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Phylogeny and taxonomy of *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981 (Monogenoidea: Monocotylidae) with a description of four new species

M.V. Domingues*[†] and F.P.L Marques

Laboratório de Helmintologia Evolutiva, Departamento de Zoologia,
Instituto de Biociências, Universidade de São Paulo, Rua do Matão,
trav. 14, n° 101 Cidade Universitária, 05508-090, São Paulo, SP, Brazil

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Abstract

The marine-derived stingrays of Potamotrygonidae, endemic to South American river systems, host an interesting parasite fauna equally related to marine lineages. Among those lineages, the diversity and phylogenetic relationships within *Potamotrygonocotyle* – a monocotylid monogenoidean specific to potamotrygonids – are poorly known, since 9 of 10 species attributed to this genus have been described in the past 3 years. Here, we readdress the diversity of *Potamotrygonocotyle* after examining the gills of 436 potamotrygonid individuals representing 12 described and 14 potentially undescribed species of freshwater stingrays from 19 major river systems of South America (i.e. sub-basins). We recognized 12 valid species within the parasite genus, of which four are described in this study. Our taxonomic decisions were based on the phylogenetic analysis of 14 ingroup terminal taxa and 12 morphological characters, which resulted in the following hypothesis of sister-group relationships: ((*P. dromedarius*, *P. tatianae* sp. nov.), (*P. rionegrense*, *P. auriculocotyle* sp. nov., ((*P. quadracotyle*, *P. umbella*), (*P. septemcotyle* sp. nov., (*P. chisholmae*, *P. uruguayense*)), (*P. tsalickisi*, *P. eurypotamoxenus*, *P. rarum*, (*P. tocantinsense* sp. nov., *P. aramasae*))))). According to our hypothesis, the absence of autapomorphic features for some nominal species, and the re-evaluation of morphological variation among populations, led us to consider *P. eurypotamoxenus* and *P. uruguayense* as junior synonymys of *P. tsalicksi* and *P. chisholmae*, respectively. Finally, we address the importance of biogeographic and host representation, in order to fully understand the patterns of morphological variation and host specificity within this group. We found that hypotheses of species delimitation depend greatly on efforts to sample specimens throughout its distributional range and that host specificity within this genus varies dramatically among lineages.

Introduction

The rivers of South and Central America host the most diverse ichthyofauna on the planet, inhabited by ~6000 out of ~13,000 species reported throughout the world (Reis *et al.*, 2003). A considerable component of the Neotropical freshwater ichthyofauna is represented by predominantly marine groups, such as anchovies,

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*E-mail: mvdomingues71@gmail.com
[†] Present address: Universidade Federal do Pará, Campus Universitário de Bragança, Instituto de Estudos Costeiros, Alameda Leandro Ribeiro s/n, 68600-000, Bragança, PA, Brazil.

croakers, sardines, needlefishes, flatfishes, puffers and stingrays (Marlier, 1967; Géry, 1969; Roberts, 1972; Fink & Fink, 1979; Reis *et al.*, 2003). Earlier attempts to address the origin of this fauna suggested that the fluvial nature of the Amazon River (i.e. slow speed, absence of rapids and large dimensions) would favour the dispersion of marine taxa into the South American river systems (Marlier, 1967; Roberts, 1972). However, in the past three decades, other explanations for the origin and diversification of Neotropical fish of marine origin have been proposed (Brooks *et al.*, 1981; Domning, 1982; Grabert, 1983; Nuttal, 1990; Webb, 1995; Lovejoy, 1997; Lovejoy *et al.*, 1998, 2006, 2010; Albert *et al.*, 2006) and the current available data suggest that a great part of this component of the Neotropical biodiversity derived from marine regions during the sea incursions in the Miocene (Boeger & Kritsky, 2003; Lovejoy *et al.*, 2006; but see Carvalho *et al.*, 2004).

The freshwater stingrays of the family Potamotrygonidae are restricted to rivers of South America that flow into the Atlantic Ocean and Caribbean Sea. The family comprises two monotypic genera, *Paratrygon* and *Plesiotrygon*, and the specious *Potamotrygon*. For the latter, Deynat (2006) suggested that its diversity is represented by 16 species and Rosa *et al.* (2008) estimated 19–21 species. However, recent surveys of potamotrygonids from Amazonian drainages revealed that the diversity of *Potamotrygon* is underestimated (F.P.L. Marques, unpublished data; M.R. de Carvalho, pers. comm.).

The origin of the freshwater potamotrygonids has been contentious (Brooks *et al.*, 1981; Lovejoy, 1997; Hoberg *et al.*, 1998, Lovejoy *et al.*, 1998, Carvalho *et al.*, 2004). The hypothesis of Brooks *et al.* (1981), for the origin of the freshwater potamotrygonids based on their parasite fauna, contradicted earlier beliefs that the marine-derived Neotropical fish fauna had entered South American river systems through the current mouth of the Amazon, or resulted from multiple invasions of rivers, which at present open into the Atlantic and Caribbean oceans. Later reiterated by Brooks (1992, 1995), Hoberg *et al.* (1998) and Zamparo *et al.* (1999), this hypothesis entertained the idea of the Pacific origin of Neotropical freshwater stingrays, despite substantial counter-evidence from host phylogeny and biogeographical distribution (Lovejoy, 1997; Lovejoy *et al.*, 1998; Marques, 2000; but see Carvalho *et al.*, 2004) and severe critiques on the reliability of the parasite data (Caira, 1990, 1994) and biogeographical methodology used (Straney, 1982).

Host–parasite relationships have the potential to enhance our understanding of host phylogeny, coevolution and historical biogeography. Von Ihering (1891, 1902) first proposed that the relationships of parasites could provide an insight into the phylogeny of their hosts, as well as an independent source of data to reconstruct the historical connection of geographical areas (e.g. southern South America and New Zealand). This research programme has become known as the 'von Ihering method' (Metcalf, 1929). For many decades since its inception, generations of parasitologists have increasingly developed the theoretical and conceptual aspects of this research programme as well as providing ample empirical documentation of such coevolutionary processes (e.g. Metcalf, 1923; Deets, 1987; Klassen & Beverly-Burton, 1988; Hafner & Nadler, 1990; Hoberg, 1992; Klassen, 1992; Brooks & McLennan, 1993; Page & Charleston, 1998; Carreno & Hoberg, 1999; Dolezel *et al.*, 1999; Paterson *et al.*, 2000; Brooks *et al.*, 2001; Paterson & Banks, 2001). This is the context in which parasite data have been used to address the history of potamotrygonid diversification.

Historical associations between hosts and parasites require extensive and accurate information on the host and parasite lineages. As Caira *et al.* (2001) point out, high-quality and extensive taxonomic and phylogenetic data must be provided prior to addressing questions regarding the evolution of any host/parasite systems. However, despite several recent systematic reviews of potamotrygonid parasites, most on cestodes (Brooks & Amato, 1992; Marques & Brooks, 2003; Marques *et al.*, 2003; Reyda, 2007), the parasites of potamotrygonid stingrays still remain poorly understood (Luchetti *et al.*, 2008). That is particularly true for monogenoids, whose taxonomy has been neglected for decades and has just recently been better documented (Domingues & Marques, 2007; Domingues *et al.*, 2007).

Among the lineages of monogenoids reported to the Neotropical freshwater system, members of typically marine monogenoid groups are also reported from marine-derived hosts (e.g. Monocotylidae, Hexabothriidae, Diplectanidae and Microcotylidae), suggesting that the parasite diversification tracked the colonization of this derived biogeographical area. Evidence for this assertion can be found in the study of Boeger & Kritsky (2003) who proposed a hypothesis for the historical biogeography of the freshwater croakers, *Plagioscion*, based on their monogenoidean parasites (i.e. *Euryhaliotrema*), palaeogeographical history of South America and fossil records. They suggested that the colonization of this derived environment by this host–parasite system probably occurred via marine incursions through western Venezuela during the Miocene. The hypothesis advanced by Boeger & Kritsky (2003) is congruent with what has been suggested for the biogeographical history of potamotrygonids (Lovejoy *et al.*, 1998; Marques, 2000; but see Carvalho *et al.*, 2004). However, at this moment there are insufficient data to address the phylogenetic position of the monogenoidean parasites of potamotrygonids within marine monocotylids, as well as to fully assess their evolution, diversification, relationships and biology (e.g. host specificity) in the large Neotropical river system.

As a result of the most recent efforts to document the diversity of monogenoidean parasites of potamotrygonids (Domingues & Marques, 2007; Domingues *et al.*, 2007), presently we recognize ten species of monocotylids and a single species of hexabothriid for Neotropical freshwater stingrays. Here, we re-evaluate the taxonomic status of some nominal species of *Potamotrygonocotyle*, propose four new species, and provide the first phylogenetic hypothesis for its members based on morphological data.

Materials and methods

Parasitological procedures

Specimens of potamotrygonids were collected using spears, gill nets or long lines in Brazil, Argentina and Peru during 2001–2007. 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 and Wess medium to study sclerotized structures (Humason, 1979). Whole mounts of monogenoids were stained with Gomori's trichrome to examine internal features (Humason, 1979). In the Results section, measurements are all given in micrometres. 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 linear distances between extreme ends. The mean measurements are followed by ranges and number of specimens measured (*n*) in parentheses.

Illustrations were prepared with the aid of a drawing tube on an Olympus BX–51 microscope with differential interference contrast and phase-contrast optics. Specimens prepared for scanning electron microscopy (SEM) were post fixed in 1% osmium tetroxide for 1–2 h at room temperature, dehydrated in a graded ethanol series, critical point dried and sputter-coated with gold.

Basins and sub-basin nomenclature follows the Agência Nacional de Águas, Ministério do Meio Ambiente, Brazil (http://hidroweb.ana.gov.br/). Type specimens and vouchers are deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, Brazil; Coleção Helmintológica do Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, SP, Brazil; Instituto de Pesquisas da Amazônia (INPA), Manaus, AM, Brazil; the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska, USA; and the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA. Morphological terminology of monocotylid follows Chisholm *et al.* (1995). Hosts have been deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP), SP, Brazil and information about them is available online (http://www.ib.usp.br/hpc/hpc_search.php).

