

Revision of *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981 (Platyhelminthes: Monogenoidea: Monocotylidae), with descriptions of four new species from the gills of the freshwater stingrays *Potamotrygon* spp. (Rajiformes: Potamotrygonidae) from the La Plata river basin

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Abstract The only known monocotylid genus to parasitise Neotropical freshwater stingrays (Potamotrygonidae) is *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981, a monotypic genus erected to accommodate *P. tsalickisi* Mayes, Brooks & Thorson, 1981. For more than 20 years, no other species has been recognised in this genus, but new efforts to survey the diversity of parasites inhabiting potamotrygonids have revealed the existence of new species and the need to redefine the genus. Here, the generic diagnosis of *Potamotrygonocotyle* is amended, *P. tsalickisi* is redescribed and four new species are recognised and described based on samples collected from the gills of freshwater potamotrygonids from the La Plata river basin: *Potamotrygonocotyle chisholmae* n. sp. and *P. dromedarius* n. sp. from *Potamotrygon motoro*; *Potamotrygonocotyle eurypotamoxenus* n. sp. from *Potamotrygon* cf. *motoro* (type-host), *P. castexi*, *P. falkneri* and *P. histrix*; and *Potamotrygon-*

ocotyle uruguayensis n. sp. from *Potamotrygon brachyura*. *Potamotrygonocotyle* is characterised by species possessing: (1) slightly sinuous sclerotised ridges on all septa; (2) two pairs of the dorsal haptoral accessory structures associated with the four posterior peripheral loculi and with anterior dorsal haptoral accessory structure bilobate or semicircular; and (3) male copulatory organ without an accessory piece.

Introduction

Although 523 species of monogenoideans have been reported in South America (Kohn & Cohen, 1998; Kohn & Paiva, 2000), only two species are known to parasitise Neotropical freshwater stingrays. This demonstrates how little is known about the diversity of monogenoideans inhabiting potamotrygonids. These two species are from the early 1980s. Thus, for more than 20 years, the diversity of monogenoideans inhabiting potamotrygonids has been restricted to one species of the Monocotylidae Taschenberg, 1879 and one species of the Hexabothriidae Price, 1942, two families typically found on marine elasmobranchs.

Within Neotropical monocotylids, the monotypic *Potamotrygonocotyle* Mayes, Brooks &

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Thorson, 1981 was proposed by Mayes, Brooks & Thorson (1981) to accommodate *P. tsalickisi* Mayes, Brooks & Thorson, 1981 from the gills of the freshwater stingray *Potamotrygon constellata* (= *P. circularis*) from Rio Itacoai, near Atalaia do Norte, Brazil. Recently, new surveys of parasites from potamotrygonids have indicated that the diversity of monogenoideans has been greatly overlooked.

Here, we amend the diagnosis of *Potamotrygonocotyle* based on the re-examination of the type-material of the type-species and in consideration of newly collected specimens. In addition, we redescribe *P. tsalickisi* and describe four new species of *Potamotrygonocotyle* from potamotrygonids from the rivers of the La Plata river basin.

Materials and methods

Specimens of potamotrygonids were collected, using a spear gun, gill net or hook and line, from the Rio Salobra, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (56°22'42"W, 20°14'26"S), during July 2004; from the Rio Uruguay, Municipality of Porto Xavier, Rio Grande do Sul, Brazil (55°08'00"W, 27°54'00"S), during March 2005; from Paraje Santa Tecla, Municipality of Ituzaingó, Province of Corrientes, Argentina (56°24'05.1"W, 27°36'34.4"S); and from Presa de Yacyretá, Municipality of Ituzaingó, Province of Corrientes, Argentina (56°39'15.2"W, 27°29'31.8"S) during May 2005. The gills were removed and placed in plastic bags containing heated (65°C) 4% formalin solution. Unstained helminths were mounted in Hoyer's or Gray & Wess medium to study sclerotised structures (Humason, 1979). Whole-mounts of monogenoideans were stained with Gomory's trichrome to determine the internal features (Humason, 1979). Measurements, all in micrometres, were made according to Mizelle & Klucka (1953). Dimensions of the organs and other structures represent the greatest measurement in dorsoventral view; lengths of curved or bent structures (anchors and male copulatory organ) represent straight line distances between the extreme ends; and for two-dimensional measurements length is given first. The average measurements are

followed by the range and number of specimens measured (n) in parentheses. Illustrations were prepared with aid of a drawing tube on a Olympus BX-51 microscope with differential interference contrast (DIC). Specimens prepared for scanning electron microscopy (SEM) were hydrated in a graded ethanol series, 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.

The following museum specimens were examined: holotype and three paratypes of *P. tsalickisi* from the United States National Parasite Collection (USNPC), Beltsville, MD, 20705, USA, USNPC nos 77157 and 77158; 11 paratypes of *P. tsalickisi* from the Harold W. Manter Laboratory of Parasitology, Lincoln, NE, 68588–0514, USA, HWML no. 21390; and five specimens of *Potamotrygonocotyle* sp., HWML no. 21390. The monocytylid morphological terminology follows Chisholm, Wheeler & Beverley-Burton (1995). Material was deposited in these and other collections: Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, SP, 04263–000, Brazil; Coleção Helminológica do Instituto Pswaldo Cruz (CHIOC), Rio de Janeiro, RJ, 21045–900, Brazil; Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, 69083–000, Brazil.

Polyonchoinea Bychowsky, 1937 Monocotylidae Taschenberg, 1879 Heterocotylinae Chisholm, Wheeler & Beverley-Burton, 1995

***Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981**

Amended generic diagnosis

Monocotylidae, Heterocotylinae. Body fusiform, flattened dorsoventrally, comprising cephalic region, trunk and haptor. Cephalic lobes poorly developed; 3 pairs of anterolateral head organs open via single pore on either side of head; cephalic glands unicellular, lateral or posterolateral to pharynx with rod-shaped secretion (*sensu* Cribb, Whittington & Chisholm, 1997); anteromedian

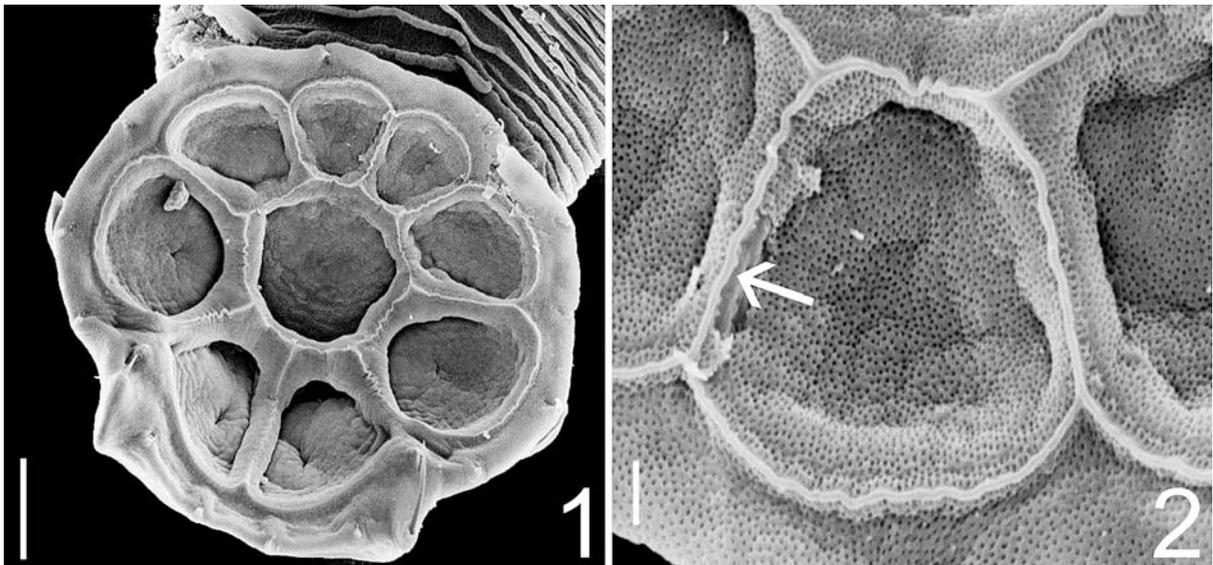
gland located anterior to pharynx with granular secretion (*sensu* Cribb et al., 1997). Eye-spots present, anterodorsal to pharynx. Mouth subterminal, mid-ventral, surrounded by sclerotised lines. Pharynx comprising muscular and glandular bulb; oesophagus short; 2 intestinal caeca, non-confluent, lacking diverticula. Haptor with 1 central and 8 peripheral loculi. Sinuous sclerotised ridge single on all septa. Dorsal surface of haptor with 2 pairs of haptor accessory structures, each associated with 1 of 4 posterior peripheral loculi; each dorsal haptor accessory structure with sclerotised margins; anterior pair of dorsal haptor accessory structures bilobate or semicircular; posterior pair of dorsal haptor accessory structures rounded or fan-like. Common genital pore mid-ventral near level of vaginal aperture. Gonads intercaecal, tandem. Vas deferens intercaecal; seminal vesicle a simple dilatation of vas deferens, forming S-shaped loop. Male copulatory organ sclerotised, directed posteriorly; accessory piece absent. Germarium tubular; pretesticular; oviduct loops the right intestinal caecum; oötype muscular. Vagina not sclerotised. Egg ovate. Type-species: *P. tsalickisi* Mayes, Brooks & Thorson, 1981.

