* closely resembles *P. quadracotyle* in comparative morphology of the posterior pair of dorsal haptoral accessory structures. Features distinguishing this species from *P. quadracotyle* and other congeners include presence of an arcuate male copulatory organ, tapered tube with distal acute tip, and a haptoral disc approximately 2/3 of the body length.

Potamotrygonocotyle rarum sp. n. Figs. 18–21, 35

Description (based on 6 specimens): Body fusiform, total length excluding haptor 460 (430–490; n = 2); 195 (180–210; n = 2) wide; widest at the level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs converging to unique pore; cephalic glands posterolateral to pharynx. Anteromedial gland not observed. Dispersed pigment granules laterodorsally to pharynx, infrequently absent. Mouth surrounded by slight sclerotisations. Pharynx elongate ovate, 90 (88–93; n = 2) long, 53 (50–55; n = 2) wide; oesophagus short; two intestinal caeca, nonconfluent, lacking diverticula. Haptor circular, haptoral disc approximately half of body length, 203 (185–220; n = 2) long, 208 (195–220; n = 2) wide, with one central and eight peripheral loculi (two anterior, four lateral, two posterior); septa ventrally surmounted by slightly sinuous sclerotized ridge. Dorsal pairs of haptoral accessory structures associated with lateral and posterior peripheral loculi; each pad with sclerotized margins. Anterior pair of dorsal haptoral accessory structures bilobate, with poorly separated lobes; each lobe semicircular, posterior pair of dorsal haptoral accessory structures semicircular (Fig. 35). Anchors 65 (n = 1) long, base 27

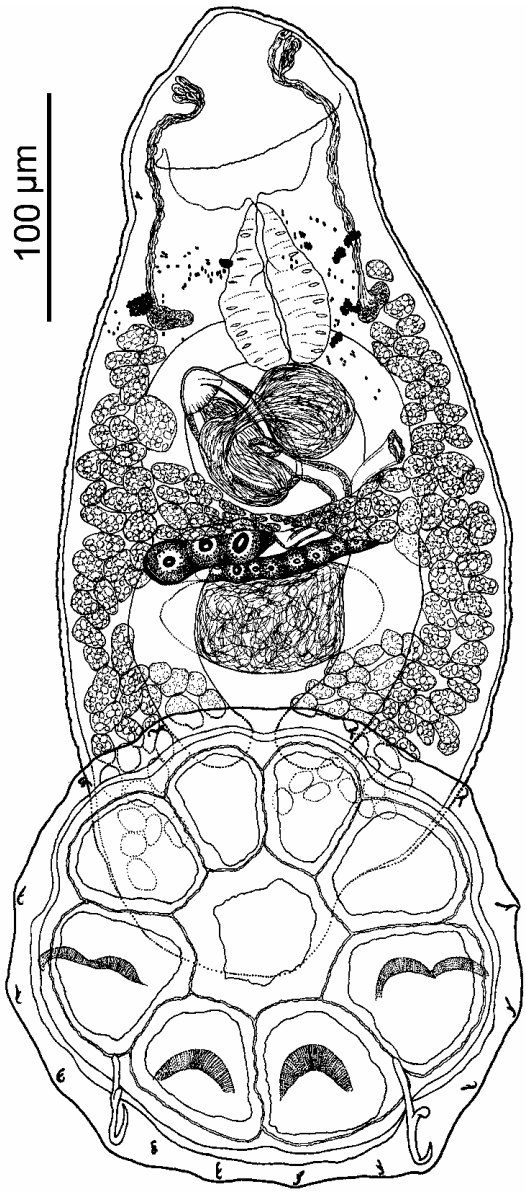
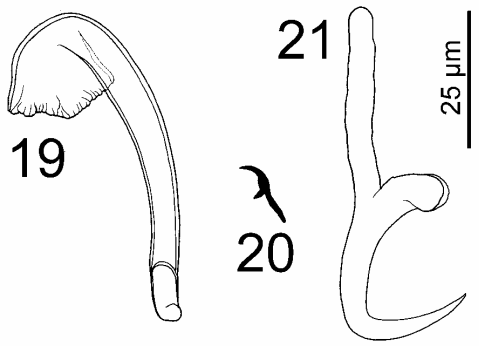
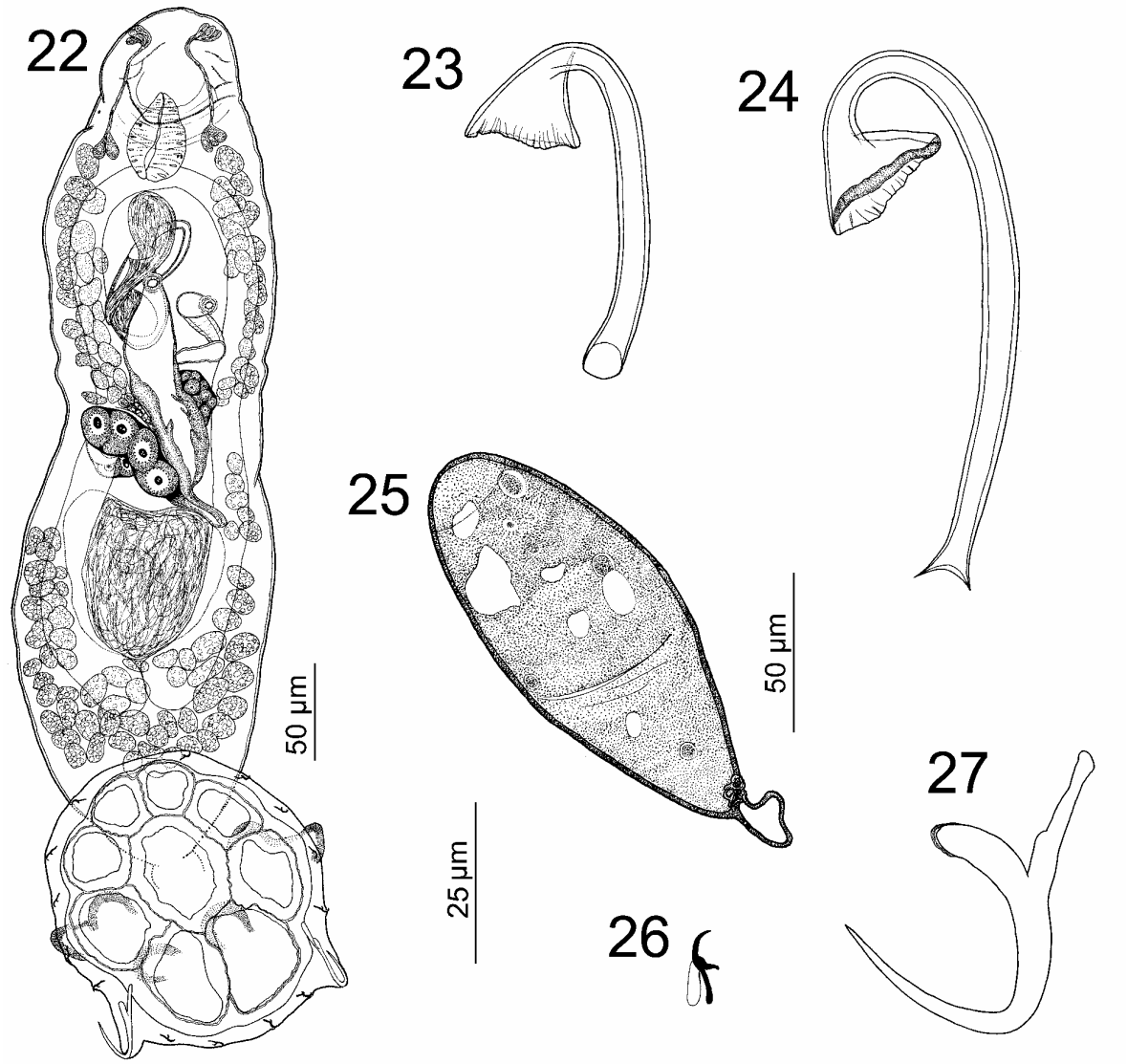