Phylogenetic analysis

Twelve characters were used for the phylogenetic analysis of 16 terminals, which included 14 species of *Potamotrygonocotyle*, and *Heliocotyle* and *Myliocotyle* used as outgroups to root the cladogram. The codings for the outgroup terminals represent summaries of the following nominal species: *Myliocotyle pteromylaei* Neifar, Euzet & Ben Hassine, 1999 (Muséum National d'Histoire Naturelle (Paris), MNHN no. 644 HF–Tk 178, 645 HF–Tk180) and *Heliocotyle kartasi* Neifar, Euzet & Ben Hassine, 1999 (MNHN no. 580 HF–Tk 97–98), as well as information available in the literature (i.e. Neifar *et al.*, 1999a, b; Chisholm & Whittington, 2000, 2004). The choice of the outgroup taxa was based on an ongoing, more inclusive phylogenetic study of Monocotylidae (M.V. Domingues, unpublished data). Characters and character states were defined based on light microscopy and SEM examination of type specimens and/or vouchers. The data matrix (table 1) was constructed using the program Windows Notepad version 5.1. The phylogenetic analysis was performed with the program T.N.T. (Goloboff *et al.*, 2008), using implicit enumeration, as the tree search algorithm, and collapsing unsupported branches after search. Character optimization was verified using Winclada (version 1.00.08; Nixon, 1999–2002). All characters were considered unordered and equally weighted.

Results

Character analysis

Characters used in the analysis are described as follows: character definition, consistency indices (CI; Kluge & Farris, 1969) and retention indices (RI; Farris, 1989) between square brackets, and assigned character states (codes within parentheses). The character matrix

Table 1. Character matrix to reconstruct evolutionary relationships of *Potamotrygonocotyle* spp. Abbreviations: 1–12 refer to characters (see *Character analysis* in Results section for character definition); '?' refers to an unknown character state; '–' refers to an inapplicable state; numbers in square brackets refer to polymorphic characters.

	Characters											
Taxa	1	2	3	4	5	6	7	8	9	10	11	12
Heliocotyle	[02]	0	0	0	0	–	0	0	–	0	0	0
Myliocotyle	0	0	0	1	2	–	1	0	–	0	0	[01]
P. tsalickisi	[02]	1	0	1	1	0	1	1	0	1	0	0
P. chisholmae	2	0	0	1	1	0	1	1	1	1	1	0
P. dromedarius	1	0	0	1	2	–	0	1	0	1	0	1
P. eurypotamoxenus	2	1	0	1	1	0	1	1	0	1	0	0
P. uruguayense	2	0	0	1	1	0	1	1	1	1	1	0
P. quadracotyle	1	0	0	2	1	1	2	1	0	1	0	0
P. umbella	2	0	0	1	1	1	2	1	0	1	0	0
P. rarum	[02]	1	0	1	1	0	1	1	0	?	?	1
P. rionegrense	0	0	0	1	1	0	1	1	0	1	0	0
P. amarasae	2	1	1	1	[12]	–	1	1	0	1	0	0
P. tatianae sp. nov.	1	0	0	1	2	–	1	1	0	1	0	1
P. tocantinsense sp. nov.	1	1	1	1	1	0	1	1	0	1	1	0
P. septemcotyle sp. nov.	2	0	0	0	1	0	1	1	0	1	1	0
P. auriculocotyle sp. nov.	0	0	0	1	1	0	0	1	0	?	?	0

used for this analysis is presented in the table 1. In the following, 'fig.' indicates a figure in the present paper and 'Fig.', a figure in cited references.

Character 1: Male copulatory organ [CI = 50; RI = 60]
(0) Inverted 'J'-shaped tube (e.g. fig. 8B; Neifar *et al.*, 1999a, Fig. 4; Neifar *et al.*, 1999b, Fig. 3; Chisholm & Whittington, 2004, Figs 4 and 7; Domingues *et al.*, 2007, Figs 23–24); (1) short, straight/arcuate tube (e.g. see figs 4B, 5B; Domingues & Marques, 2007, Fig. 16; Domingues *et al.*, 2007, Fig. 2); (2) long, straight/arcuate tube (e.g. fig. 6B; Chisholm & Whittington, 2000, Fig. 2B; Domingues & Marques, 2007, Figs 11, 21–22, 27; Domingues *et al.*, 2007, Figs 13–14, 29). The definition of short and long tubes is made by the comparison between the size of base of the male copulatory organ (MCO) and its length (fig. 4B). For instance, long MCOs possess length > three times the length of the base, whereas short MCOs possess length ≤ three times the length of the base. This character is polymorphic for *Heliocotyle* (i.e. *H. kartasi*, inverted 'J'-shaped MCO, versus *H. ewingi*, long, straight/arcuate MCO), *Potamotrygonocotyle tsalickisi* and *P. rarum*.

Character 2: Distal portion of the male copulatory organ [CI = 100; RI = 100]
(0) Terminal (e.g. figs 4B, 6B, 8B; Neifar *et al.*, 1999a, Fig. 4; Neifar *et al.*, 1999b, Fig. 3; Chisholm & Whittington, 2000, Fig. 2B; Chisholm & Whittington, 2004, Figs 4 and 7; Domingues & Marques, 2007, Figs 11, 16, 27); (1) subterminal (e.g. fig. 5B; Domingues & Marques, 2007, Figs 6, 21–22; Domingues *et al.*, 2007, Figs 19, 29).

Character 3: Anterior cavities in the ejaculatory bulb [CI = 100; RI = 100]
(0) Absent; (1) present (e.g. fig. 5A; Domingues *et al.*, 2007, Fig. 31).

Character 4: Number of peripheral loculi in the haptor [CI = 66; RI = 0]
(0) Seven (e.g. figs 6A, 7; Neifar *et al.*, 1999b, Figs 1–2; Chisholm & Whittington, 2000, Figs 1, 3A); (1) eight (e.g. figs 4A, 5A, 8A; Neifar *et al.*, 1999a, Figs 1–2; Chisholm & Whittington, 2004, Figs 1, 2, 5; Domingues & Marques, 2007, Figs 1, 5, 10, 15, 20, 26; Domingues *et al.*, 2007, Figs 12, 18, 22, 28); (2) four (e.g. Domingues *et al.*, 2007, Figs 1, 6, 10).

Character 5: Dorsal haptoral accessory structure associated with the posterolateral loculi [CI = 100; RI = 100]
(0) Absent; (1) bilobate (e.g. figs 3B, 9B–D); (2) semicircular (e.g. figs 3D, 4A, 9A; Chisholm & Whittington, 2004, Figs 1, 2, 5; Domingues & Marques, 2007, Figs 15, 32). This character is polymorphic for *Potamotrygonocotyle aramasae*.

Character 6: Dorsal haptoral accessory structure associated with the posterolateral loculi (when bilobate) [CI = 100; RI = 100]
(0) Semicircular lobes (e.g. fig. 9B–D; Domingues & Marques, 2007, Figs 31, 33–34; Domingues *et al.*, 2007, Figs 35–37); (1) elongate lobes (e.g. Domingues *et al.*, 2007, Figs 7, 34).

Character 7: Dorsal haptoral accessory structure associated with the posterior loculi [CI = 50; RI = 33]
(0) Semicircular (e.g. fig. 9D; Domingues & Marques, 2007, Fig. 32); (1) elongate laterally (e.g. figs 3B, 3D, 9A–C; Domingues & Marques, 2007, Figs 31, 33–34; Domingues *et al.*, 2007, Figs 35–37); (2) elongate anteroposterior (e.g. Domingues *et al.*, 2007, Figs 7, 34).

Character 8: Mouth sclerotization [CI = 100; RI = 100]
(0) Absent; (1) present (fig. 1). Sclerotization of the mouth is defined by the presence of a sclerotized line, anterior and posterior to the mouth. Using SEM, Chisholm & Whittington (2000) reported an anterior

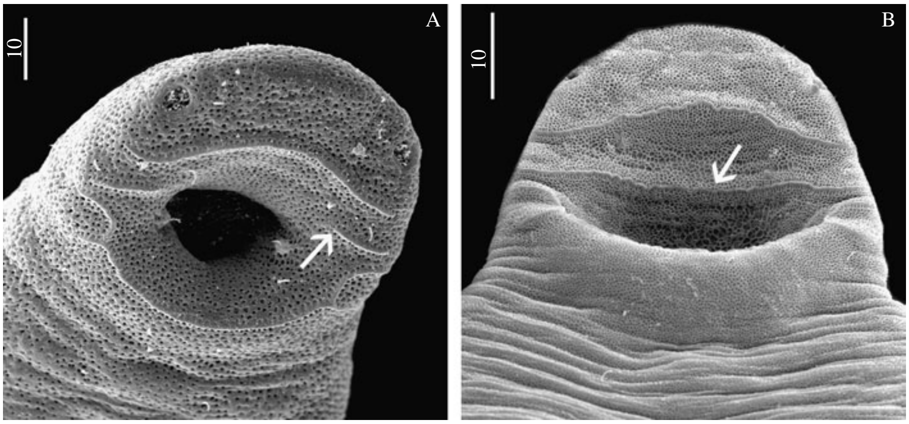

Fig. 1. Morphology of the anterior region of *Potamotrygonocotyle*. (A) Mouth of *P. umbella* Domingues, Pancera & Marques, 2007, showing the discontinuous medial sclerotization; (B) mouth of *P. chisholmae* Domingues, & Marques, 2007, showing the continuous medial sclerotization. Arrows show the medial sclerotization. Scale bars in μm.

ridge (line) on the mouth of *Heliocotyle ewingi* Chisholm & Whittington, 2000; however, these authors were not sure whether this structure was muscular or sclerotized. However, we examined *H. kartasi* (MHNH no. 580 HF–Tk 97–98) under differential interference contrast (DIC) and phase-contrast light microscopy, and the ridge does not look similar to the ridge observed on all septa that we know to be sclerotized. Thus, *Heliocotyle* was coded as not having this feature.