Remarks

The original diagnosis of *Potamotrygonocotyle* by Mayes et al. (1981) and the amended generic diagnosis proposed by Chisholm et al. (1995) did not include the presence of a single sinuous sclerotised ridge on all septa. Examination of the type-specimens (holotype, paratypes) of *P. tsalickisi* and new species of *Potamotrygonocotyle* confirms the presence of this feature (Figs. 1, 2). We also include in the generic diagnosis the morphology of the egg (Figs. 3, 12, 17, 23, 28) and the presence of sclerotised lines around the mouth (Fig. 4).

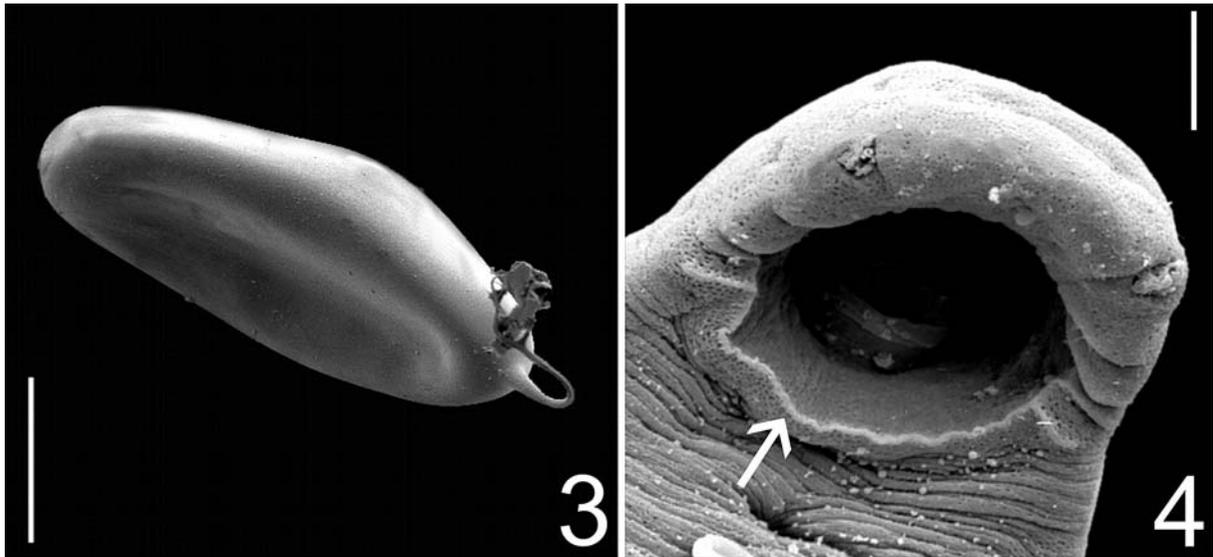
Monocotylids have been traditionally described as having tetrahedral eggs (Chisholm et al., 1995; Kearns, 1986). The only exceptions for this pattern were described for *Dictyocotyle coeliaca* Nybelin, 1941 and *Squalotrema llewellyni* Kearns & Green, 1983 (see Kearns, 1986). Mayes et al. (1981) did not describe the egg for *Potamotrygonocotyle tsalickisi*. However, specimens of *P. tsalickisi*, as well as members of the new species described below, clearly indicate the presence of ovate eggs.

The presence of sclerotised lines around the mouth was observed for *Potamotrygonocotyle*



Figs. 1–2 General morphology of haptor structures of *Potamotrygonocotyle* spp. under SEM. 1. Ventral view of haptor showing loculi. 2. Detail of haptor septa; arrow

shows the sclerotised sinuous single ridges on the septa. Scale-bars: 1, 30 μ m; 2, 5 μ m



Figs. 3–4 General morphology of structures of *Potamotrygonocotyle* spp. under SEM. 3. Egg. 4. Detail of anterior region, arrow shows the sclerotisation around the mouth. Scale-bars: 3, 30 μ m; 4, 15 μ m

specimens from the La Plata river basin under SEM (Fig. 4). However, we were unable to detect this feature on the specimens of *Potamotrygonocotyle* studied by Mayes et al. (1981) (USNPC 77157 and 77158; HWML 21390) because the observation of these specimens was limited to light microscopy.

Potamotrygonocotyle can be distinguished from other heterocotyline by the combined presence of: (1) a slightly sinuous sclerotised ridges on all septa; (2) two pairs of dorsal haptoral accessory structures with sclerotised margins associated with the four posterior peripheral loculi and with the anterior pair of dorsal haptoral accessory structures being bilobate or semicircular; and (3) the male copulatory organ without an accessory piece.

Chisholm et al. (1995) proposed a cladistic analysis for species of the Monocotylidae. In their analysis, *Potamotrygonocotyle* is more closely related to (*Spinuris* (*Nonacotyle* + *Neoheterocotyle*)) than to *Heterocotyle* based on the presence of six dorsal sclerites (= dorsal haptoral accessory structures). However, our studies suggest that species of *Potamotrygonocotyle* have only four dorsal haptoral accessory structures. These authors also overlooked the presence of the slightly sinuous sclerotised ridge on all septa.

Both characters are observed in *Heterocotyle*, indicating that a new cladistic analysis is required to access the phylogenetic position of *Potamotrygonocotyle* within the Heterocotylineae.

***Potamotrygonocotyle tsalickisi* Mayes, Brooks & Thorson, 1981**

Type-host: *Potamotrygon constellata* (Vaillant) (syn. *P. circularis* Garman).

Type-locality: Itacoai River, 5 km SE Atalaia do Norte, Brazil (July 1976; July 1978).

Site of infection: Gills.

Comparative measurements: Table 1.

Redescription (Figs. 5–9)

[Based on the holotype and paratypes from USNPC and HWML.] Body fusiform 760 (630–900; n = 5) \times 270 (230–310; n = 6) wide, widest at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs; cephalic glands not observed. Dispersed pigment granules (eye-spots) prominent laterodorsally to pharynx. Pharynx subspherical to elongate ovate, 86 (83–88; n = 7) \times 62 (55–70;

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

	Body		Pharynx		Haptor		Anchor		Shape of anterior *DHAS		Shape of posterior *DHAS		Hooksr		**MCO		**MCO		Egg	
	length	width	length	width	length	width	length	base width	anterior	posterior	length	length	aperture	Shape	length	length	length	width	length	width
<i>P. tsalickisi</i>	630–900	230–310	83–88	55–70	225–270	240–270	49–65	20–25	bilobate	rounded	10–13	50–66	subterminal	arcuate	130–185	90–125	125–138	43–125		
<i>P. chisholmae</i>	260–590	110–280	33–75	25–55	120–215	125–220	42–66	17–27	trilobate	fan-like	10–15	33–75	terminal	straight	48–118	50–98	108–175	35–78		
<i>P. dromedarius</i>	310–550	170–430	50–63	38–40	190–270	215–285	37–55	16–20	semicircular	rounded	9–12	22–33	terminal	cornet/straight	100–163	75–150	no data	no		
<i>P. euryptoma xenus</i>	260–530	110–240	45–78	30–50	140–230	140–245	42–60	16–28	bilobate	elongate/rounded	10–13	30–64	subterminal	arcuate	75–150	58–95	110–158	45–78		
n. sp.																				
<i>P. uruguayensis</i>	310–550	100–170	48–55	23–30	145–210	145–185	51–60	20–25	bilobate/divergent	fan-like	12–14	67–78	terminal	straight	50–75	53–63	105–198	50–70		
n. sp.																				