Fig. 18. *Potamotrygonocotyle rarum* sp. n. Whole mount (holotype).



Figs. 19–21. *Potamotrygonocotyle rarum* sp. n. **Fig. 19.** Male copulatory organ. **Fig. 20.** Hook. **Fig. 21.** Anchor.



Figs. 22–27. *Potamotrygonocotyle rionegrense* sp. n. **Fig. 22.** Whole mount (holotype). **Fig. 23.** Male copulatory organ. **Fig. 24.** Male copulatory organ (bent). **Fig. 25.** Egg. **Fig. 26.** Hook. **Fig. 27.** Anchor.

(n = 1) wide, with heavily diverging roots, deep root twice as big as superficial root; evenly curved shaft and point. Hooks similar, 12 (10–13; n = 12) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with dilated proximal portion and curved point. Male copulatory organ sclerotized, arcuate, proximal portion bent dorsally, distal aperture subterminal, 58 (51–63; n = 3) long. Accessory piece absent. Testis transversally ovate, 60 (53–68; n = 2) long, 94 (88–100; n = 2) wide; vas deferens not observed; seminal vesicle C-shaped, entering dorsally at posterior region of ejaculatory bulb. Ejaculatory bulb muscular, ovate with two anterior ovate internal chambers; glands associated with ejaculatory bulb not observed. Germarium tubular, unbranched, looping right intestinal caecum; distal end ascendant; Mehlis' glands not observed. Vagina and vaginal canal muscular. Vaginal pore sinistroventral at level of common genital pore; seminal receptacle not observed. Oötype well developed. Vitellaria coextensive with gut, absent in the regions of reproductive organs. Egg not observed. For comparative measurements see Table 1.