Character 9: Mouth sclerotization (medial sclerotization) [CI = 100; RI = 100]

(0) Medial sclerotization discontinuous (fig. 1A); (1) medial sclerotization continuous (fig. 1B).

Character 10: Egg [CI = 100; RI = 100]

(0) Tetrahedral (e.g. Neifar *et al.*, 1999a, Fig. 5a); (1) ovate (e.g. Domingues & Marques, 2007, Fig. 3).

Character 11: Egg filament [CI = 50; RI = 66]

(0) Short (e.g. fig. 4C; Domingues & Marques, 2007, Figs 7, 17, 23; Domingues *et al.*, 2007, Figs 3, 15, 25, 30); (1) long (e.g. figs 5C, 6C; Domingues & Marques, 2007, Figs 12, 28). A long egg filament is defined by the length of the filament being ≥ 0.5 times the length of the egg, whereas a short egg filament has a length < 0.5 times the length of the egg.

Character 12: Roots of the anchor [CI = 50; RI = 50]

(0) Deep root equal to or 1.5 times bigger than superficial root (e.g. figs 5E, 6E, 8D; Domingues & Marques, 2007, Figs 9, 14, 25, 30; Domingues *et al.*, 2007, Figs 5, 17, 27, 33); (1) deep root twice as big as the superficial root (e.g. fig. 4E; Domingues & Marques, 2007, Fig. 19; Domingues *et al.*, 2007, Fig. 21). This character is polymorphic for *Myliocotyle* (i.e. *M. borneoensis* Chisholm & Whittington, 2004 – deep root twice as big as the superficial root – versus *M. pteromylaei* and *M. multicrista* Chisholm & Whittington, 2004 – deep root twice as big as the superficial root).

Phylogenetic analysis

The phylogenetic analysis based on 12 morphological characters (table 1) for 16 terminals resulted in a single most parsimonious topology 23 steps long with a CI = 69 and RI = 72 (fig. 2). *Potamotrygonocotyle* can be defined by two unambiguous synapomorphies: (1) the presence of sclerotizations around the mouth (character 8), and (2) the presence of ovate eggs (character 10). As a result of the restricted number of characters we were able to compile for this dataset, 30% of the nodes remained unresolved after phylogenetic analysis. We also observed that some nominal species of *Potamotrygonocotyle* resulted in polytomic nodes (e.g. node H in fig. 2) or as sister taxa of other nominal species (e.g. node G in fig. 2) without any autapomorphies that could diagnose them. Based on this systematic evidence, we have made some taxonomic decisions that will be discussed in detail below.

Systematics

POLYONCHOINEA Bychowsky, 1937
MONOCOTYLIDAE Taschenberg, 1879
HETEROCOTYLINAE Chisholm, Wheeler & Beverley-Burton, 1995
Potamotrygonocotyle Mayes, Brooks & Thorson, 1981

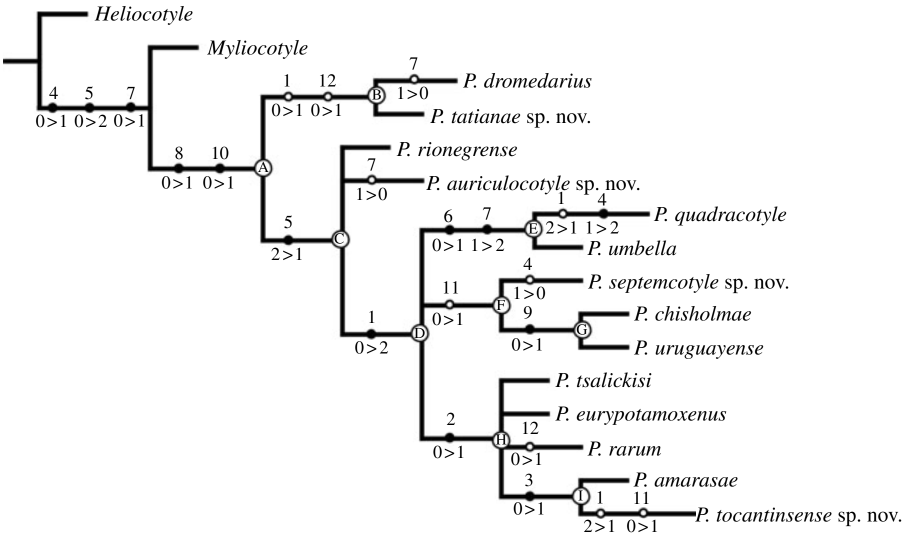

Fig. 2. Phylogenetic hypothesis for 14 terminals for *Potamotrygonocotyle*, including ten nominal species and four newly described taxa, based on 12 morphological characters. *Heliocotyle* and *Myliocotyle* are presented as outgroups. Tree length = 23; consistency index = 69; retention index = 72. Numbers above the branches indicate respective characters. Numbers below the branches refer to postulated evolutionary changes. Open circles on the branches indicate a homoplastic character state. Filled circles on the branches indicate a synapomorphic or autapomorphic character state. Open circle with Roman alphabet characters on the nodes of the ingroups are used to indicate grouping information available in the text.

Type species. *P. tsalickisi* Mayes, Brooks & Thorson, 1981.

Remarks. *Potamotrygonocotyle* was revised by Domingues & Marques (2007).

Potamotrygonocotyle tsalickisi Mayes, Brooks & Thorson, 1981

Synonymy. Potamotrygonocotyle eurypotamoxenus Domingues & Marques, 2007

Type host and type locality. Potamotrygon constellata (Vaillant, 1880), Rio Itacoaí, 5 km SE Atalaia do Norte, Brazil, July 1976; July 1978.

Other hosts and localities. *Potamotrygon castexi* Castello & Yagolkowski, 1969, Rio Salobra, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (56°22′42″W, 20°14′26″S), 19–24 July 2004 (Host nos MZUSP MS04–02, MS04–05–06, MS04–11, MS04–21–22, MS04–26; MS04–39 and MS04–44); *P.* cf. *castexi* Rio Madre de Dios, Municipality of Boca Manu, Madre de Dios, Peru (12°17′047″W, 70°53′086″S), May 2001 (Host no. MZUSP PU–19); *P. falkneri* Castex & Maciel, 1963, Rio Salobra, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (56°22′42″W, 20°14′26″S), 19–24 July 2004 (Host no. MZUSP MS04–03); *P. histrix* (Müller & Henle, 1834), Rio Salobra, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (56°22′42″W, 20°14′26″S), 19–24 July 2004 (Host nos MZUSP MS04–09, MS04–31, MS04–41, MS04–43); *P. motoro* (Müller & Henle, 1841), Rio Salobra, District of Salobra, Municipality of Miranda, Mato Grosso do Sul, Brazil (56°22′42″W, 20°14′26″S), 19–24 July 2004 (Host nos MZUSP MS04–13, MS04–23, MS04–34); *P. motoro* from Rio Paraná, Paraje Santa Tecla, Municipality of Ituzaingó, Province of Corrientes (56°24′05.1″W, 27°36′34.4″S) and Rio Paraná, Presa de Yacyretá, Municipality of Ituzaingó, Province of Corrientes, Argentina (56°39′15.2″W, 27°29′31.8″S), May 2005; *P.* cf. *motoro*, Rio Mutum, Municipality of Barão de Melgaço, Mato Grosso, Brazil (11°51′19″S, 60°43′10″W), 8 July 2006 (Host nos MZUSP AC06–017–021); *P. orbygnyi* (Castelnau, 1855), Rio Yavari, Municipality of Benjamin Constant, Amazonas, Brazil (4°18′25″S, 70°4′31″W), 5 September 2006 (Host no. MZUSP TA06–18); *P. orbignyi*, Rio Tarauacá, Lago Arara, Municipality of Tarauacá, Acre, Brazil (11°51′19″S, 60°43′10″W), 22 July 2006 (Host no. MZUSP AC06–094); *Potamotrygon* sp., Rio Paraguay, Igarapé do Padre Inácio, Municipality of Cáceres, Mato Grosso, Brazil (16°00′09″S, 57°42′22″W), 29 June 2007 (Host no. MZUSP MT07–01); *Potamotrygon* sp. 'tar_1', Rio Tarauacá, Lago Arara, Municipality of Tarauacá, Acre, Brazil (11°51′19″S, 60°43′10″W), 22–23 July 2006 (Host nos MZUSP AC06–088, AC06–092, AC06–95, AC06–105, AC06–108–109, AC06–111); *Potamotrygon humerosa* Garman, 1913, Rio Abacaxis, Municipality of Borba, Amazonas, Brazil (4°23′05″S, 59°35′44″W), 9–26 January 2007 (Host nos MZUSP AM07–03, AM07–06, AM07–21); *Potamotrygon* sp., Rio Amazonas, Iquitos, Peru (3°43′22″S, 73°12′42″W) 18 April 2005 (Tomáš Scholz no. 05PI 199).

Material examined. USNPC nos 77 157, 77 158, 103685-103687, HWML nos 21 390, 49338-49339, MZUSP nos 6356-6359, 6364, 6873-6888, CHIOC nos 37443-37447, INPA nos 575-577.

Comparative measurements. Table 2A, B.

Remarks. *Potamatrygonocotyle tsalickisi* is the type species of the genus and it was recently redescribed by Domingues & Marques (2007) based on type material. The phylogenetic position of this species is in a polytomy (node H, fig. 2) with *P. eurypotamoxenus, P. rarum* and (*P. aramasae* + *P. tocantinsense* sp. nov.), based on the presence of the distal portion of the male copulatory organ being subterminal (character 2, table 1 and fig. 2). Except for some morphometric differences (table 2), which we no longer consider as evidence of interspecific variation, this species is very similar to *P. eurypotamoxenus* by sharing the same morphology of the MCO (long male copulatory organ with subterminal aperture). Additionally, throughout character analysis, we were unable to find any autapomorphy for either of these two nominal species. Thus, we consider *P. eurypotamoxenus* a junior synonymy of *P. tsalicksi*.