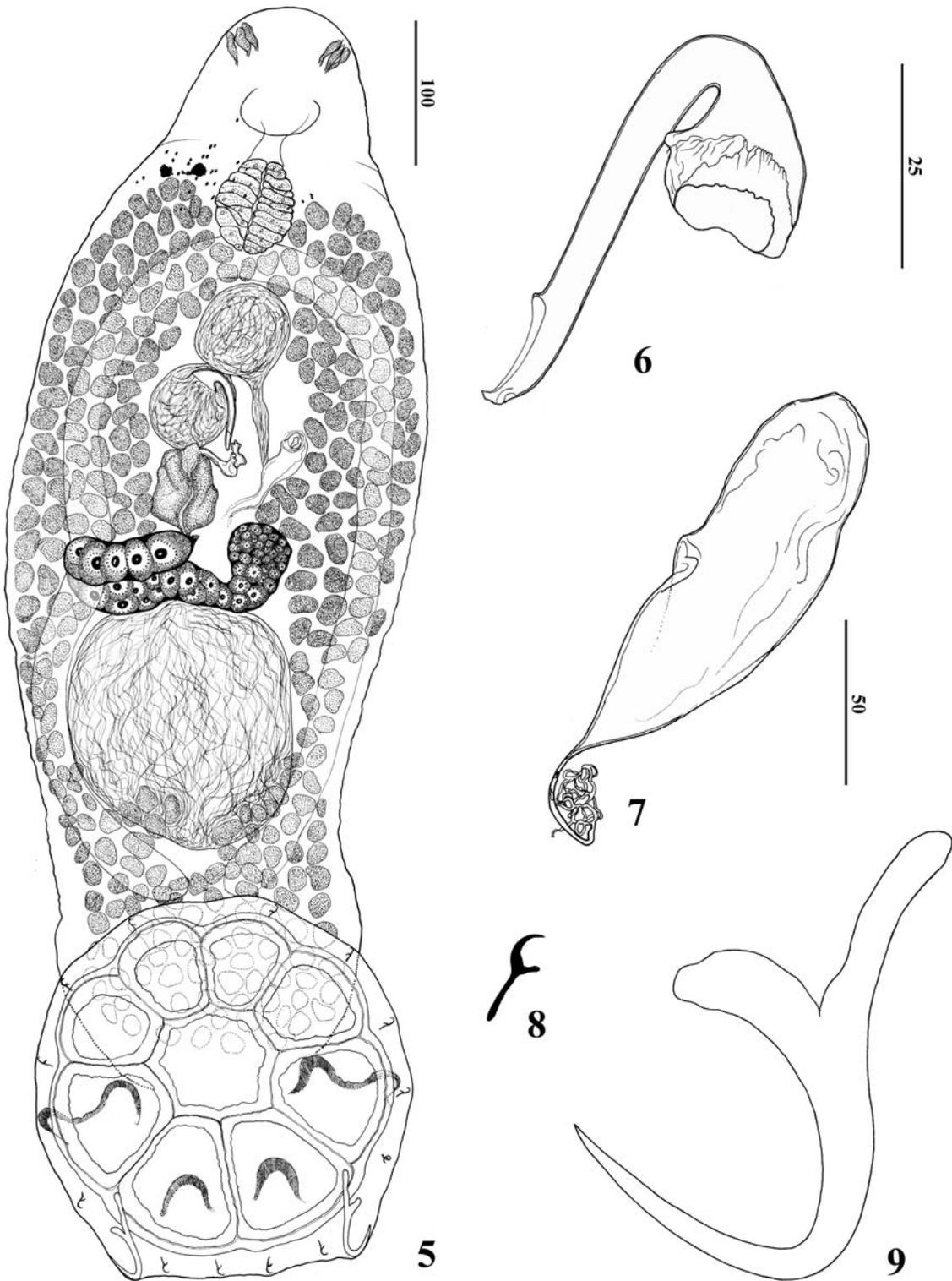
* DHAS, dorsal haptoral accessory structure

** MCO, male copulatory organ

n = 7); oesophagus short. Haptor subcircular, 247 (225–270; n = 5) × 252 (240–270; n = 5) wide; septa surmounted ventrally by slightly sinuous sclerotised ridge. Anterior pair of dorsal haptoral accessory structures bilobate; each anterior dorsal haptoral accessory structure with concave separation between lobes; posterior pair rounded. Anchors 58 (49–65; n = 10) long, base 22 (20–25; n = 3) wide, with heavily diverging roots, evenly curved shaft and point. Hooks similar 11 (10–13; n = 12), distributed on marginal membrane of haptor, with thumb erect, proximal portion of shaft dilate and point curved. Male copulatory organ arcuate, 55 (50–66; n = 7) long; distal aperture subterminal. Testis ovate, 153 (130–185; n = 6) × 138 (90–180; n = 6); seminal vesicle composed of 2 spherical portions; distal portion appears to represent ejaculatory bulb. Germarium unbranched, distal end ascendant; Mehlis' gland not observed. Vagina and vaginal canal not sclerotised. Vaginal pore sinistroventral at level of common genital pore. Oötype well developed, with ascendant limb. Vitelline follicles coextensive with gut. Egg ovate, 133 (125–138; n = 3) × 75 (43–125; n = 3); proximal filament 1/3 of egg size; distal end reticulate.

Remarks

The original description of *Potamostrongylocotyle tsalickisi*, while basically adequate, lacked drawings of the hooks and the detailed male copulatory organ, which are provided herein. Comparison of the type-specimens (USNPC 77157 and 77158; HWML 21390) indicated that some specimens did not agree with the original description and may represent a different species. Thus, of the 16 slides examined, we considered 11 to be similar to the holotype of *P. tsalickisi*, whereas the other five appear to be an undescribed species of *Potamostrongylocotyle*. The holotype of *P. tsalickisi* can be distinguished from these five specimens by the morphology of the male copulatory organ and anterior pair of dorsal haptoral accessory structures. Specimens which conform to our concept of *P. tsalickisi*, and hence the holotype, possess a larger j-shaped male copulatory organ, and the anterior pairs of the dorsal haptoral accessory structures are bilobate, whereas the remaining



Figs. 5–9 *Potamotrygonocotyle tsalickisi*. 5. Holotype whole-mount. 6. Male copulatory organ. 7. Egg. 8. Hook. 9. Anchor. Scale-bars: 5, 100 µm; 6, 8, 9, 25 µm; 7, 50 µm

specimens in the type-series of Mayes et al. (1981) have a, short, straight copulatory organ and semi-circular anterior dorsal haptor accessory structures. Thus, these five specimens will be hereafter referred to as *Potamotrygonocotyle* sp. A.

***Potamotrygonocotyle chisholmae* n. sp.**

Holotype, type-host and type-locality: MZUSP no. 6352; *Potamotrygon motoro* (Müller & Henle), Salobra River, District of Salobra, Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004) (56°22'42"W, 20°14'26"S).

Other specimens: Paratypes, MZUSP no. 6354 a-ak, CHIOC no. 36699 a-e, INPA no. 489 a-e, HWML no. 48395, USNPC no. 98534, Salobra River, District of Salobra, Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004) (56°22'42"W, 20°14'26"S); vouchers, MZUSP nos 6362 a-c, 6363, CHIOC nos 36700, 36801 a-b, INPA no. 490, HWML no. 48396, USNPC no. 98535, Paraje Santa Tecla, Municipality of Ituzaingó, Province

of Corrientes, Argentina (56°24'05.1"W, 27°36'34.4"S) and Presa de Yacyretá, Municipality of Ituzaingó, Province of Corrientes, Argentina (56°39'15.2"W, 27°29'31.8"S) during May 2005.

Site of infection: Gills.

Etymology: The specific name is for Dr Leslie Chisholm, The University of Adelaide, Australia, in recognition of her valuable work on the Monocotylidae.

Comparative measurements: Tables 1, 2.