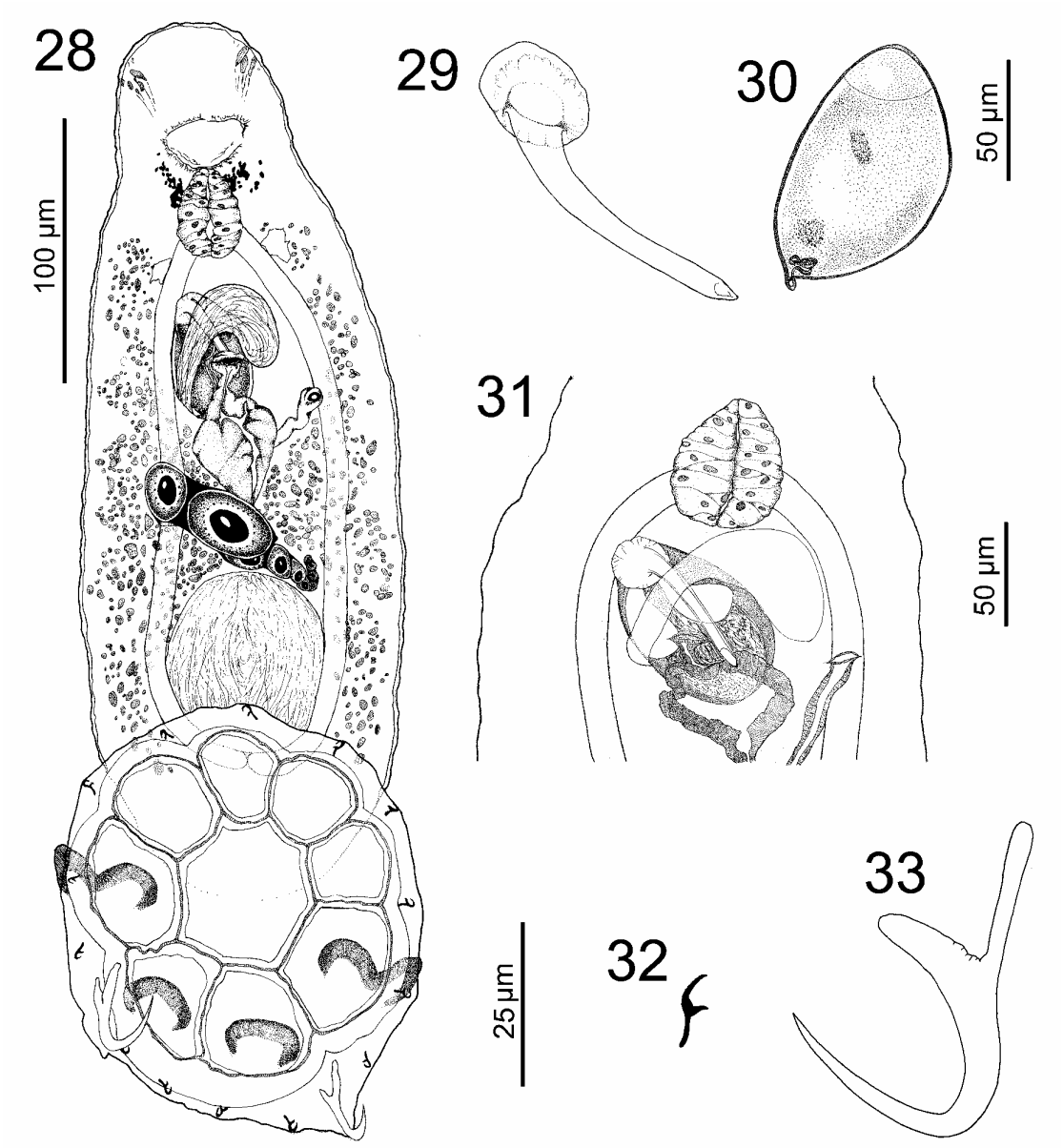
Holotype, type host, type locality: MZUSP 6371; *Potamotrygon schroederi* Fernández-Yépez, 1958, Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), 25 January 2004.

Other specimens: 5 paratypes; 4 MZUSP 6372a–d, 1 IPCR M-453; Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), January and February 2005.

Site of infection: Gills.

Etymology: The specific name is from Latin (*rarum* = rare) and it refers to the fact that only a few specimens were collected from 13 host specimens in the Negro River, January and February 2005.

Remarks. *Potamotrygonocotyle rarum* resembles *P. tsalickisi* Mayes, Brooks et Thorson, 1981 by having an ejaculatory bulb with two anterior internal chambers



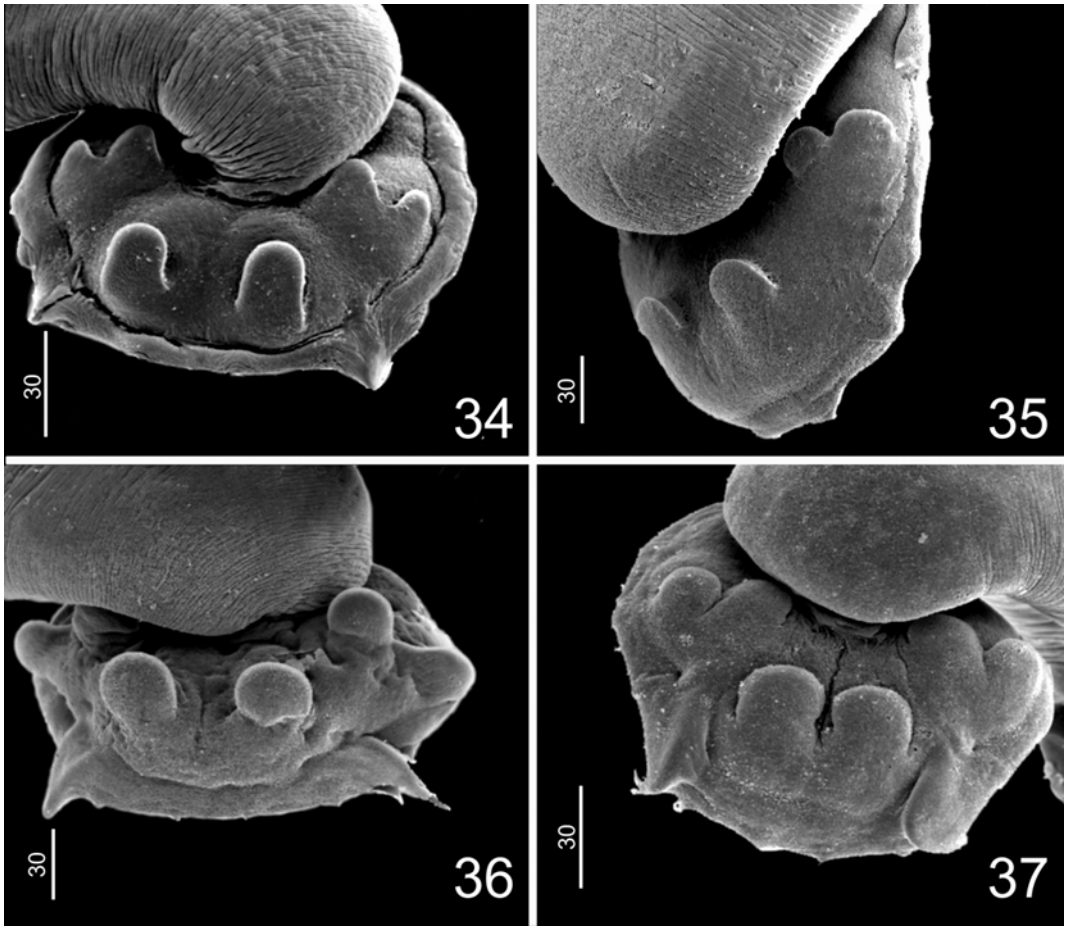
Figs. 28–33. *Potamotrygonocotyle aramasae* sp. n. **Fig. 28.** Whole mount (composite drawing). **Fig. 29.** Male copulatory organ. **Fig. 30.** Egg. **Fig. 31.** Detail of reproductive structures. **Fig. 32.** Hook. **Fig. 33.** Anchor.