Potamotrygonocotyle aramasae Domingues, Pancera & Marques, 2007

Type host and type locality. Paratrygon aiereba (Müller & Henle, 1841), Rio Negro, Municipality of Barcelos, Amazonas, Brazil (62°55′13.64″W, 0°58′11.72″S), 26 January 2005 (Host no. MZUSP RN05–33).

Other localities. Rio Paranã, Municipality of Paranã, Tocantins, Brazil (12°37′48″S, 52°57′36″W), 3 August 2004 (Host no. MZUSP TO04–11); Rio Tapajós, Municipality of Santarém, Pará, Brazil (2°16′47″S, 55°0′0″W), 3 and 9 October 2005 (Host nos MZUSP TJ05–02; TJ05–29); Rio Araguaia, Municipality of São Miguel do Araguaia, Goiás, Brazil (12°56′51″S, 50°31′32″W), 6 June 2006 (Host nos MZUSP TO05–02–04; TO05–08–12; TO05–29; TO05–80); Rio Araguaia, Municipality of Caseara, Tocantins, Brazil (9°16′11.9994″S, 49°57′36″W), 6 June 2006 (Host no. MZUSP TO05–45); Rio Tocantins, Municipality of Ipueiras, Tocantins, Brazil (11°18′36″S, 48°27′36″W), 14 June 2005 (Host no. MZUSP TO05–29); Rio Xingú, Municipality of São Félix do Xingú, Pará, Brazil (6°39′36″S, 52°0′0″W), 6 July 2005 (Host no. MZUSP TO05–80); Rio Yavari, Municipality of Benjamin Constant, Amazonas, Brazil (4°18′25″S, 70°4′31″W), 4–5 September 2006 (Host nos MZUSP TA06–14–16); Rio Urariquera, Municipality of Boa Vista, Brazil (3°22′51.9594″N, 60°35′44.1594″W), 24 February 2007 (Host no. MZUSP AM07–48).

Material examined. MZUSP nos 6373–6374, 6848–6864, CHIOC nos 37448-37451, INPA nos 575–577, USNPC nos 103688–103691, HWML nos 49340–49342.

Comparative measurements. Table 3.

Remarks. *Potamotrygonocotyle aramasae* is widespread in the Amazon Basin and seems to be host specific to *Paratrygon aiereba*. Two forms of *Potamotrygonocotyle aramasae* were recognized based on the morphology of the anterior dorsal haptoral accessory structure (ADHAS) and presence/absence of the sclerotization around the mouth (fig. 3A–D). One morphotype is characterized by

Table 2. Comparative measurements (µm) of *Potamotrygonocotyle tsalickisi* Mayes, Brooks & Thorson, 1981 from potamotrygonids in Brazil and Peru. *N*, Number of specimens measured; AM, Amazonas; PR, Paraná; PE, Peru.

A

	**P. constellata*	*N*	*P. castexi* (PR)	*N*	*P.* cf.*castexi* (PE)	*N*	*P. falkneri* (PR)	*N*	*P. histrix* (PR)	*N*	*P. motoro* (PR)	*N*	*P. motoro* (AM)	*N*
Body														
Length	760 (630–900)	5	485 (390–530)	8	–	–	350 (320–380)	2	330 (260–400)	5	453 (360–500)	8	–	–
Width	270 (230–310)	6	194 (130–240)	8	–	–	185 (180–190)	2	140 (110–160)	5	167 (150–180)	8	–	–
Pharynx														
Length	86 (83–88)	7	67 (60–75)	7	–	–	58	1	50 (45–58)	5	50 (45–78)	6	–	–
Width	62 (55–70)	7	43 (35–50)	7	–	–	38	1	38 (30–43)	5	39 (35–45)	6	–	–
Haptor														
Length	247 (225–270)	5	204 (190–230)	8	–	–	193 (190–195)	2	160 (140–180)	4	170 (160–205)	8	–	–
Width	252 (240–270)	5	195 (150–245)	8	–	–	170 (140–200)	2	185 (150–200)	4	203 (180–220)	8	–	–
Anchor														
Length	58 (49–65)	10	54 (50–60)	33	50	1	51 (45–55)	13	52 (42–56)	25	54 (50–57)	16	52 (50–54)	3
Width base	22 (20–25)	3	22 (19–26)	20	–	–	23 (20–28)	15	23 (20–25)	21	20 (16–24)	11	21 (20–22)	2
Hooks														
Length	11 (10–11)	26	11 (10–13)	220	11 (10–12)	5	11 (10–12)	108	11 (10–13)	171	12 (11–13)	83	11 (10–11)	13
MCO														
Length	55 (50–66)	7	48 (34–58)	28	55	1	42 (40–47)	14	45 (30–52)	23	50 (35–65)	11	37 (35–38)	3
Testis														
Length	153 (130–185)	6	128 (110–150)	3	–	–	100	1	81 (75–88)	3	107 (93–125)	5	–	–
Width	138 (90–180)	6	73 (58–93)	3	–	–	87	1	62 (58–65)	3	86 (75–95)	5	–	–
Egg														
Length	133 (125–138)	3	145 (110–158)	14	125	1	–	–	143	1	155	1	–	–
Width	75 (43–125)	3	67 (45–75)	13	38	1	–	–	78	1	65	1	–	–

B

	P. cf. *motoro* (AM)	*N*	*P. orbignyi* (AM)	*N*	*Potamotrygon* sp.'tar_1'	*N*	*P. humerosa*	*N*	*Potamotrygon* sp. (PE)	*N*	*Potamotrygon* sp. (PR)	*N*
Body												
Length	–	–	–	–	–	–	–	–	–	–	–	–
Width	–	–	–	–	–	–	–	–	–	–	–	–
Pharynx												
Length	–	–	–	–	–	–	–	–	–	–	–	–
Width	–	–	–	–	–	–	–	–	–	–	–	–
Haptor												
Length	–	–	–	–	–	–	–	–	–	–	–	–
Width	–	–	–	–	–	–	–	–	–	–	–	–
Anchor												
Length	46 (38–56)	8	54 (51–57)	4	57 (55–59)	3	50	3	60 (60–61)	2	50 (49–53)	4
Width base	22 (20–23)	12	21 (20–22)	4	23 (23–24)	3	20 (21–22)	2	23 (22–24)	2	21 (20–21)	2
Hooks												
Length	11 (10–12)	41	11 (10–12)	24	12	9	11 (10–12)	9	12 (10–13)	8	11 (10–12)	13
MCO												
Length	46 (38–52)	12	51 (46–56)	4	49 (45–56)	3	47 (44–53)	3	56 (53–60)	3	44 (41–45)	3
Testis												
Length	–	–	–	–	–	–	–	–	–	–	–	–
Width	–	–	–	–	–	–	–	–	–	–	–	–
Egg												
Length	139 (138–140)	2	143	1	–	–	–	–	–	–	–	–
Width	68	2	73	1	–	–	–	–	–	–	–	–

*Type host and type locality.

Table 3. Comparative measurements (µm) of *Potamotrygonocotyle aramasae* Domingues, Pancera & Marques, 2007 from eight localities. *N*, Number of specimens measured; AC, Acre; AM, Amazonas; PA, Pará; RR, Roraima; TO, Tocantins.

	*RioNegro (AM)	*N*	Rio Araguaia (TO)	*N*	Rio Paranã (TO)	*N*	RioTapajós (AM)	*N*	Rio Tocantins (TO)	*N*	Rio Xingú (PA)	*N*	Rio Yavari (AM)	*N*	Rio Tarauacá (AC)	*N*	Rio Urariquera (RR)	*N*
Body																		
Length	255 (170–360)	15	336 (260–450)	16	370	1	285 (200–370)	11	408 (330–530)	5	330	1	437 (360–530)	19	390 (300–430)	4	353 (350–360)	3
Width	136 (100–180)	14	129 (90–200)	14	–	–	131 (80–200)	13	178 (170–200)	5	170	1	158 (100–270)	19	173 (100–260)	4	140 (120–160)	3
Pharynx																		
Length	50 (38–65)	11	40 (28–55)	9	48	1	50 (38–73)	5	65 (53–70)	5	50	1	71 (50–90)	18	67 (58–75)	3	61 (60–63)	2
Width	30 (25–38)	11	28 (25–35)	9	40	1	34 (25–50)	5	36 (30–43)	5	38	1	39 (30–45)	18	45 (33–58)	3	63	1
Haptor																		
Length	165 (130–180)	15	158 (95–245)	18	215	1	158 (115–200)	12	199 (185–225)	5	160	1	213 (165–245)	18	209 (200–235)	4	188 (180–200)	3
Width	148 (115–170)	13	140 (125–190)	12	235	1	139 (105–160)	10	175 (155–215)	5	175	1	195 (140–260)	13	211 (190–255)	4	183 (150–215)	3
Anchor																		
Length	50 (48–53)	15	48 (39–66)	22	55	1	48 (39–56)	16	56 (52–60)	13	53 (49–56)	2	54 (44–69)	15	52	1	58 (57–60)	3
Width base	19 (17–25)	10	22 (18–25)	9	25	1	21 (11–27)	11	22 (17–26)	10	23	1	24 (17–28)	8	22	1	22	1
Hook																		
Length	10 (10–11)	89	11 (9–12)	133	10 (9–10)	14	10 (9–11)	89	10 (9–11)	80	10 (9–11)	8	10 (9–11)	72	10 (9–11)	21	11 (11–12)	15
MCO																		
Length	54 (47–60)	14	44 (37–52)	19	53 (46–60)	2	50 (33–67)	9	51 (46–63)	13	55 (52–58)	3	49 (32–65)	16	46 (36–53)	4	57 (55–60)	3
Testis																		
Length	72 (58–90)	9	72 (40–113)	9	83	1	63	1	106 (80–125)	4	–	–	84 (60–93)	10	113	1	77 (62–90)	3
Width	58 (38–73)	8	55 (25–90)	8	133	1	58	1	75	2	–	–	66 (50–90)	10	70	1	78 (55–92)	3
Egg																		
Length	88	1	–	–	–	–	105	1	81 (75–88)	2	105	1	94 (68–118)	10	88	1	112	1
Width	53	1	–	–	–	–	70	1	49 (40–58)	2	63	1	56 (48–73)	10	80	1	52	1

*Type host and type locality.