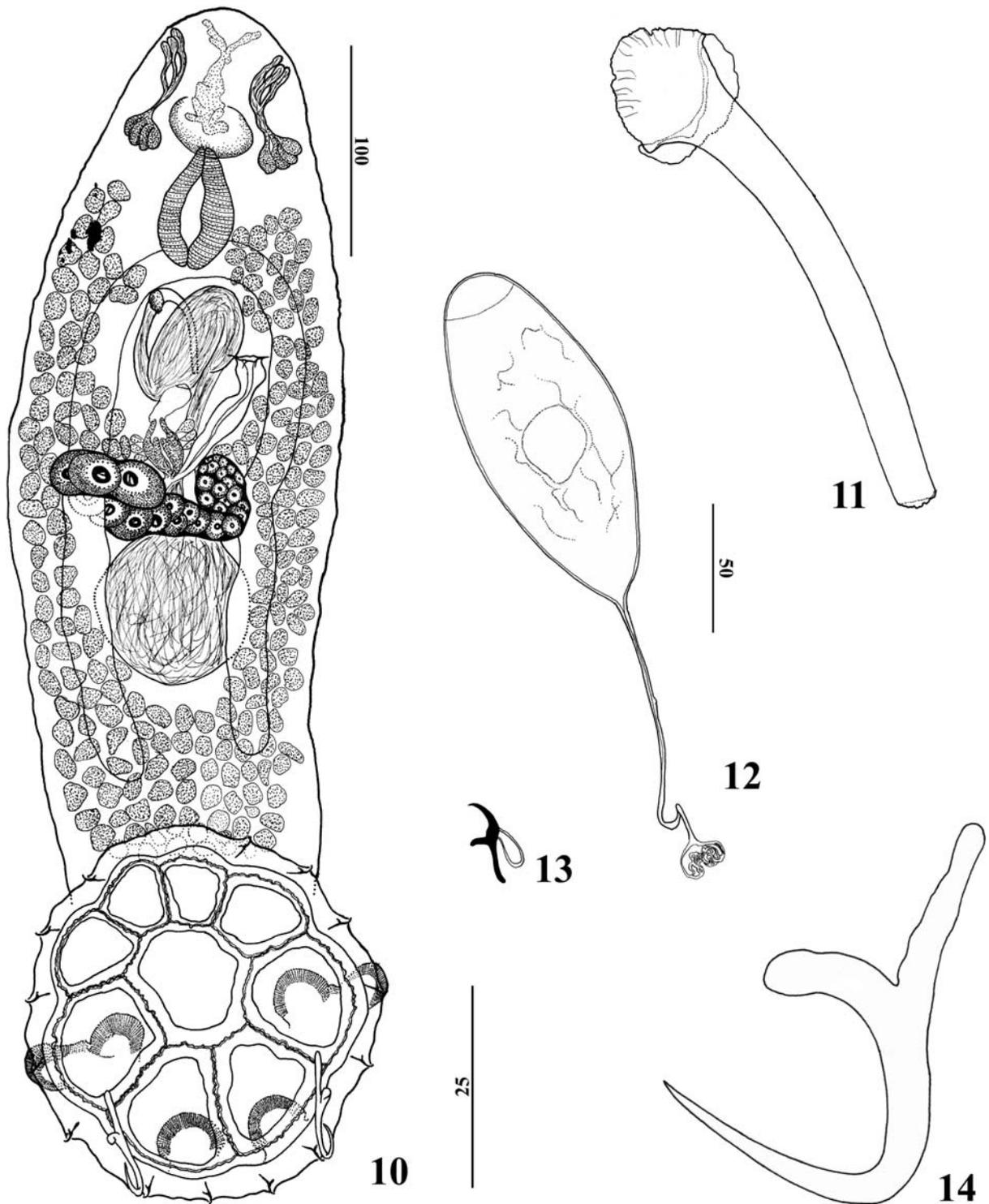
Description (Figs. 10–14, 31)

[Based on 50 specimens.] Body fusiform 432 (300–590; n = 17) long; 194 (140–280; n = 16) wide; widest at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs and cephalic glands anterolateral to pharynx. Anteromedial gland present. Dispersed pigment granules (eye-spots) prominent dorsolateral to pharynx. Mouth surrounded by slight sclerotisations. Pharynx

Table 2 Comparative measurements (μm) of specimens of *Potamotrygonocotyle chisholmae* n. sp. from *Potamotrygon motoro* in Brazil and Argentina

	<i>Potamotrygon motoro</i> (BR)	N	<i>Potamotrygon motoro</i> (AR)	N
Body				
Length	432 (300–590)	17	300 (260–340)	2
Width	194 (140–280)	16	120 (110–130)	2
Pharynx				
Length	55 (38–75)	17	33	1
Width	38 (25–55)	16	38	
Haptor				
Length	181 (140–215)	17	133 (120–145)	2
Width	187 (150–220)	16	140 (125–155)	2
Anchor				
Length	52 (43–66)	43	45 (42–47)	12
Base width	21 (17–27)	20	19 (17–21)	7
Hook lengths				
Pair 1	12 (10–14)	23	12 (11–12)	9
Pair 2	12 (10–14)	26	12 (10–12)	9
Pair 3	12 (10–13)	24	12 (11–12)	8
Pair 4	12 (11–13)	28	12 (11–12)	10
Pair 5	12 (10–13)	29	12 (10–12)	10
Pair 6	12 (10–15)	34	12 (10–12)	11
Pair 7	12 (10–15)	31	12 (11–12)	8
Copulatory organ				
Length	63 (33–75)	41	52 (47–55)	10
Testis				
Length	92 (68–118)	10	55 (48–63)	2
Width	72 (50–98)	8	56 (50–63)	2
Egg				
Length	142 (108–175)	15	–	–
Width	63 (35–78)	14	–	–

BR, Brazil; AR, Argentina



Figs. 10–14 *Potamotrygonocotyle chisholmae* n. sp. 10. Whole-mount. 11. Male copulatory organ. 12. Egg. 13. Hook. 14. Anchor. Scale-bars: 10, 100 μm ; 11, 13, 14, 25 μm ; 12, 50 μm

subspherical to elongate-oval, 55 (38–75; n = 17) × 38 (25–55; n = 16); oesophagus short. Haptor subcircular, 181 (140–215; n = 17) × 187 (150–220; n = 16); septa surmounted ventrally by slightly sinuous sclerotised ridge. Anterior pair of dorsal haptor accessory structures trilobate, with median lobe as slight projection; each lobe fan-like; posterior pair of dorsal haptor accessory structures fan-like. Anchors 52 (43–66; n = 43) long, base 21 (17–27; n = 20) wide, with heavily diverging roots, evenly curved shaft and point. Hooks similar, 12 (10–15; n = 195), distributed on marginal membrane of haptor, with depressed thumb, shaft with dilate proximal portion and curved point. Male copulatory organ a long straight slightly tapered tube, 63 (33–75; n = 41) long; distal aperture terminal. Testis subspherical, 92 (68–118; n = 10) × 72 (50–98; n = 8); seminal vesicle sigmoid; ejaculatory bulb not observed. Germarium unbranched; distal end ascendant; Mehlis' gland not observed. Vagina and vaginal canal muscular. Vaginal pore sinistroventral at level of common genital pore. Oötype well developed, with ascendant limb. Vitelline follicles coextensive with gut. Egg ovate, 142 (108–175; n = 15) × 63 (35–78; n = 14), with proximal filament same size as egg; distal end reticulate.

Remarks

Potamotrygonocotyle chisholmae n. sp. shares with *P. tsalickisi* most of its morphometric attributes except that the new species tends to have a smaller body length in comparison to the type-species (260–590 vs 630–900 µm, respectively; Table 2). *P. chisholmae* further differs from the type-species by having a trilobed anterior dorsal haptor accessory structure and fan-like posterior ones (Fig. 31), the presence of a long straight male copulatory organ, and the aperture of the distal end of male copulatory organ terminal (Table 2).

Potamotrygonocotyle dromedarius n. sp.

Holotype, type-host, type-locality: MZUSP no. 6354, *Potamotrygon motoro* (Müller & Henle), Salobra River, District of Salobra, Miranda, Mato

Grosso do Sul, Brazil (19–24 July 2004) (56°22'42"W, 20°14'26"S).

Other specimens: paratypes, MZUSP no. 6355 a-g, CHIOC no. 36802, INPA no. 491, HWML no. 48397, USNPC no. 98536, Salobra River, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004) (56°22'42"W, 20°14'26"S).