and in the general morphology of the male copulatory organ. However, it differs from *P. tsalickisi* by having anchors with deep roots twice as long as the superficial roots.

Potamotrygonocotyle rionegrense sp. n.

Figs. 22–27, 36

Description (based on 42 specimens): Body fusiform, with constriction at midlength, total length excluding haptor 356 (300–480; n = 11); 142 (140–205; n = 11) wide; greatest width usually in posterior trunk. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs converging to unique pore; cephalic glands posterolateral to pharynx. Anteromedial gland not observed. Pigment granules usually absent, infrequently in cephalic, trunk regions. Mouth surrounded by slight sclerotisations. Pharynx elongate ovate, 49 (43–58; n = 9) long, 32 (25–38; n = 9) wide; oesophagus short; two intestinal caeca, nonconfluent, partially overlapping, lacking diverticula. Haptor circular, 171 (140–205; n = 9) long, 169 (135–200; n = 9) wide, with one central and eight peripheral loculi (two anterior, four lateral, two posterior); septa ventrally surmounted by slightly sinuous sclerotized ridge. Dorsal pairs of haptoral accessory structures associated with lateral and posterior peripheral loculi; each pad with sclerotized margins. Anterior pair of dorsal haptoral accessory structures bilobate, with well separated lobes, each lobe circular; posterior pair of dorsal haptoral accessory structures circular (Fig. 36). Anchors 46 (42–



Figs. 34–37. General morphology of dorsal haptoral accessory structures of *Potamotrygonocotyle* spp., scanning electron micrographs. **Fig. 34.** *Potamotrygonocotyle umbella* sp. n. **Fig. 35.** *Potamotrygonocotyle rarum* sp. n. **Fig. 36.** *Potamotrygonocotyle rionegrense* sp. n. **Fig. 37.** *Potamotrygonocotyle aramasae* sp. n. Scale bars in µm.

51; n = 23) long, base 21 (18–24; n = 17) wide, with heavily diverging roots, evenly curved shaft and point. Hooks similar, 12 (10–13; n = 116) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with dilated proximal portion and curved point. Male copulatory organ sclerotized, arcuate, 70 (58–92; n = 21) long, proximal portion bent dorsally, directed posteriorly; distal aperture terminal, concave; base with small proximal flap. Accessory piece absent. Testis transversally ovate, 60 (50–80; n = 10) long, 80 (68–98; n = 10) wide; vas deferens not observed; seminal vesicle sigmoid, entering dorsally at posterior region of ejaculatory bulb. Ejaculatory bulb muscular, ovate without distinct internal chambers; glands associated with ejaculatory bulb not observed. Germarium tubular, unbranched, looping right intestinal caecum; distal end ascendant; Mehlis' glands not observed. Vagina and vaginal canal muscular. Vaginal pore sinistroventral at level of common genital pore; seminal receptacle spherical. Oötype well developed. Vitellaria coextensive with gut, absent in the regions of reproductive organs and near body midlength. Egg ovate, 110 (n = 1) long, 40 (n = 1) wide, with short filament; distal end reticulate. For comparative measurements see Table 1.

Holotype, type host, type locality: MZUSP 6373; *Potamotrygon* sp., Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), 21 January 2005.

Other specimens: 41 paratypes; 21 MZUSP 6374a–u, 4 CHIOC 36885a–d, 4 HWML 48543–48546, 5 INPA 503a–e, 6 USNPC 99795–99797, 1 IPCR M-454; Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), January and February 2005.

Site of infection: Gills.

Etymology: The specific name refers to the Negro River, from where the type host was collected.

Remarks. *Potamotrygonocotyle rionegrense* differs from all other species of the genus by having a sclerotized arcuate male copulatory organ with the proximal portion bent dorsally and then directed posteriorly and by the terminal, concave distal aperture.

Potamotrygonocotyle aramasae sp. n.