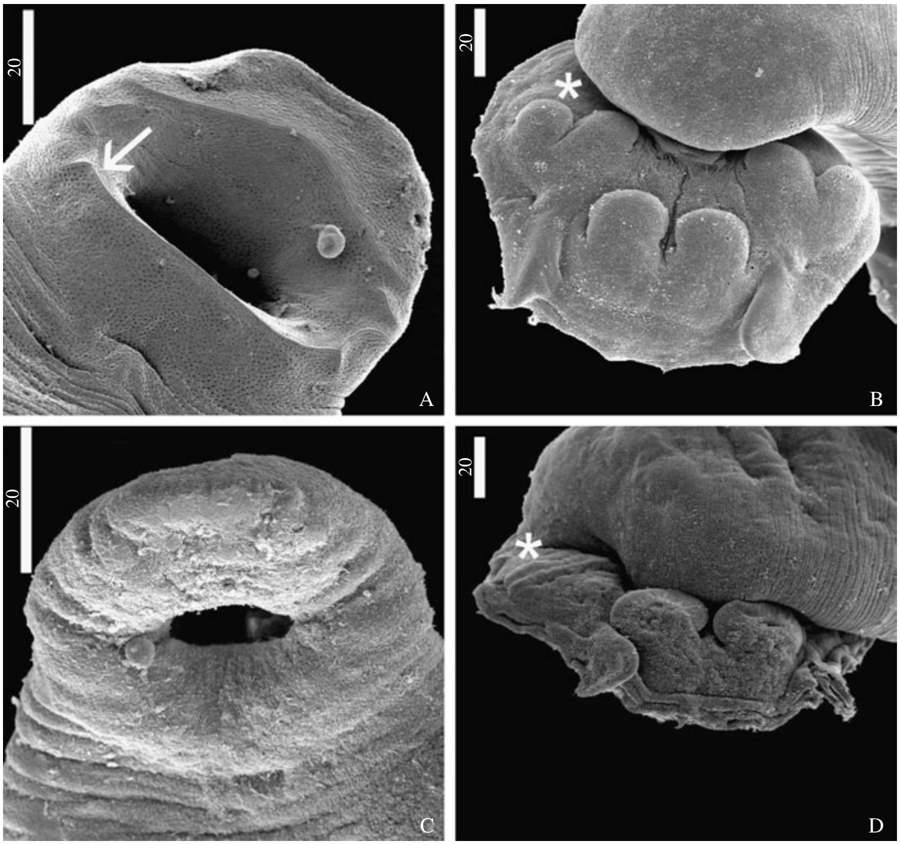

Fig. 3. Morphology of *Potamotrygonocotyle aramasae* Domingues, Pancera & Marques, 2007. (A) Anterior region of *P. aramasae* forma 'A', arrow shows the sclerotization around the mouth; (B) haptor (dorsal view) of *P. aramasae* forma 'A', asterisk shows the bilobate anterior dorsal haptoral accessory structure; (C) anterior region of *P. aramasae* forma 'B'; (D) haptor of *P. aramasae* forma 'B', asterisk shows the semicircular anterior dorsal haptoral accessory structure. Scale bars in μm.

having a bilobate ADHAS (fig. 3B) and is reported from Rio Araguaia (TO), Rio Paranã (TO), Rio Negro (AM, type-locality) and Rio Xingú (PA). The other morphotype is characterized by possessing a semicircular ADHAS (fig. 3D) and is reported from Rio Tocantins (TO), Rio Yavari (AM), Rio Tarauacá (AC) and Rio Tapajós (PA). Specimens from Rio Tapajós also lack the sclerotizations around their mouth (fig. 3C). Since this character is considered a synapomorphy for the genus (see *Character analysis* section), the absence of sclerotizations around the mouth is considered a secondary loss for the Rio Tapajós population. We believe that future phylogeographic studies will help us to define the real taxonomic status of these populations.

A comparison of morphometric attributes (table 3), as well as the comparative morphology of the ejaculatory bulb, male copulatory organ and haptoral structures (e.g. anchor, hook) do not reveal significant morphologic differences among the morphotypes, which we would consider as evidence for different lineages. The variation observed possibly represents an intraspecific, maturity or seasonal variation, suggesting that specimens of *Potamotrygonocotyle* that parasitize *Paratrygon aiereba* from different Amazonas and Tocantins sub-basins are representatives of *Potamotrygonocotyle aramasae*.

This species is sister to *P. tocantinsense* sp. nov. (node I, fig. 2). Both species share the presence of the ejaculatory bulb with two anterior ovate cavities (character 3, table 1 and fig. 2). However, *P. aramasae* can be distinguished from *P. tocantinsense* sp. nov. by the shape of the male copulatory organ (long MCO versus short MCO, respectively; character 1, table 1 and fig. 2) and by the size of the egg filament (short versus long, respectively; character 11, table 1 and fig. 2).

Potamotrygonocotyle chisholmae Domingues & Marques, 2007

Synonymy. Potamotrygonocotyle uruguayense Domingues & Marques, 2007.

Type host and type locality. Potamotrygon motoro, Rio Salobra, District of Salobra, Miranda, Mato Grosso do Sul, Brazil (56°22′42″W, 20°14′26″S), 19–24 July 2004 (Host no. MZUSP MS04–13).

Other hosts and localities. *P. brachyura* (Günther, 1880), Rio Uruguay, Municipality of Porto Xavier, Rio Grande do Sul, Brazil (27°53′52″S, 55°13′26″W), 24–31 March 2005 (Host nos MZUSP UR05–01–02, UR05–04); *P. brachyura*, Rio Mutum, Municipality of Barão de Melgaço, Mato Grosso, Brazil (11°51′19″S, 60°43′10″W), 5–7 July 2006 (Host nos MZUSP AC06–007–008, AC06–011); *P. henlei* (Castelnau, 1855), Rio Araguaia, Municipality of Caseara, Tocantins, Brazil (9°16′11″S, 49°57′47″W), 25 June 2005 (Host no. MZUSP TO05–70); *P. leopoldi* Castex & Castello, 1970, Rio Xingú, Municipality of São Félix do Xingú, Pará, Brazil (6°39′36″S, 52°0′0″W), 1–6 July 2005 (Host nos MZUSP TO05–68, TO05–71, TO05–75, TO05–83–87, TO05–89–90); *P. motoro* Rio Shilive, Municipality of Boca Ishiriwe, Madre de Dios, Peru (12°29′24.31″S, 70°35′41.77″W), May 2001 (Host no. MZUSP PU–14); *P. motoro*, Rio Paraná, Paraje Santa Tecla, Municipality of Ituzaingó, Province of Corrientes, Argentina (56°24′05.1″W, 27°36′34.4″S) and Rio Paraná, Presa de Yacyretá, Municipality of Ituzaingó, Province of Corrientes, Argentina (56°39′15.2″W, 27°29′31.8″S), May 2005; *P. motoro*, Rio Mutum, Municipality of Barão de Melgaço, Mato Grosso, Brazil (11°51′19″S, 60°43′10″W), 5 July 2006 (Host nos MZUSP AC06–001, AC06–003); *P. motoro,* Rio Purus, Lago Novo, Municipality of Boca do Acre, Acre, Brazil (8°44′27″S, 67°22′52″W), 17 July 2006 (Host nos MZUSP AC06–074–75); *P. motoro*, Rio Amazonas, Iquitos, Peru (3°43′22″S, 73°12′42″W), 5–8 September 2006; *P. motoro*, Rio Paraguay, Igarapé do Padre Inácio, Municipality of Cáceres, Mato Grosso, Brazil (16°00′09″S, 57°42′22″W), 29 June 2007 (Host no. MZUSP MT07–02); *P. motoro*, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (1°00′36″S, 48°57′36″W), 28 August 2007 (Host no. MZUSP PA07–50); *P. motoro*, Rio Tocantins, Baía de Marajó, Municipality of Colares, Pará, Brazil (0°55′45″S, 48°17′29″W), 29 August 2007 (Host no. MZUSP PA07–82); *P.* cf. *motoro,* Rio Tapajós, Municipality of Santarém, Pará, Brazil (2°16′47″S, 55°0′0″W), 4 October 2005 (Host nos MZUSP AC06–017, AC06–20–21); *P.* cf. *motoro*, Rio Solimões, Municipality of Tabatinga, Amazonas, Brazil (4°20′22″S, 69°53′18″W), 25–26 August 2006 (Host nos MZUSP TA06–02–04); *P. orbignyi*, Rio Tarauacá, Lago Arara, Municipality of Tarauacá, Acre, Brazil (11°51′19″S, 60°43′10″W), 22 July 2006 (Host no. MZUSP AC06–094); *P. scobina* Garman, 1913, Rio Tocantins, Baía de Marajó, Municipality of Colares, Pará, Brazil (0°55′45″S, 48°17′29″W), 16, 22 and 29 August 2007 (Host nos MZUSP PA07–07, PA07–46, PA07–83); *Potamotrygon* sp. ‘1’, *Potamotrygon* sp. ‘2’, and *Potamotrygon* sp. ‘3’, Rio Solimões, Municipality of Tabatinga, Amazonas, Brazil (4°20′22″S, 69°53′18″W), 25–26 August 2006 and 1 September 2006 (Host nos MZUSP TA06–01, TA06–06 and TA06–09, respectively); *Potamotrygon* sp. ‘tpj_1’, Rio Tapajós, Municipality of Pimental, Pará, Brazil (4°33′35″S, 56°15′35″W), 10 October 2005 (Host nos MZUSP TJ05–36, TJ05–40–41), Rio Teles Pires, Municipality of Alta Floresta, Mato Grosso, Brazil (8°52′48″S, 57°22′48″W), 2 and 5 December 2005 (Host nos MZUSP MT05–05, MT05–17, MT05–26–27), and Rio Jamanxim, Municipality of Novo Progresso, Pará, Brazil (7°08′24″S, 55°22′48″W), 13 December 2005 (Host nos MZUSP MT05–26–27); *Potamotrygon* sp. ‘tar_1’, Rio Tarauacá, Lago Arara, Municipality of Tarauacá, Acre, Brazil (11°51′19″S, 60°43′10″W), 22–23 July 2006 (Host nos MZUSP AC06–088, AC06–092, AC06–095, AC06–105, AC06–108–109, AC06–111); *Potamotrygon* sp. ‘tar_2’, Rio Tarauacá, Lago Arara, Municipality of Tarauacá, Acre, Brazil (11°51′19″S, 60°43′10″W), 22–23 July 2006 (Host nos MZUSP AC06–103–104; AC06–110); *Potamotrygon* sp. ‘mar_1’, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (1°00′36″S 48°57′36″W) 28 August 2007 (Host nos MZUSP PA07–51, PA07–62–63, PA07–74, PA07–84–85); *Potamotrygon* sp. ‘toc_2’, Rio Tocantins, Municipality of Ipueiras, Tocantins, Brazil (11°18′36″S, 48°27′36″W), 11–15 June 2005 (Host nos MZUSP TO05–16; TO05–18; TO05–20; TO05–23–28; TO05–30–32).