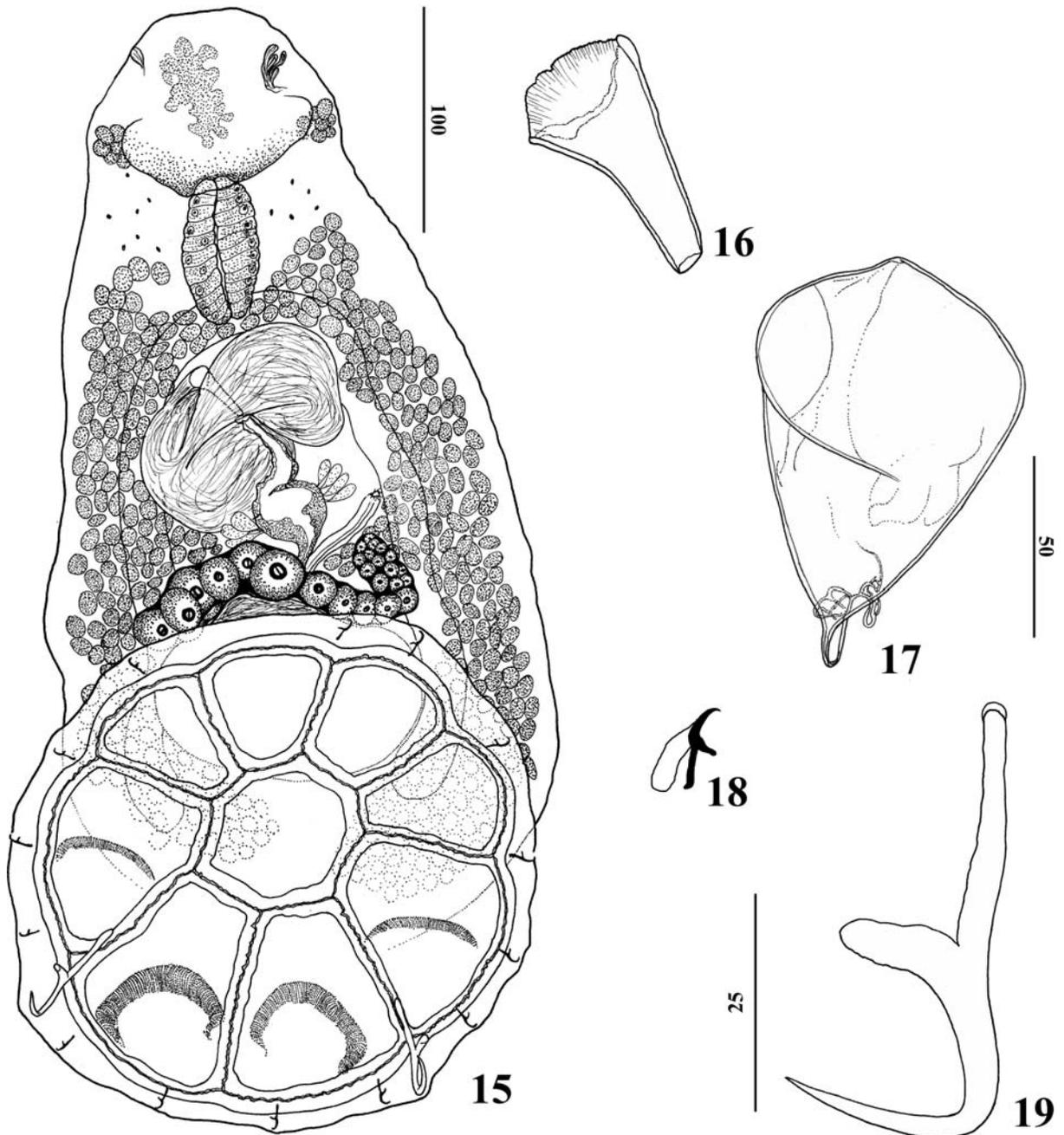
Site of infection: Gills.

Etymology: The specific name refers to the presence of semicircular anterior dorsal haptor accessory structures, rather than bilobate, as in the other species described herein. This name is treated as a noun.

Comparative measurements: Table 1.

Description (Figs. 15–19, 32)

[Based on 15 specimens.] Body fusiform 417 (310–550; n = 9) long; 240 (170–430; n = 9) wide, greatest width at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs; cephalic glands anterolateral to pharynx. Anteromedial gland present. Dispersed pigment granules (eye-spots) prominent laterodorsally to pharynx. Mouth surrounded by slight sclerotisations. Pharynx elongate-oval, 55 (50–63; n = 7) × 38 (38–40; n = 7); oesophagus short. Haptor subcircular; septa ventrally surmounted by slightly sinuous sclerotised ridge. Haptor disc $c.1/2$ body length, 242 (190–270; n = 6) × 259 (215–285; n = 7). Anterior pair of dorsal haptor accessory structures semicircular; posterior pair of dorsal haptor accessory structures rounded. Anchors 48 (37–55; n = 14) long, base 19 (16–20; n = 6) long, with greatly divergent roots; deep root twice as large as superficial root; evenly curved shaft and point. Hooks similar, 10 (9–12; n = 64) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with dilate proximal portion and curved point. Male copulatory organ 29 (22–33; n = 11) long, cornet-shaped, comprising short straight slightly tapered tube, with distal aperture terminal. Testis ovate, 125 (100–163; n = 7) × 94 (75–150; n = 6); seminal vesicle composed of 2 subspherical portions; ejaculatory bulb not observed. Germarium unbranched, with ascendant



Figs. 15–19 *Potamotrygonocotyle dromedarius* n. sp. 15. Whole-mount. 16. Male copulatory organ. 17. Egg (bent). 18. Hook. 19. Anchor. Scale-bars: 15, 100 µm; 16, 18, 19, 25 µm; 17, 50 µm

distal end; Mehlis' gland present. Vagina and vaginal canal muscular. Vaginal pore sinistro-ventral at mid-level of oötype. Oötype well

developed, with ascendant limb. Vitelline follicles coextensive with gut. Egg ovate, with short proximal filament; distal end reticulate.

Remarks

Potamotrygonocotyle dromedarius n. sp. closely resembles *Potamotrygonocotyle* sp. A. from *Potamotrygon constellata* (see comments above) based on the presence of semicircular anterior dorsal haptoral accessory structures. Because of the poor condition of the voucher slides of *Potamotrygonocotyle* sp. A. (HWML no. 21390), we cannot discern whether these specimens should be considered *P. dromedarius* or a new species.

Among the species described above, there is no morphometric attribute that distinguish specimens of *Potamotrygonocotyle dromedarius* from those assigned to *P. chisholmae* n. sp. (Table 2). Members of this species also possess a terminal male copulatory organ aperture. However, *P. dromedarius* can be distinguished from *P. chisholmae* by having semicircular anterior dorsal haptoral accessory structures and rounded posterior ones (Fig. 32), and the copulatory organ as a straight cornet-shaped structure (Table 2). *P. dromedarius* differs from *P. tsalickisi* by possessing a smaller body length, a different morphology of the accessory dorsal haptoral accessory structures, in the morphology of the copulatory organ, and in the position of the aperture of the copulatory organ.

Potamotrygonocotyle eurypotamoxenus n. sp.

Holotype, type-host, type-locality: Holotype, MZUSP no. 6356, *Potamotrygon* cf. *motoro* (Müller & Henle), Salobra River, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004) (56°22′42″W, 20°14′26″S). **Other specimens:** Paratypes, MZUSP no. 6357 a-l, CHIOC no. 36803, INPA no. 492 a,b, HWML no. 48398, USNPC no. 98537, *Potamotrygon* cf. *motoro* (Müller & Henle), Salobra River, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004) (56°22′42″W, 20°14′26″S); vouchers, MZUSP no. 6358 a-p, CHIOC no. 36804 a-d, INPA no. 493 a-e, HWML no. 48399, USNPC no. 98538, *P. castexi* Castello & Yagolkowski, Salobra River, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004); MZUSP no. 6359 a-s, CHIOC no. 36805 a-e, INPA no. 494 a-e, HWML

no. 48400, USNPC no. 98539; *P. hystrix* (Müller & Henle), Salobra River, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004); MZUSP no. 6360 a-g, 6361 a-h, CHIOC no. 36803 a-b, INPA no. 495 a,b, HWML no. 48401; *P. falkneri* Castex & Maciel, Salobra River, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (19–24 July 2004); MZUSP no. 6364; *P. motoro*, Paraje Santa Tecla, Municipality of Ituzaingó, Province of Corrientes (56°24′05.1″W, 27°36′34.4″S) and Presa de Yacyretá, Municipality of Ituzaingó, Province of Corrientes, Argentina (during May 2005) (56°39′15.2″W, 27°29′31.8″S).

Site of infection: Gills.

Etymology: The specific epithet is from Greek (*eurý* = broad + *potamo* = river *xen/o* = guest) and refers to the wide occurrence of this parasite within freshwater stingrays (Potamotrygonidae). This name is treated as a noun.

Comparative measurements: Tables 1, 3.