Figs. 28–33, 37

Description (based on 28 specimens): Body fusiform, total length excluding haptor 255 (170–360; n = 15); 136 (100–180; n = 14) wide; greatest width usually at level of germarium. Tegument smooth. Cephalic lobes absent; 3 pairs of head organs converging to unique pore; cephalic glands posterolateral to pharynx. Anteromedial gland not observed. Dispersed pigment granules laterodorsally to pharynx, infrequently absent. Mouth surrounded by slight sclerotisations. Pharynx elongate ovate, 50 (38–65; n = 11) long, 30 (25–38; n = 11) wide; oesophagus short; two intestinal caeca, nonconfluent, lacking diverticula. Haptor subcircular, 165 (130–180; n = 15) long, 148 (115–170; n = 13) wide, with one central and eight peripheral loculi (two anterior, four lateral, two posterior); septa ventrally surmounted by slightly sinuous sclerotized ridge. Dorsal pairs of haptoral accessory structures associated with lateral and posterior peripheral loculi; each pad with sclerotized margins. Anterior pair of dorsal haptoral accessory structures bilobate, with well separated lobes, each lobe semicircular; posterior pair of dorsal haptoral accessory structures ovate (Fig. 37). Anchors 50 (48–53; n = 15) long, base 19 (17–25; n = 10) wide, with heavily diverging roots and evenly curved shaft and point. Hooks similar, 10 (10–11; n = 89) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with dilated proximal portion and curved point. Male copulatory organ sclerotized, arcuate, 54 (47–60; n = 14) long, with conical expanded base, acute distal portion, directed posteriorly; distal aperture subterminal. Accessory piece absent. Testis elongate ovate, 72 (58–90; n = 9) long, 58 (38–73; n = 8) wide; vas deferens not observed; seminal vesicle sigmoid, entering dorsally at posterior region of ejaculatory bulb. Ejaculatory bulb muscular, ovate with two anterior ovate internal chambers (Fig. 31); glands associated with ejaculatory bulb not observed. Germarium tubular, unbranched, looping right intestinal caecum; distal end ascendant; Mehlis' glands not observed. Vagina and vaginal canal muscular. Vaginal pore sinistroventral at level of common genital pore; seminal receptacle spherical. Oötype well developed. Vitellaria coextensive with gut, absent in regions of reproductive organs. Egg ovate, 88 (n = 1) long, 53 (n = 1) wide, with short filament; distal end reticulate. For comparative measurements see Table 1.

Holotype, type host, type locality: MZUSP 6375; *Paratrygon aiereba*, Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), 26 January 2005.

Other specimens: 27 paratypes; 11 MZUSP 6376a–k, 4 CHIOC 36886–36887a–c, 2 HWML 48547–48548, 5 INPA 504ab–507, 4 USNPC 99798–99800, 1 IPCR M-455; Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), January and February 2005.

Table 1. Comparative measurements (in μm) and morphological characters used to distinguish species of *Potamotrygonocotyle*.

	Body		Pharynx		Haptor		Anchor		Anterior DHAS shape	Posterior DHAS shape	Hooks length	MCO length	MCO shape	MCO aperture	Chamber EB	Testes		Egg		Egg filament
	length	width	length	width	length	width	length	base								length	width	length	width	
P. szidati	630–900	230–310	83–88	55–70	225–270	240–270	49–65	20–25	bilobate	rounded	10–13	50–66	arcuate	subterminal	present	130–185	90–180	125–138	43–125	short
P. chisholmae	260–590	110–280	33–75	25–55	120–215	125–220	42–66	17–27	trilobate	fan–like	10–15	33–75	straight	terminal	absent	48–118	50–98	108–175	35–78	long
P. dromedarius	310–550	170–430	50–63	38–40	190–270	215–285	37–55	16–20	semicircular	rounded	9–12	22–33	straight	terminal	absent	100–163	75–150	no data	no data	short
P. eurypotamonensis	260–530	110–240	45–78	30–50	140–230	140–245	42–60	16–28	bilobate	rounded	10–13	30–64	arcuate	subterminal	absent	75–150	58–95	110–158	45–78	short
P. uruguayense	310–550	100–170	48–55	23–30	145–210	145–185	51–60	20–25	bilobate	fan–like	12–14	67–78	straight	terminal	absent	50–75	53–63	105–198	50–70	long
P. quadricoxyle sp. n.	220–380	100–180	28–48	25	113–160	95–125	36–47	20–25	bilobate	elongate	10–14	30–44	straight	terminal	absent	30–35	43–73	120–133	43–65	short
P. umbella sp. n.	200–300	80–130	35–40	25–30	155–180	135–190	34–39	14–17	bilobate	elongate	8–11	34–38	arcuate	terminal	absent	33–50	48–68	110–130	43–58	short
P. rarum sp. n.	430–490	180–210	88–93	50–55	185–220	195–220	65	27	bilobate	rounded	10–13	51–63	arcuate	subterminal	present	53–68	88–100	no data	no data	short
P. tionegrense sp. n.	300–480	140–205	43–58	25–38	140–205	135–200	42–51	18–24	bilobate	rounded	10–13	58–92	arcuate	terminal	absent	50–80	68–98	110	40	short
P. aramasae sp. n.	170–360	100–180	38–65	25–38	130–180	115–170	48–53	17–25	bilobate	rounded	10–11	47–60	arcuate	subterminal	present	58–90	38–73	88	53	short

DHAS – dorsal haptoral accessory structure; MCO – male copulatory organ; EB – ejaculatory bulb.

Site of infection: Gills.

Etymology: The specific epithet refers to the local common name of the host, "aramaçã".

Remarks. *Potamotrygonocotyle aramasae* differs from its congeneric species by having a male copulatory organ with an acute distal tip, a subterminal aperture, and an ejaculatory bulb with two anterior ovate cavities.