Material examined. MZUSP nos 6352, 6354 a–ak, 6365–6366 a–v, 6902–6956, CHIOC nos 37412–37436, INPA nos 552–566, USNPC nos 103658–103677, HWML nos 49321–49333.

Comparative measurements. Table 4A, B.

Remarks. *Pamotrygonocotyle chisholmae* was sister to *P. uruguayense* (node G, fig. 2) by sharing continuous medial sclerotization on the mouth (character 9, table 1 and figs 1B and 2). Domingues & Marques (2007) distinguished *P. chisholmae* from *P. uruguayense* on the basis of the shape of the ADHAS (trilobate versus bilobate, respectively). However, re-evaluation of this character based on the collection of new material from different hosts and localities suggested no clear-cut distinction between trilobate and bilobate ADHAS that could be assigned to any of these nominal species unequivocally. We recognize that this character displays a continuum of variation among populations once credited to different lineages. In addition, throughout character analysis, we were unable to determine any autapomorphy for either of these species. Thus, we consider *P. uruguayense* as a junior synonymy of *P. chisholmae.*

Potamotrygonocotyle dromedarius Domingues & Marques, 2007

Type host and type locality. *Potamotrygon motoro*, Rio Salobra, District of Salobra, Miranda, Mato Grosso do Sul, Brazil (56°22′42″W, 20°14′26″S), 21 July 2004 (Host no. MZUSP MS04–13).

Other hosts and localities. *P. henlei,* Rio Araguaia, Municipality of Caseara, Tocantins, Brazil (9°16′11″S, 49°57′47″W), 25 June 2005 (Host no. MZUSP TO05–70); *P. leopoldi,* Rio Xingú, Municipality of São Félix do Xingú, Pará, Brazil (6°39′36″S, 52°0′0″W), 3 and 6 July 2005 (Host nos MZUSP TO05–75, TO05–89); *P. motoro*, Rio Shilive, Municipality of Boca Ishiriwe, Madre de Dios, Peru (12°29′24.31″S, 70°35′41.77″W), May 2001 (Host no. MZUSP PU–14); *P. motoro*, Rio Mutum, Municipality of Barão de Melgaço, Mato Grosso, Brazil (11°51′19″S, 60°43′10″W), 7 July 2006 (Host no. MZUSP AC06–009); *P. motoro*, Rio Paraguay, Igarapé do Padre Inácio, Municipality of Cáceres, Mato Grosso, Brazil (16°00′09″S, 57°42′22″W), 29 June 2007 (Host no. MZUSP MT07–02); *Potamotrygon* sp. ‘toc_2’, Rio Tocantins, Municipality of Ipueiras, Tocantins, Brazil (11°18′36″S, 48°27′36″W), 14–15 June 2005 (Host nos MZUSP TO05–28, TO05–16, TO05–18, TO05–20, TO05–23–28, TO05–30–32).

Material examined. MZUSP nos 6354–6355, 6865–6872, CHIOC nos 37437-37442, INPA nos 567–570, USNPC nos 103678–103684, HWML nos 49334–49337.

Table 4. Comparative measurements (µm) of *Potamotrygonocotyle chisholmae* Domingues & Marques, 2007 from potamotrygonids in Brazil and Peru. *N*, Number of specimens measured; AM, Amazonas; PR, Paraná; PE, Peru.

A

	*P. motoro (PR)	*N*	*P. motoro* (AM)	*N*	*P. motoro* (PR)	*N*	*P.* cf. *motoro* (AM)	*N*	*P.* cf. 1 *motoro* (AM)	*N*	*P.* cf. 2 *motoro* (AM)	*N*	*P. motoro* (PE)	*N*	*P. leopoldi*	*N*	*P. henlei*	*N*	*Potamotrygon* sp. '1'	*N*
Body																				
Length	432 (300–590)	17	–	–	420	1	368 (260–470)	9	–	–	–	–	–	–	–	–	416 (410–430)	3	503 (360–610)	6
Width	194 (140–280)	16	–	–	280	1	133 (100–240)	9	–	–	–	–	–	–	–	–	160 (150–180)	3	240 (170–290)	6
Pharynx																				
Length	55 (38–75)	17	–	–	–	–	40	1	–	–	–	–	–	–	–	–	56 (50–65)	3	74 (63–88)	4
Width	38 (25–55)	16	–	–	–	–	33	1	–	–	–	–	–	–	–	–	39 (35–45)	3	45 (38–50)	4
Haptor																				
Length	181 (140–215)	17	–	–	–	–	159 (135–190)	9	–	–	–	–	–	–	–	–	217 (190–245)	3	217 (175–250)	6
Width	187 (150–220)	16	–	–	–	–	157 (135–175)	8	–	–	–	–	–	–	–	–	192 (175–210)	3	214 (165–260)	4
Anchor																				
Length	52 (43–66)	43	47 (40–51)	18	54 (50–65)	4	47 (40–51)	19	47 (46–48)	3	60 (52–67)	2	46 (43–52)	10	51	1	46 (41–55)	3	50 (48–53)	10
Width base	21 (17–27)	20	21 (16–23)	18	21 (20–22)	4	21 (19–25)	17	22 (21–23)	3	22 (22–23)	3	20 (18–22)	9	22	1	21 (20–22)	3	23 (21–26)	10
Hook																				
Length	12 (10–15)	195	11 (10–12)	80	11 (10–12)	23	11 (10–12)	88	11 (11–12)	21	11 (11–12)	18	12 (11–13)	36	10	7	11	13	11 (10–12)	68
MCO																				
Length	63 (33–75)	41	57 (40–70)	18	63 (55–70)	4	58 (52–63)	22	49 (45–52)	2	63 (60–68)	3	58 (50–65)	10	–	–	–	–	–	–
Testis																				
Length	92 (68–118)	10	–	–	–	–	73 (55–88)	7	–	–	–	–	–	–	–	–	–	–	–	–
Width	72 (50–98)	8	–	–	–	–	64 (45–80)	7	–	–	–	–	–	–	–	–	–	–	–	–
Egg																				
Length	142 (108–175)	15	129 (118–140)	5	144 (138–150)	2	149 (147–150)	2	–	–	–	–	–	–	–	–	–	–	–	–
Width	63 (35–78)	14	60 (45–65)	5	65	2	64 (63–65)	2	–	–	–	–	–	–	–	–	–	–	–	–

B

	Potamotrygon sp. '2'	*N*	*Potamotrygon* sp. 'tpj_1'	*N*	*Potamotrygon* sp.'tar_1'	*N*	*Potamotrygon* sp. 'tar_2'	*N*	*Potamotrygon* 'sp_mar1'	*N*	*Potamotrygon* sp. 'toc_2'	*N*
Body												
Length	–	–	394 (350–470)	5	–	–	–	–	332 (250–410)	5	460 (380–570)	22
Width	–	–	172 (140–200)	5	–	–	–	–	106 (90–130)	5	164 (110–210)	22
Pharynx												
Length	–	–	58 (50–65)	5	–	–	–	–	41 (38–43)	4	58 (48–75)	20
Width	–	–	39 (38–43)	5	–	–	–	–	25 (23–33)	4	36 (28–50)	20
Haptor												
Length	–	–	190 (175–210)	5	–	–	–	–	150 (140–160)	2	207 (155–245)	20
Width	–	–	181 (165–205)	4	–	–	–	–	140 (135–145)	2	203 (165–255)	23
Anchor												
Length	50 (50–51)	4	47 (40–51)	15	47 (45–50)	7	46 (45–47)	6	42 (40–46)	4	52 (48–58)	29
Width base	22 (20–25)	4	20 (17–22)	13	21 (20–23)	7	21 (21–22)	5	21 (19–22)	2	22 (20–24)	22
Hook												
Length	11 (11–12)	18	11 (10–12)	63	11 (10–12)	31	11 (10–12)	27	11 (11–13)	21	11 (10–13)	146
MCO												
Length	64 (62–65)	3	59 (50–65)	10	54 (50–60)	7	53 (50–56)	6	48 (46–50)	2	68 (58–75)	30
Testis												
Length	–	–	72 (63–88)	3	–	–	–	–	43 (30–50)	4	78 (55–100)	17
Width	–	–	63 (50–75)	3	–	–	–	–	46 (30–55)	4	70 (50–112)	17
Egg												
Length	163	1	124 (113–140)	4	130	1	–	–	143	1	141 (125–163)	11
Width	65	1	71 (63–80)	4	63	1	–	–	65	1	59 (43–70)	11

*Type host and type locality.

Table 5. Comparative measurements (µm) of *Potamotrygonocotyle dromedarius* Domingues & Marques, 2007 from potamotrygonids in Brazil and Peru. *N*, Number of specimens measured; AM, Amazon; PR, Paraná; PE, Peru.