Description (Figs. 20–25, 33)

[Based on 20 specimens from type-series.] Body fusiform 453 (360–500; n = 7) long; 167 (150–180; n = 7) wide, greatest width at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs; anteromedial glands not observed. Dispersed pigment granules (eye-spots) prominent laterodorsally to pharynx. Mouth surrounded by slight sclerotisations. Pharynx elongate-oval, 66 (53–78; n = 5) × 39 (35–45; n = 5); oesophagus short. Haptor subcircular, 198 (185–205; n = 7) × 204 (180–220; n = 7), with septa surmounted ventrally by slightly sinuous sclerotised ridge. Anterior pair of dorsal haptoral accessory structures bilobate; each dorsal haptoral accessory structure with concave separation between lobes; posterior pair of dorsal haptoral accessory structures elongate and rounded. Anchors 54 (50–57; n = 16) long, base 20 (16–24; n = 11) long, with greatly divergent roots, evenly curved shaft and point. Hooks similar, 12 (11–13; n = 76) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ 53 (45–65; n = 10) long, arcuate; distal

Table 3 Comparative measurements (μm) of specimens of *Potamotrygonocotyle eurypotamoxenus* n. sp. from five species of *Potamotrygon* in Brazil and Argentina

	<i>Potamotrygon</i> cf. <i>motoro</i> (BR)	N	<i>Potamotrygon</i> <i>motoro</i> (AR)	N	<i>Potamotrygon</i> <i>castexi</i>	N	<i>Potamotrygon</i> <i>falkneri</i>	N	<i>Potamotrygon</i> <i>histris</i>	N
Body										
Length	453 (360–500)	7	380	1	485 (390–530)	8	350(320–380)	2	330 (260–400)	5
Width	167 (150–180)	7	150	1	194 (130–240)	8	185 (180–190)	2	140 (110–160)	5
Pharynx										
Length	66 (53–78)	5	45	1	67 (60–75)	7	58	1	50 (45–58)	5
Width	39 (35–45)	5	38	1	43 (35–50)	7	38	1	38 (30–43)	5
Haptor										
Length	198 (185–205)	7	160	1	204 (190–230)	8	193 (190–195)	2	160 (140–180)	4
Width	204 (180–220)	7	180	1	195 (150–245)	8	170 (140–200)	2	185 (150–200)	4
Anchor										
Length	54 (50–57)	16	46 (42–50)	2	54 (50–60)	33	51 (45–55)	13	52 (42–56)	25
Base width	20 (16–24)	11	20	1	22 (19–26)	20	23 (20–28)	15	23 (20–25)	21
Hook lengths										
Pair 1	13 (11–13)	8	12	1	11 (10–13)	27	11 (10–12)	15	11 (10–13)	26
Pair 2	12 (12–13)	11	11	1	11 (10–12)	32	11 (10–12)	15	11 (10–12)	25
Pair 3	12 (11–13)	10	12	1	11 (10–12)	35	11 (10–12)	14	11 (10–12)	23
Pair 4	12 (12–13)	11	12	1	11 (10–13)	32	11 (10–12)	16	11 (10–12)	26
Pair 5	12 (11–13)	10	12	1	11 (10–13)	32	11 (10–12)	16	11 (10–12)	24
Pair 6	12 (11–13)	13	12	1	11 (10–13)	33	11 (10–12)	15	11 (10–12)	25
Pair 7	12 (12–13)	13	12	1	12 (10–13)	29	11 (10–12)	17	11 (10–13)	22
Copulatory organ										
Length	53 (45–65)	10	35	1	48 (34–58)	28	42 (40–47)	14	45 (30–52)	23
Testis										
Length	107 (93–125)	5	–	–	128 (110–150)	3	100	1	81 (75–88)	3
Width	86 (75–95)	5	–	–	73 (58–93)	3	87	1	62 (58–65)	3
Egg										
Length	155	1	–	–	145 (110–158)	14	–	–	143	1
Width	65	1	–	–	67 (45–75)	13	–	–	78	1

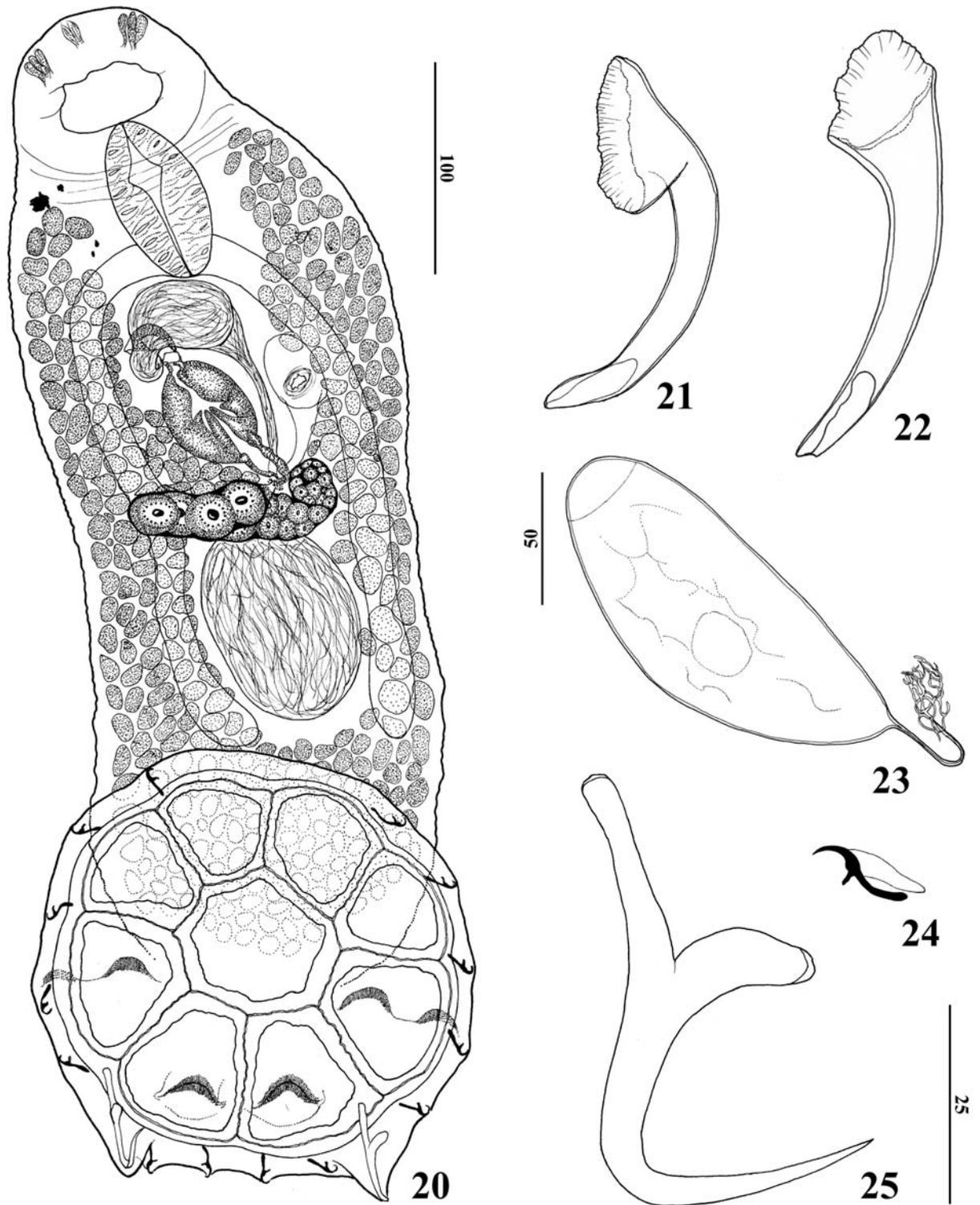
BR, Brazil; AR, Argentina

aperture subterminal. Testis ovate, 107 (93–125; $n = 5$) \times 86 (75–95; $n = 5$); seminal vesicle composed of single subspherical portion; ejaculatory bulb not observed. Germarium unbranched; distal end ascendant; Mehlis' gland not observed. Vagina and vaginal canal not sclerotised; vaginal vestibule present; vaginal pore sinistroventral, posterior to common genital pore. Oötype well developed, with ascendant limb. Vitelline follicles coextensive with gut. Egg ovate, 155 ($n = 1$) \times 65 ($n = 1$), with short proximal filament; distal end reticulate.

Remarks

Potamotrygonocotyle eurypotamoxenus n. sp. is most similar to *P. tsalickisi* with which it shares

most of its morphometric attributes, a bilobed anterior dorsal haptor accessory structure, an arcuate copulatory organ and a subterminal copulatory organ aperture (Fig. 33; Table 2). However, members of this new species differ from those assigned to the type-species by having a smaller body length (260–530 vs 630–900 μm , respectively) and the shape of the posterior dorsal haptor accessory structure (rounded and elongated vs rounded, respectively; Table 2). In addition, *P. eurypotamoxenus* can be distinguished from *P. chisholmae* n. sp. and *P. dromedarius* n. sp. by possessing an arcuate rather than straight copulatory organ, and a subterminal rather than terminal copulatory organ aperture (Table 2).