Hexabothriidae Price, 1942

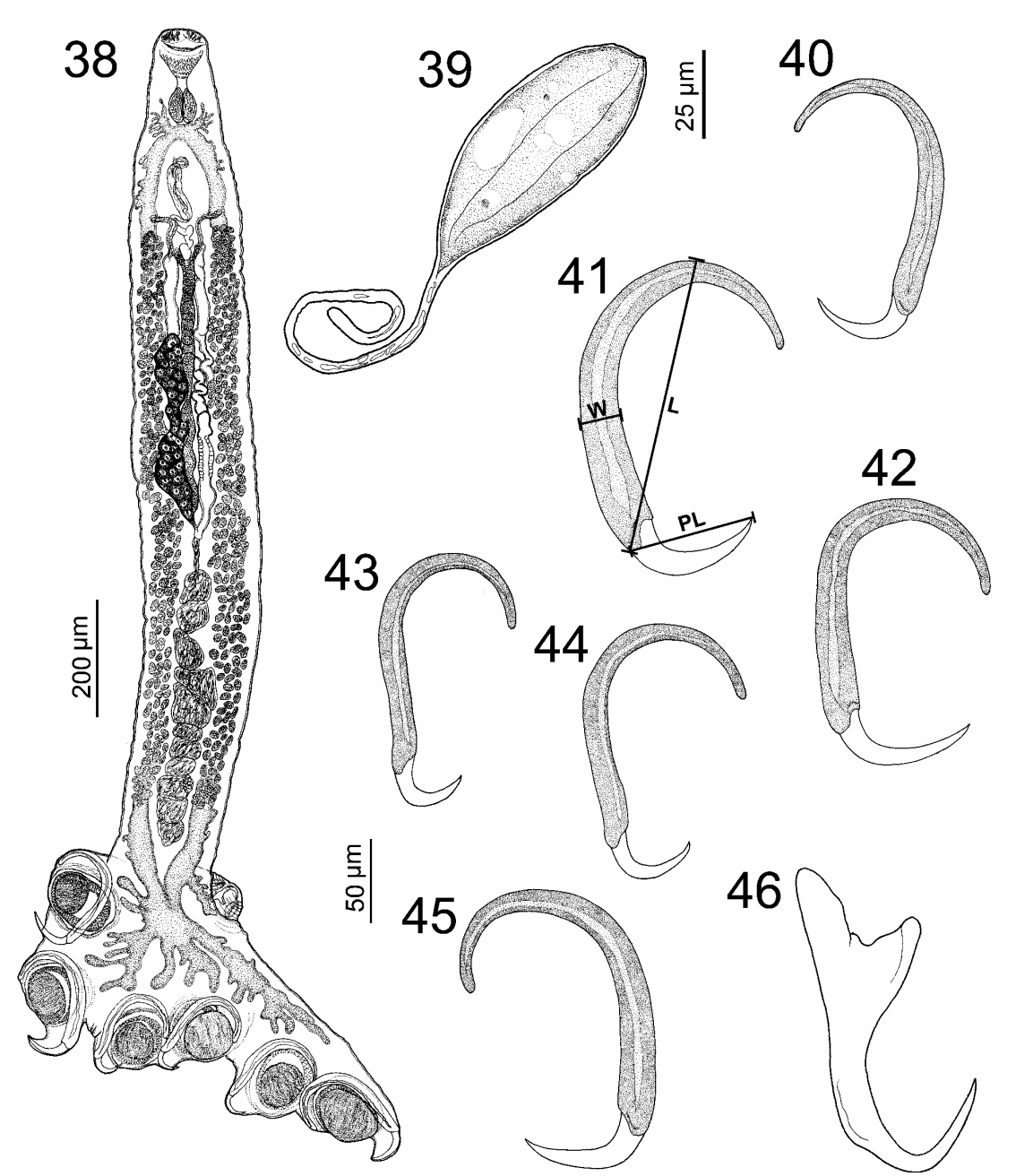
Paraheteronchocotyle Mayes, Brooks et Thorson, 1981

Emended diagnosis. Body elongate, comprising oral region, trunk, peduncle and haptor. Tegument thin, smooth. Eyes absent. Mouth subterminal, pharynx muscular, glandular; two intestinal caeca with diverticula, caeca confluent in peduncle posterior to testes, branching into haptor, haptoral appendix. Haptor asymmetrical, sucker complexes linear except complex 1 adjacent to base of haptoral appendix; sucker sclerite 1, 1' similar; haptoral appendix marginal, originating from dorsal haptoral surface lateral to body midline, armed with 0 or 1 anchor, 2 terminal suckers. Cirrus unarmed, comprising two portions: distal part elongate, bulbous; proximal part non-dilated; prostatic region not observed. Testes numerous, irregular; vas deferens sinuous, dorsal to vitelline commissure, with small looping proximal to entrance into cirrus, delicate distal wall of vas deferens. Genital pore at level of gut bifurcation. Germarium lobate anteriorly, sinuous descending germarium branch; ascending germarium branch absent, seminal receptacle not observed; oviduct originating from posterior end of germarium; oötype smooth; uterus lateral to germarium, dorsal to vitellinic channel. Vagina parallel, comprising two segments; distal portion glandular, proximal portion delicate. Vaginal pores on ventral surface about midway between body midline and lateral margin on each side of cirrus. Vitellaria comprising two bilateral bands extending from level of vaginal pores into level of posterior testes; vitelline commissure lateral to germarium, genitointestinal canal dorsal to germarium. Eggs with one polar filament. Parasites of Potamotrygonidae. Type species: *Paraheteronchocotyle amazonense* Mayes, Brooks et Thorson, 1981.

Remarks. *Paraheteronchocotyle* was erected by Mayes et al. (1981) to accommodate *P. amazonense* collected from the freshwater stingray *Potamotrygon constellata* from the upper Amazon River. The diagnostic characters used to define *Paraheteronchocotyle* included the uniform-sized suckers, unequal-sized sucker sclerites, and haptoral appendix without anchors. Examination of the type material from USNPC and HWML confirmed the diagnostic features used by Mayes et al. (1981) to recognize *P. amazonense*. The comparison of the type material and voucher specimens collected during the present study suggested that these specimens are conspecific. However, upon study of the voucher specimens, we were able to detect that some specimens have anchors (usually one) associated with the haptoral appendix, suggesting that the absence of haptoral anchors may be related to secondary loss as already reported within many groups of Monogenoidea (for discussion see Kritsky and Boeger 1989, Desdevises 2001). In addition, we noticed that Mayes et al.