	**P. motoro* (PR)	*N*	*P. henlei*	*N*	*P. leopoldi*	*N*	*P. motoro* (AM)	*N*	*P. motoro* (PR)	*N*	*P. motoro* (PE)	*N*	*Potamotrygon* sp. 'toc_2'	*N*
Body														
Length	417 (310–550)	9	365 (320–450)	4	300	1	–	–	–	–	–	–	427 (350–530)	7
Width	240 (170–430)	9	175 (140–200)	4	180	1	–	–	–	–	–	–	190 (140–220)	7
Pharynx														
Length	55 (50–63)	7	56 (50–70)	4	–	–	–	–	–	–	–	–	62 (53–75)	6
Width	38 (38–40)	7	38 (33–48)	4	–	–	–	–	–	–	–	–	40 (33–45)	6
Haptor														
Length	242 (190–270)	6	260 (250–285)	4	250	1	–	–	–	–	–	–	253 (235–270)	6
Width	259 (215–285)	7	260 (250–285)	4	255	1	–	–	–	–	–	–	254 (230–280)	7
Anchor														
Length	48 (37–55)	14	51 (49–55)	5	45 (45–46)	2	44 (43–44)	3	49	1	50	1	51 (49–53)	7
Width base	19 (16–20)	6	19 (18–20)	3	17 (17–18)	2	17 (15–19)	3	21 (19–22)	2	17	1	18 (16–21)	7
Hook														
Length	10 (9–12)	64	10 (9–10)	25	10 (9–11)	19	10 (10–11)	18	10	10	10 (10–11)	4	10 (9–11)	29
MCO														
Length	29 (22–33)	11	28 (25–30)	6	29 (27–30)	2	24 (22–25)	3	25	2	25	1	29 (28–30)	6
Testis														
Length	125 (100–163)	7	68 (57–75)	4	65	1	–	–	–	–	–	–	88 (75–100)	6
Width	94 (75–150)	6	80 (68–88)	4	83	1	–	–	–	–	–	–	84 (63–105)	6
Egg														
Length	–	–	135	1	–	–	–	–	–	–	–	–	118	1
Width	–	–	–	–	–	–	–	–	–	–	–	–	90	1

* Type host and type locality.

Comparative measurements. Table 5.

Remarks. *Potamotrygonocotyle dromedarius* was sister to *P. tatianae* sp. nov. based on two homoplastic characters: the male copulatory organ as a short tube (character 1, table 1 and fig. 2), and the deep root twice as large as the superficial root (character 12, table 1 and fig. 2). However, it differs from *P. tatianae* sp. nov. by possessing a semicircular posterior dorsal haptoral accessory structure (DHAS), which is elongate laterally in *P. tatianae* sp. nov. (character 7, table 1 and fig. 2).

Potamotrygonocotyle quadracotyle Domingues Pancera & Marques, 2007

Type host and type locality. *Potamotrygon* sp. 'cururu', Rio Negro, Municipality of Barcelos, Amazonas, Brazil (62°55′13″W, 0°58′11″S), January–February 2005 (Host nos MZUSP RN05–02, RN05–10, RN05–40, RN05–68, RN05–97).

Material examined. MZUSP nos 6367–6368.

Remarks. *Potamotrygonocotyle quadracotyle* is restricted to *Potamotrygon* sp. 'cururu', an undescribed species locally referred as 'cururu', from Rio Negro. This species is closely related to another species endemic to Rio Negro, *P. umbella,* (node E, fig. 2) based on the presence of the DHAS associated with the posterolateral and posterior loculi with elongate lobes (characters 6 and 7, respectively; table 1 and fig. 2). However, this species is unique among *Potamotrygonocotyle* by possessing a haptor with one central and four peripheral loculi (character 4, table 1 and fig. 2). Many genera within Monocotylidae have been established on the basis of haptor loculi configuration (e.g. *Nonacotyle, Decacotyle, Euzetia*), which would suggest that *P. quadracotyle* could well be assigned to a different genus. However, that would make *Potamotrygonocotyle* paraphyletic. Our results call for a re-evaluation of haptoral loculi configuration as the justification for erecting genera within Monocotylidae and show the relevance of phylogenetic studies within this family to guide taxonomic decisions.

Potamotrygonocotyle rarum Domingues Pancera & Marques, 2007

Type host and type locality. *Potamotrygon schroederi* Fernández-Yépez, 1958, Rio Negro, Municipality of Barcelos, Amazonas, Brazil (62°55′13.64″W, 0°58′11.72″S), 25–26 January 2005 (Host nos MZUSP RN05–01, RN05–12, RN05–21, RN05–23, RN05–36).

Material examined. MZUSP nos 6371–6372.

Remarks. *Potamotrygonocotyle rarum* is restricted to *Potamotrygon schroederi*. This species nested in a clade with *P. tsalickisi* (= *P. eurypotamoxenus*), *P. tocantinsense* sp. nov. and *P. aramasae* (node H) based on the morphology of the male copulatory organ (character 2, table 1, fig. 2; see comments on *P. tsalickisi*). However, *P. rarum* differs from the other species within the node H by having anchors with deep roots twice as big as superficial (character 12, table 1, fig. 2).

Potamotrygonocotyle rionegrense Domingues Pancera & Marques, 2007

Type host and type locality. *Potamotrygon motoro*, Rio Negro, Municipality of Barcelos, Amazonas, Brazil (62°55′13.64″W, 0°58′11.72″S), 23 and 31 January 2005 (Host nos MZUSP RN05–04–05, RN05–62).

Other hosts and localities. *Potamotrygon motoro,* Rio Purus, Lago Novo, Municipality of Boca do Acre, Acre, Brazil (8°44′27″S, 67°22′52″W), 17 July 2006 (Host nos MZUSP AC06–073–75, AC06–079); *P. motoro*, Rio Mutum, Municipality of Barão de Melgaço, Mato Grosso, Brazil (11°51′19″S, 60°43′10″W), 5–8 July 2006 (Host nos MZUSP AC06–001, AC06–003, AC06–020, AC06–022); *P.* cf. *motoro*, Rio Mutum, Municipality of Barão de Melgaço, Mato Grosso, Brazil (11°51′19″S, 60°43′10″W), 8 July 2006 (Host no. MZUSP AC06–020); *P.* cf. *motoro*, Rio Solimões, Municipality of Tabatinga, Amazonas, Brazil (4°20′22″S, 69°53′18″W), 26 August 2006 (Host no. MZUSP TA06–04); *Potamotrygon* sp., Rio Amazonas, Iquitos, Peru (3°43′22″S, 73°12′42″W) 7–8 September 2006 (Tomáš Scholz nos P235a, P312a, P334a); *P. motoro*, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (1°00′36″S 48°57′36″W), 28–29 August 2007 (Host nos MZUSP PA07–50; PA07–82); *Potamotrygon* sp., Rio Paraguay, Igarapé do Padre Inácio, Municipality of Cáceres, Mato Grosso, Brazil (16°00′09″S, 57°42′22″W), 29 June 2007 (Host no. MZUSP MT07–01); *Potamotrygon* sp. '3', Rio Solimões, Municipality of Tabatinga, Amazonas, Brazil (4°20′22″S, 69°53′18″W), 1 September 2006 (Host no. MZUSP TA06–06); *Potamotrygon* sp. 'tar_2', Rio Tarauacá, Lago Arara, Municipality of Tarauacá, Acre, Brazil (11°51′19″S, 60°43′10″W), 22–23 July 2006 (Host nos MZUSP AC06–103–104, AC06–110); *P. humerosa*, Rio Abacaxis, Municipality of Borba, Amazonas, Brazil (4°23′05″S, 59°35′44″W), 9–26 January 2007 (Host nos MZUSP AM07–03; AM07–06–07, AM07–09, AM07–16, AM07–18, AM07–20, AM07–22).

Material examined. MZUSP nos 6371–6372, 6889–6901, CHIOC nos 37452-37453, INPA no. 578, USNPC nos 103692–103694.

Comparative measurements. Table 6

Remarks. *Potamotrygonocolyle rionegrense* nested in a clade (node C, fig. 2) with *P. auriculocotyle* sp. nov. and a large group of *Potamotrygonocotyle* species, i.e. clade D (fig. 2) based on the presence of bilobate DHAS associated with the posterolateral loculi (character 5, table 1 and fig. 2). *Potamotrygonocotyle rionegrense* differs from *P. auriculocotyle* sp. nov. by the morphology of the DHAS associated with the posterior loculi (elongate laterally versus semicircular, respectively).

Potamotrygonocotyle umbella Domingues, Pancera & Marques, 2007

Type host and type locality. *Potamotrygon* sp. 'cururu', Rio Negro, Municipality of Barcelos, Amazonas, Brazil (62°55′13″W, 0°58′11″S), 23 and 26 January 2005 (Host nos MZUSP RN05– 02, RN05– 10, RN05– 40).

Table 6. Comparative measurements (μm) of *Potamotrygonocotyle rionegrense* Domingues, Pancera & Marques, 2007 from potamotrygonids in Brazil, and Peru. *N*, Number of specimens measured; AM, Amazonas; PR, Paraná; PE, Peru.

	* *P. motoro* (AM)	*N*	*P. motoro* (AM)	*N*	*P. motoro* (PR)	*N*	*P.* cf. *motoro* (AM)	*N*	*Potamotrygon* sp. (PE)	*N*	*Potamotrygon* sp. (PR)	*N*	*Potamotrygon* sp. '3'	*N*	*Potamotrygon* sp.'tar_2'	*N*	*P. humerosa*	*N*
Body																		
Length	356 (300–480)	11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width	142 (140–205)	11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pharynx																		
Length	49 (43–58)	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width	32 (25–38)	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Haptor																		
Length	171 (140–205)	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width	169 (135–200)	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Anchor																		
Length	46 (42–51)	23	43 (41–45)	4	40	1	48	1	41 (40–42)	2	42	1	55	1	41 (37–43)	6	42 (39–46)	11
Width base	21 (18–24)	17	17 (16–18)	4	16	1	20	1	18 (17–18)	2	15	1	16	1	16 (15–17)	5	17 (15–20)	9
Hook																		
Length	12 (12–13)	15	11 (10–12)	12	10	3	11 (10–12)	7	11 (10–12)	7	–	–	11 (10–11)	3	10 (10–11)	14	10 (10–11)	35
MCO																		
Length (bent)	70 (58–92)	21	78 (68–84)	5	65 (63–67)	2	75	2	54 (53–55)	2	66 (65–67)	2	75	1	72 (60–81)	6	63 (50–78)	8
Total length	96 (66–135)	11	108 (101–114)	5			109 (108–110)	2	83 (79–87)	2	87 (84–89)	2	117	1	97 (91–103)	6	95 (71–111)	7
Testis																		
Length	60 (50–80)	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Width	80 (68–98)	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Egg																		
Length	110	1	–	–	125 (105–145)	2	–	–	–	–	–	–	–	–	–	–	158	1

* Type host and type locality.