Figs. 20–25 *Potamotrygonocotyle eurypotamoxenus* n. sp. 20. Whole-mount. 21. Male copulatory organ (bent). 22. Male copulatory organ. 23. Egg. 24. Hook. 25. Anchor. Scale-bars: 20, 100 μm ; 21, 22, 24, 25, 25 μm ; 23, 50 μm

***Potamotrygonocotyle uruguayensis* n. sp.**

Holotype, type-host, type-locality: MZUSP no. 6365, *Potamotrygon brachyura* (Günther), Uruguay River, Porto Xavier, Rio Grande do Sul (055°08'00"W, 27°54'00"S), during March 2005.

Other specimens: Paratypes, MZUSP no. 6366 a-v, CHIOC no. 36807 a-d, INPA no. 496 a-d, HWML no. 48402, USNPC no. 98540, Uruguay River, Porto Xavier, Rio Grande do Sul, Brazil (055°08'00"W, 27°54'00"S), during March 2005.

Site of infection: Gills.

Etymology: The specific name refers to the Uruguay River, Rio Grande do Sul, Brazil, from which the type-material was collected.

Comparative measurements: Table 1.

Description (Figs. 26–30, 34)

[Based on 37 specimens.] Body fusiform 403 (310–550; n = 9) long; 137 (100–170; n = 9) wide; greatest width at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; 3 pairs of head organs; cephalic glands anterolateral to pharynx. Anteromedial gland present. Dispersed pigment granules (eye-spots) prominent dorsolateral to pharynx. Mouth surrounded by slight sclerotisations. Pharynx elongate ovate, 51 (48–55; n = 3) × 26 (23–30; n = 4); oesophagus short. Haptor subcircular, 171 (145–210; n = 9) × 165 (145–185; n = 8), with septa surmounted ventrally by slightly sinuous sclerotised ridge. Anterior pair of dorsal haptor accessory structures bilobate with divergent rounded lobes; each anterior dorsal haptor accessory structure with slightly concave separation between lobes; posterior pair of dorsal haptor accessory structures fan-shaped. Anchors 56 (51–60; n = 26) long, base 23 (20–25; n = 13) long, with heavily divergent roots, evenly curved shaft and point. Hooks similar, 13 (12–14; n = 126) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ 72 (67–78; n = 20) long, a long straight slightly tapered tube; distal aperture terminal. Testis ovate, 58 (50–75; n = 3) × 58 (53–63; n = 3); seminal vesicle composed of 2

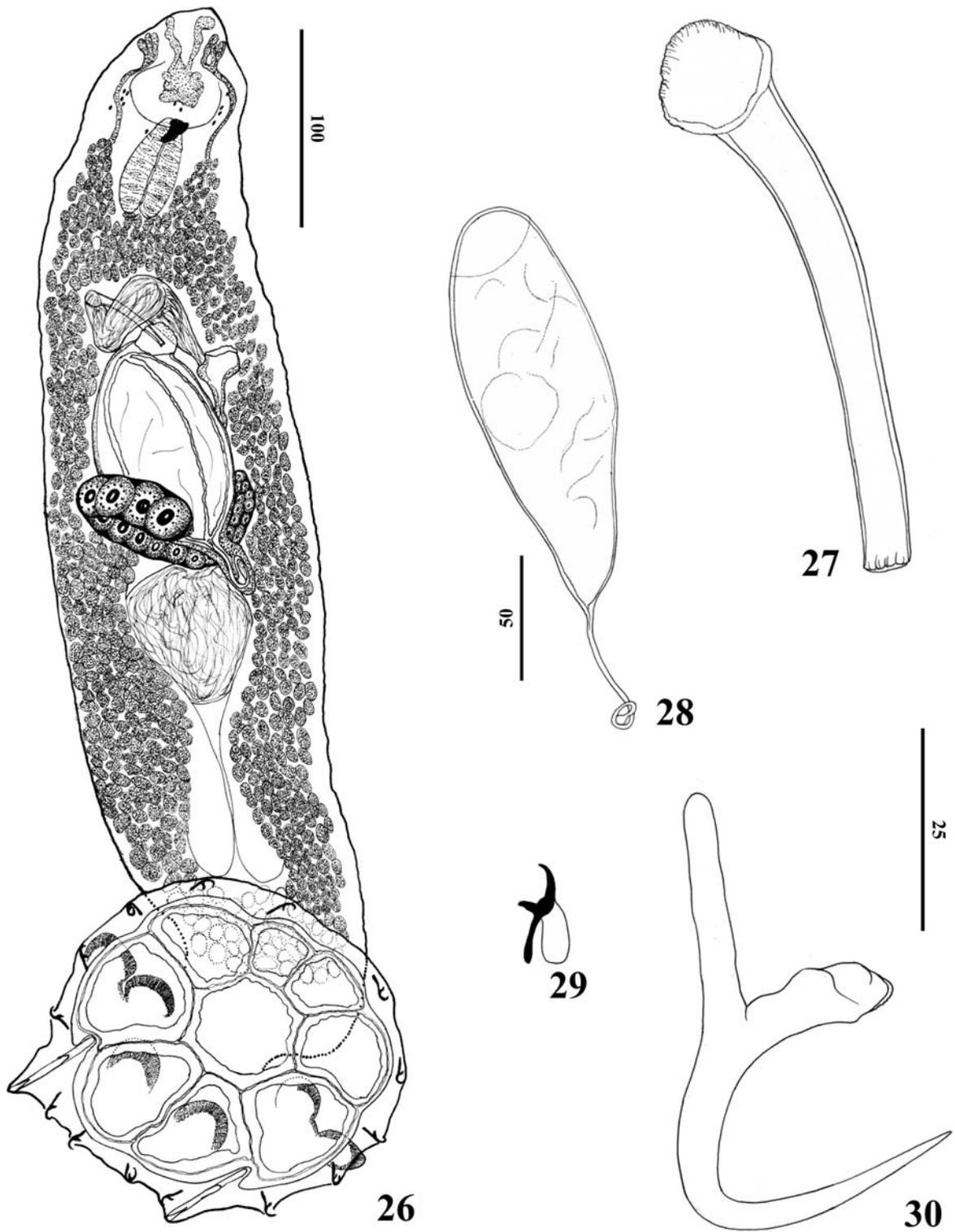
similar fusiform portions; ejaculatory bulb not observed. Germarium unbranched, with ascendant distal end; Mehlis' gland not observed. Vagina and vaginal canal not sclerotised; vaginal vestibule present; vaginal pore sinistroventral, at level of common genital pore. Seminal receptacle not observed. Oötype well developed, with ascendant limb. Vitelline follicles dense, coextensive with gut. Egg ovate, 155 (105–198; n = 8) × 65 (50–70; n = 8), with short proximal filament; distal end reticulate or pointed.