Table 2. Comparative measurements (in µm) of specimens of *Paraheteronchocotyle amazonense* from *Potamotrygon constellata* and *P. orbignyi.*

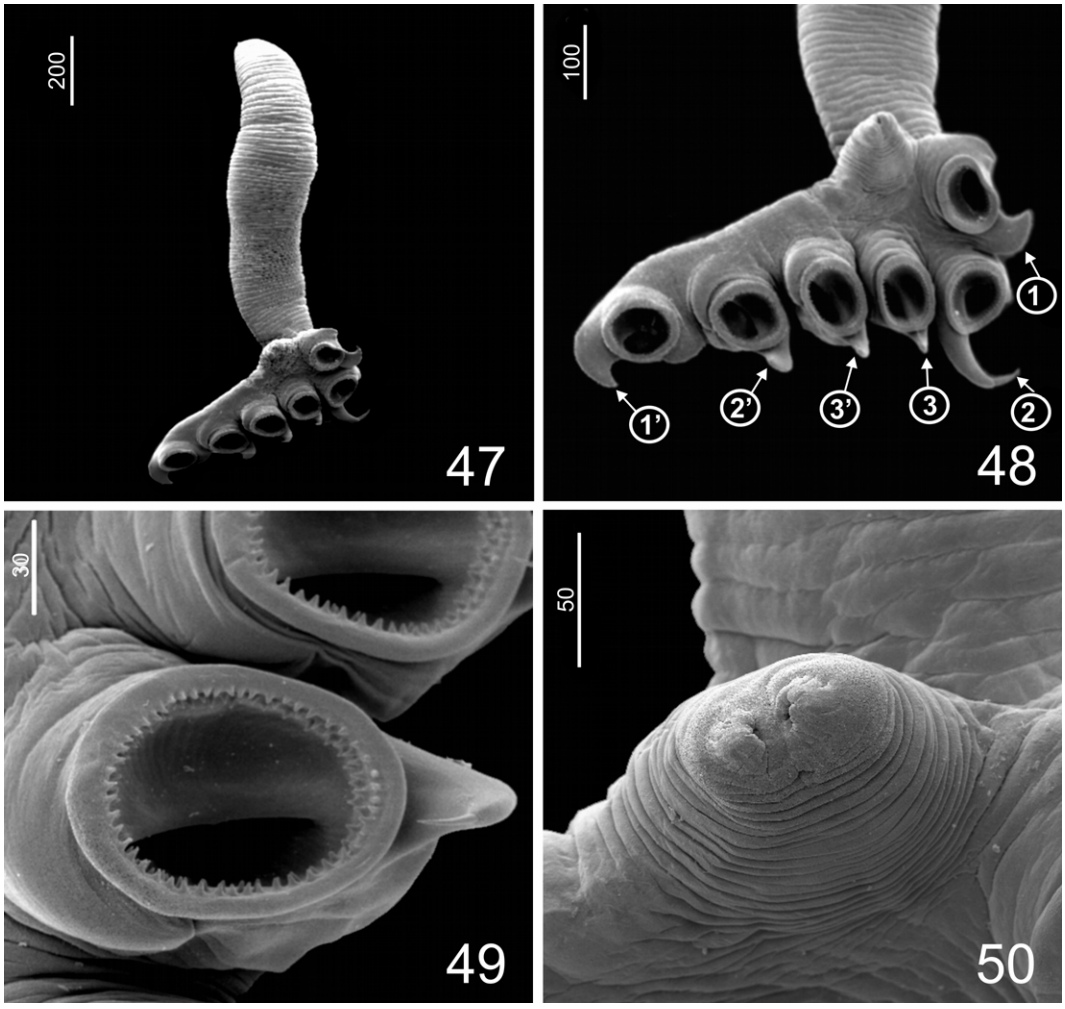
	Potamotrygon constellata	n	*Potamotrygon orbignyi*	n
Body				
length	1732 (1615–1881)	3	1450 (1200–1600)	6
width	483 (437–510)	3	208 (160–240)	6
Haptor				
length	410 (350–446)	3	314 (280–350)	7
width	1022 (919–1098)	3	777 (710–850)	7
Appendix				
length	177 (150–203)	2	138 (120–150)	5
width	194 (188–200)	2	138 (110–160)	6
Appendix sucker				
length	109 (88–131)	2	69 (50–95)	6
width	70 (56–85)	2	40 (30–50)	6
Oral sucker				
length	88 (75–100)	2	100 (88–108)	6
width	146 (113–180)	2	94 (88–100)	6
Pharynx				
length	82 (78–87)	2	78 (63–95)	6
width	77 (76–78)	2	63 (53–70)	6
Copulatory organ				
length	96 (88–105)	2	118 (105–125)	3
width	33 (28–38)	2	34 (30–38)	3
Testes field				
length	–	–	550	1
width	–	–	85 (80–90)	2
Germarium				
length	606	1	91 (75–100)	4
width	144	1	14 (13–15)	4
Egg				
length	141	1	99 (88–115)	3
width	58	1	38	3
Anchor				
length	–	–	56 (50–63)	3
base width	–	–	26 (25–28)	2
Sucker sclerite 1				
length	243 (234–253)	3	153 (123–170)	7
width	34 (32–36)	3	20 (17–22)	7
point length	95 (78–105)	3	72 (64–78)	7
Sucker sclerite 2				
length	270 (261–277)	5	182 (157–204)	7
width	37 (32–42)	5	27 (20–32)	7
point length	100 (88–110)	5	79 (74–85)	7
Sucker sclerite 3				
length	199 (181–222)	4	140 (131–151)	7
width	21 (20–22)	4	16 (13–20)	7
point length	41 (37–47)	3	45 (32–57)	7
Sucker sclerite 1'				
length	273 (251–287)	4	186 (170–208)	7
width	37 (33–42)	4	25 (20–29)	7
point length	101 (100–101)	3	75 (70–79)	7
Sucker sclerite 2'				
length	213 (199–234)	3	154 (140–167)	7
width	23 (22–26)	3	17 (15–19)	7
point length	46 (43–48)	2	40 (22–54)	7
Sucker sclerite 3'				
length	205 (193–227)	3	147 (138–157)	7
width	22 (16–30)	3	16 (14–20)	7
point length	26	1	43 (31–53)	7