Remarks

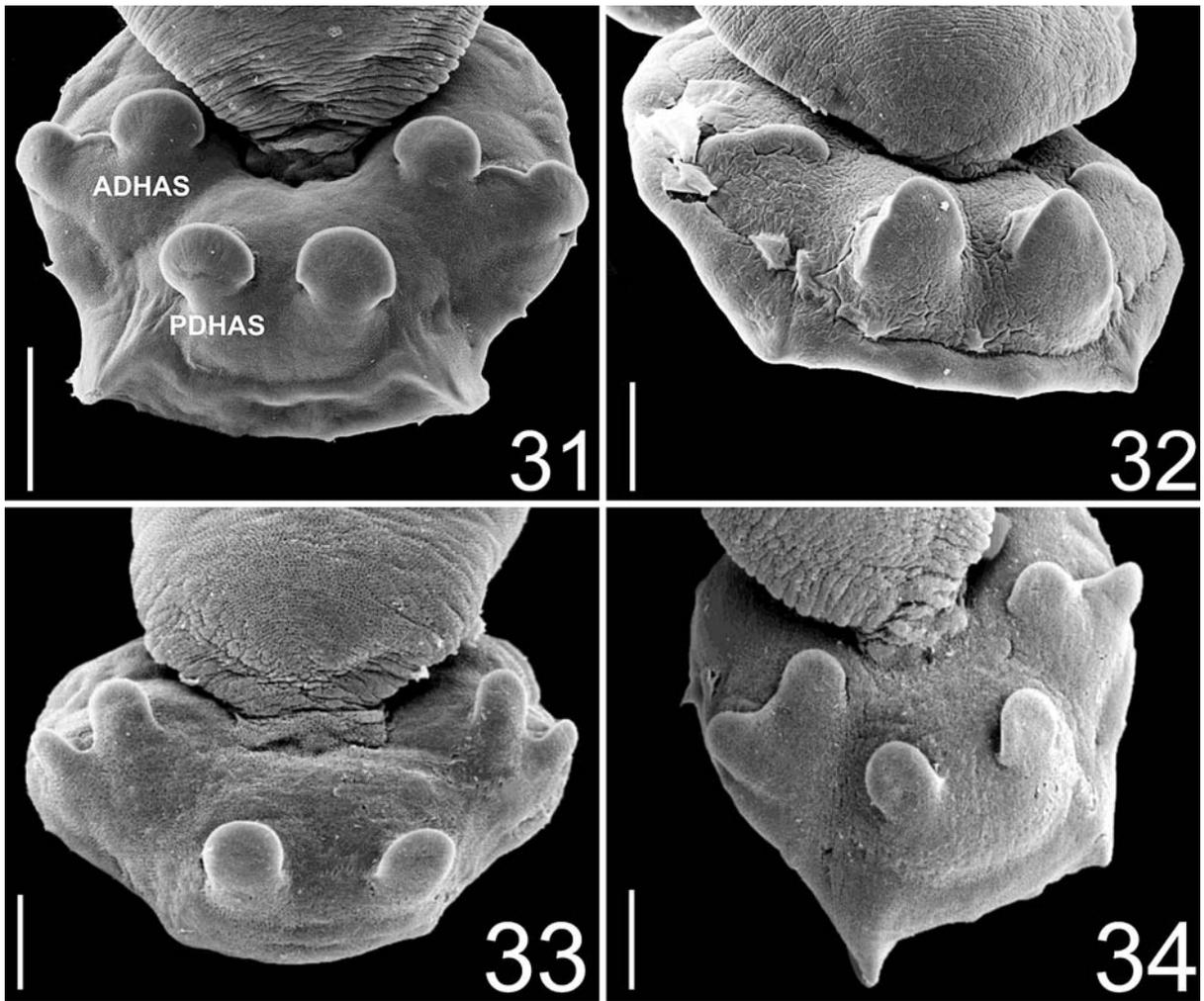
Potamotrygonocotyle uruguayensis n. sp. is most similar to *P. chisholmae* n. sp., since both share overlapping morphometric attributes and a similar morphology of the posterior dorsal haptor accessory structure, copulatory organ and position of the genital aperture (Table 2). However, specimens of *P. uruguayensis* can be distinguished from *P. chisholmae* by the morphology of the anterior dorsal haptor accessory structure (bilobate and divergent vs trilobate, respectively; Fig. 34, Table 2). Furthermore, the morphology of the anterior dorsal haptor accessory structure and the male copulatory organ distinguish member of this new species from those allocated to the remaining species (Table 2).

Key to *Potamotrygonocotyle* species (see also Table 2)

1. Haptor with semicircular anterior dorsal haptor accessory structure (DHAS); posterior DHAS rounded; male copulatory organ a straight cornet-shape with a terminal aperture *P. dromedarius* n. sp.
- Haptor with bilobate or trilobate anterior DHAS; posterior DHAS elongate, fan-like or rounded; male copulatory organ arcuate or straight, with a terminal or subterminal aperture 2
2. Haptor with trilobate anterior DHAS and fan-shaped posterior DHAS; male copulatory organ straight, with terminal aperture *P. chisholmae* n. sp.



Figs. 26–30 *Potamotrygonocotyle uruguayensis* n. sp. 26. Whole-mount. 27. Male copulatory organ. 28. Egg. 29. Hook. 30. Anchor. Scale-bars: 26, 100 μm ; 27, 29, 30, 25 μm ; 28, 50 μm



Figs. 31–34 General morphology of dorsal haptoral accessory structures of *Potamotrygonocotyle* spp. under SEM. 31. *P. chisholmae* n. sp. 32. *P. dromedarius* n. sp. 33. *P. eurypotamoxenus* n. sp. 34. *P. uruguayensis* n. sp. *Abbre-*

viations: ADHAS, anterior dorsal haptoral accessory structure; PDHAS, posterior dorsal haptoral accessory structure. *Scale-bars*: 30 μ m

- Haptor with bilobate anterior DHAS and posterior DHAS elongate, fan shaped or rounded; male copulatory organ arcuate or straight, with terminal or subterminal aperture 3
- 3. Haptor with bilobate/divergent anterior DHAS and fan-shaped posterior DHAS; male copulatory organ straight, with terminal aperture *P. uruguayensis* n. sp.
- Haptor with bilobate anterior DHAS, posterior DHAS rounded and elongate or

- rounded; male copulatory organ with subterminal aperture 4
- 4. Haptor with bilobate anterior DHAS and elongate, rounded posterior DHAS; male copulatory organ with subterminal aperture; body length 260–530 μ m *P. eurypotamoxenus* n. sp.
- Haptor with bilobate anterior DHAS and rounded posterior DHAS; male copulatory organ with subterminal aperture; body length 630–900 μ m..... *P. tsalickisi* Mayes, Brooks & Thorson, 1981.

Discussion

Potamotrygonocotyle is recognisable as a member of the Heterocotylinae due to the presence of a dorsal haptoral accessory appendix ('dorsal haptoral accessory sclerites' *sensu* Chisholm et al., 1995). Combined features distinguishing *Potamotrygonocotyle* from the other genera in the subfamily include: (1) the presence of a bilobate or semicircular anterior dorsal haptoral accessory structure; and (2) a male copulatory organ without an accessory piece.

The phylogenetic hypothesis proposed by Chisholm et al. (1995) suggested that *Potamotrygonocotyle* is the sister group of the clade (*Spinuris* (*Nonacotyle*, *Neoheterocotyle*)). This relationship is supported by the presence of a haptor with dorsal sclerites. However, the new interpretation of the haptoral structures (see generic diagnosis) and the inclusion of new taxa (e.g. *Myliocotyle* Neifar, Euzet & Ben Hassine, 1999 and *Heliocotyle* Neifar, Euzet & Ben Hassine, 1999) suggests that the relationships of the taxa allocated to the Heterocotylinae at the generic level should be reassessed.

Rohde (1993) reported that species of the Monogenoidea have the highest degree of host-specificity among parasites of marine fishes. This author found that 78% of 435 species of monogenoideans from several seas are restricted to one host species, 89% to one genus, 96% to one family and 98% to one order. However, Chisholm and Whittington (1996) found some species of monocotylids occurring on more than one host (e.g. *Heterocotyle chinensis* Timofeeva, 1983 on *Dasyatis akajei*, *D. fluviorum* and *Himantura uarnak*), suggesting that *Heterocotyle* spp. may not be strictly host-specific. Similar patterns of low host specificity are observed for other monocotylids, such as *Neoheterocotyle* Hargis, 1955, *Spinuris* Doran, 1953, *Calicotyle* Diesing, 1850 and *Empruthotrema* Johnston & Tieg, 1922 (see Chisholm & Whittington, 1997; Neifar, Euzet & Ben Hassine, 2000; Gomez del Prado & Euzet, 1999). However, a strict host-specificity is reported for some monocotylid species. Neifar et al. (2000) reported a high host-specificity for species of the Heterocotylinae and Merizocotylinae parasitising Myliobatiformes

off the Tunisian coast. In the present work, we observed different patterns of host-specificity for *Potamotrygonocotyle*, where more than one species occur on one host (e.g. *P. chisholmae* n. sp., *P. dromedarius* n. sp. and *P. eurypotamoxenus* n. sp. on *Potamotrygon motoro*); one species occurs on more than one host (e.g. *Potamotrygonocotyle eurypotamoxenus* on *Potamotrygon castexi*, *P. hixtrix*, *P. falkneri*, *P. motoro* and *P. cf. motoro*); and one species of parasite is restricted to one host species (e.g. *Potamotrygonocotyle tsalickisi* and *P. uruguayensis* on *Potamotrygon constellata* and *P. brachyura*, respectively).

The high host-specificity of monogenoideans appears to be related to ecological selection, reproduction strategies (a tendency toward K-strategies), a direct life-cycle and complex attachment structures specialised for specific sites on the host (see Kearn, 1994; Rohde, 1993). Chisholm & Whittington (1997) suggested that the puzzling question of host-specificity of the monocotylids is not easily resolved and that this problem is probably due to uncertainties in the identification of the hosts and their parasites.

The discovery of four new species of monogenoideans from potamotrygonids in the southern basins suggests that new species will probably be found during future fieldwork in other basins of South America. We also expect that more collections will undoubtedly provide more information on the host-specificity of these worms.

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