Figs. 38–46. *Paraheteronchocotyle amazonense* Mayes, Brooks et Thorson, 1981. **Fig. 38.** Whole mount (composite drawing). **Fig. 39.** Egg. **Fig. 40.** Sucker sclerite 1. **Fig. 41.** Sucker sclerite 2. **Fig. 42.** Sucker sclerite 3. **Fig. 43.** Sucker sclerite 1'. **Fig. 44.** Sucker sclerite 2'. **Fig. 45.** Sucker sclerite 3'. **Fig. 46.** Haptoral appendix anchor. L – sucker sclerite length; PL – point length; W – sucker sclerite width.

(1981) misinterpreted the relative position of testes in the diagnosis of *Paraheteronchocotyle*. According to them, in *Paraheteronchocotyle* species the testes were pre-ovarian (= pre-germarian), but examination of the type material and voucher specimens revealed that the testes are postgermarian in the genus.

Boeger and Kritsky (1989) suggested in their cladistic analysis that *Paraheteronchocotyle* is closely related to *Heteronchocotyle* based on the morphology of the haptor (sucker complexes linear except complex 1 adjacent to base of haptoral appendix and complex sclerites dissimilar in shape and size). *Paraheteronchocotyle* differs from *Heteronchocotyle* by similar sucker sclerites 1, 1' (sclerite 1 dissimilar from 1' in *Heteronchocotyle*) and eggs with one elongate polar filament (eggs with two flat, elongate polar filaments in *Heteronchocotyle*). However, their cladistic analysis has many missing data for *Paraheteronchocotyle* due to the poor conditions of the material used for analysis, suggesting that a new analysis should readdress the phylogenetic position of the genus considering the complete coding for this taxon.



Figs. 47–50. General morphology of structures of *Paraheteronchocotyle amazonense* Mayes, Brooks et Thorson, 1981, scanning electron micrographs. **Fig. 47.** Whole specimen (dorsal view). **Fig. 48.** Detail of haptor. **Fig. 49.** Detail of haptoral sucker. **Fig. 50.** Detail of haptoral appendix. Circled numbers identify sucker sclerites in the haptor. Scale bars in μm.

Paraheteronchocotyle amazonense Mayes, Brooks et Thorson, 1981 Figs. 38–50

Redescription (based on the holotype, USNPC 77159, 3 paratypes, USNPC 77160 and HWML 21391, and 8 newly collected specimens – see below): Body elongate. Tegument with annulations throughout trunk. Oral sucker with papillae on inner wall; pharynx ovate elongate; intestinal caeca with anterior diverticula lateral to pharynx. Haptor asymmetrical with 3 pairs of equal-sized suckers armed with unequal-sized sucker sclerites and dorsal haptoral appendix. Papillae present on inner wall of haptoral suckers (Fig. 46). Haptoral appendix bearing two terminal, unarmed suckers (Fig. 47). Single anchor when present with wide base, short roots, medially curved shaft and short point. Testes 10–12 in number. Common genital aperture nonpapillate. Egg ovate elongate, polar filament same size as egg; 1–50 eggs recorded per specimen. For comparative measurements see Table 2.

Type host: *Potamotrygon constellata* (Vaillant, 1880) (= *P. circularis*).

Type locality: Itacoaí River, 5 km SE of Atalaia do Norte, Brazil (July 1976 and July 1978).

Site of infection: Gills.

Newly collected specimens: 8 vouchers; 4 MZUSP 6377a–d, 1 CHIOC 36888, 1 INPA 510, 1 USNPC 99801, 1 IPCR M-456; *Potamotrygon orbignyi* (Castelnau, 1855), Negro River, Municipality of Barcelos, Amazonas, Brazil (0°58'11"S, 62°55'13"W), January and February 2005.

Remarks. The examination of the specimens of *Paraheteronchocotyle* collected from the gills of *Potamotrygon orbignyi* from the Negro River and type specimens of *P. amazonense* from the gills of *P. constellata* indicates that they are conspecific. Except for the presence of anchors (see generic diagnosis) and some morphometric differences (Table 2), they share similar morphology of the haptoral and reproductive structures.

The intraspecific morphometric variation detected in specimens of *P. amazonense* collected from different localities and hosts does not support the proposal of a new species of *Paraheteronchocotyle.* Among ectoparasitic monogenoideans, fluctuations of abiotic (e.g. salinity, temperature) and biotic (e.g. host size) conditions have been shown to influence morphometric variation observed in some haptoral structures (Boeger and Kritsky 1988, Thoney 1988, Mo 1991, Rohde 1991, Perera 1992).

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