Material examined. MZUSP nos 6369–6370 a–k.

Remarks. *Potamotrygonocotyle umbella* is restricted to *Potamotrygon* sp. 'cururu'. This parasite species is sister with another endemic monocotylid from Rio Negro, *P. quadracotyle* (node E, fig. 2), based on the presence of the DHAS associated with the posterior and posterolateral loculi with elongate lobes (characters 6 and 7, respectively; table 1, and fig. 2). *P. umbella* is easily distinguished from *P. quadracotyle* by eight peripheral loculi in the haptor, whereas specimens of *P. quadracotyle* possess four.

Potamotrygonocotyle tatianae sp. nov

Description. Based on 15 specimens (fig. 4). Body fusiform, total length – excluding haptor – 406 (360–470; $n = 7$); 151 (120–180; $n = 7$) wide at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; three pairs of head organs converging to unique pore; cephalic glands unicellular, posterolateral to pharynx, with rod-shaped secretion. Anteromedial gland present. Dispersed pigment granules dorsal to pharynx, infrequently absent. Mouth surrounded by sclerotized ridges, medial sclerotization of the mouth discontinuous. Pharynx elongate ovate, 49 (45–55; $n = 7$) long, 33 (28–33; $n = 7$) wide; oesophagus short; two intestinal caeca, non-confluent, lacking diverticula; distal ending close to testis. Haptor circular, 203 (200–205; $n = 4$) long, 191 (175–205; $n = 4$) wide, with one central and eight peripheral loculi (two anterior, four lateral, two posterior); septa surrounded by slightly sinuous sclerotized ridge. Dorsal surface of haptor with two 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 associated with

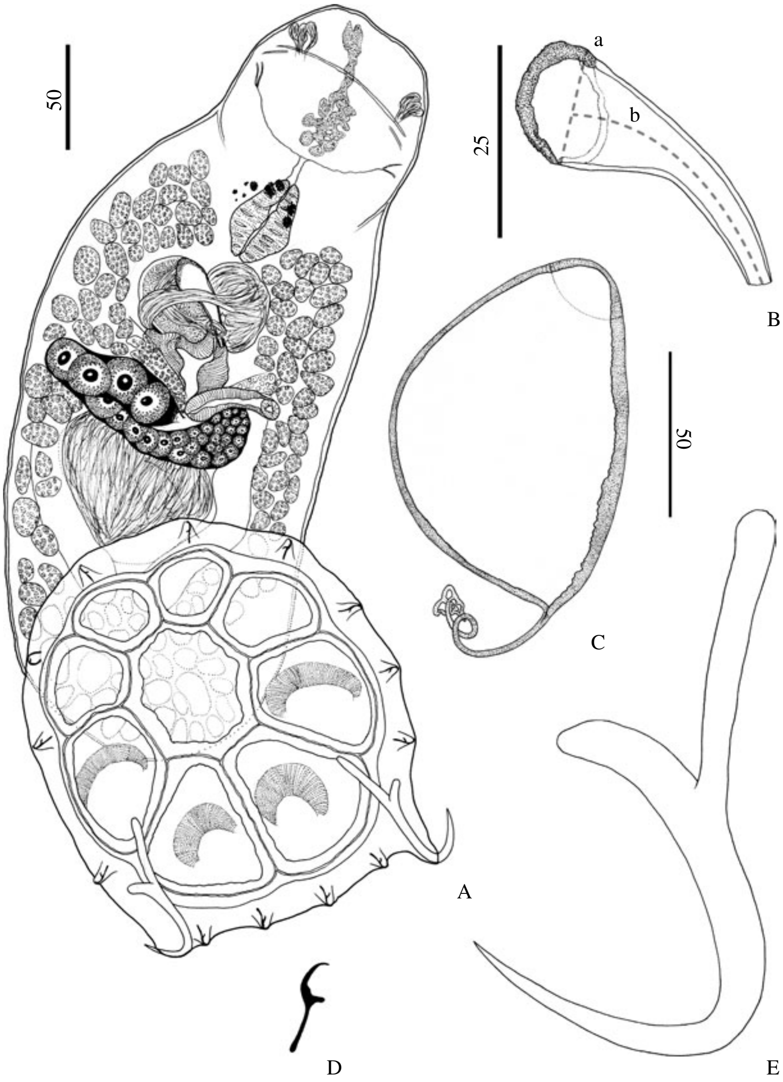

Fig. 4. *Potamotrygonocotyle tatianae* sp. nov. (A) Whole mount (holotype); (B) male copulatory organ (MCO); a, length of the base of the MCO; b, total length of the MCO; (C) egg; (D) hook; (E) anchor. Scale bars in μm.

posterolateral loculi semicircular; posterior pair of dorsal haptoral accessory structures associated with posterior loculi semicircular (see fig. 9A). Anchors 71 (62–76; $n = 13$) long, base 24 (15–26; $n = 12$) wide, with heavy diverging roots; deep root twice as large as superficial root; evenly curved shaft and point. Hooks similar, 10 ($n = 24$) long, distributed on marginal membrane of haptor, with slightly depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ sclerotized, short slightly arcuate tapered tube, 45 (43–47; $n = 7$) long, aperture terminal. Accessory piece absent. Testis cordiform or transversally ovate, 73 (38–90; $n = 7$) long, 88 (75–100; $n = 7$) wide; vas deferens not observed; seminal vesicle lateral to ejaculatory bulb, 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 heavily muscular. Vaginal pore sinistroventral at level of proximal portion of germarium; seminal receptacle not observed. Oötype well developed. Vitellarium coextensive with gut, absent in regions of reproductive organs. Egg ovate, 114 (113–115; $n = 2$) long, 83 (78–88; $n = 2$) wide, with short filament; distal end of filament reticulate.

Holotype, type host and type locality. MZUSP no. 6835; *Paratrygon* sp. '1', Rio Yavari, Municipality of Benjamin Constant, Amazonas, Brazil (4°18′25″S, 70°4′31″W), 5 September 2006 (Host no. MZUSP TA06–17).

Other specimens. 14 paratypes: 9 MZUSP no. 6836a–i, 1 CHIOC no. 37404, 1 HWML no. 49318, 2 INPA no. 548a–b, 1 USNPC no. 103650, *Paratrygon* sp. '1', Rio Yavari, Municipality of Benjamin Constant, Amazonas, Brazil (4°18′15.1194″S, 70°4′19.56″W), 5 September 2006 (Host no. MZUSP TA06–17).

Site of infection. Gills.

Etymology. The specific name is attributed after Tatiana Raso de Moraes Possato (*in memoriam*; born 1 April 1978, died 30 April 2006), a young researcher who dedicated her short life to the study of elasmobranchs.

Remarks. *Potamotrygonocotyle tatianae* sp. nov. is sister species to *P. dromedarius* (node B, fig. 2). Both species share the presence of an anchor with a deep root twice as large as the superficial root (character 12, table 1 and fig. 2), a homoplastic character also found in *P. rarum*, and a short male copulatory organ (character 1, table 1 and fig. 2), another homoplastic character that can be observed in *P. tocantinsense* sp. nov. However, *P. tatianae* sp. nov. can be distinguished from its sister species in comparative morphology of the dorsal haptoral accessory structure associated with the posterior loculi (elongate laterally versus semicircular, respectively).

Potamotrygonocotyle tocantinsense sp. nov

Description. Based on 19 specimens (fig. 5). Body fusiform, total length – excluding haptor – 670 (580–800; $n = 7$), 193 (140–270; $n = 7$) wide at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; three pairs of head organs converging to unique pore; cephalic glands unicellular, posterolateral to pharynx, with rod-shaped secretion. Anteromedial gland present. Dispersed pigment granules laterodorsal to pharynx, infrequently absent. Mouth surrounded by sclerotized ridges, medial sclerotization of the mouth discontinuous. Pharynx elongate ovate, 76 (70–88; $n = 7$) long, 47 (43–50; $n = 7$) wide; oesophagus short; two intestinal caeca, non-confluent, lacking diverticula; distal ending at midpoint between testis and posterior body ending. Haptor circular, 247 (235–265; $n = 7$) long, 47 (225–250; $n = 7$) wide, with one central and eight peripheral loculi (two anterior, four lateral, two posterior); septa ventrally surrounded by slightly sinuous sclerotized ridge. Dorsal surface of haptor with two 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 associated with posterolateral loculi bilobate, with slightly concave separation between lobes; posterior pair of dorsal haptoral accessory structures associated with posterior loculi semicircular (see fig. 9B). Anchors 55 (53–58; $n = 8$) long, base 25 (23–27; $n = 10$) wide, with diverging roots, evenly curved shaft and point. Hooks similar, 12 (10–12; $n = 44$) long, distributed on marginal membrane of haptor, with depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ sclerotized, short, arcuate, tapered tube, 42 (28–51; $n = 13$) long, distal portion slightly expanded laterally, aperture subterminal. Accessory piece absent. Testis ovate, 118 (83–148; $n = 7$) long, 81 (63–118; $n = 7$) wide; vas deferens not observed; seminal vesicle C-shaped, entering dorsal surface of posterior region of ejaculatory bulb. Ejaculatory bulb muscular, ovate with distinct internal chambers; glands associated with ejaculatory bulb not observed. Germarium tubular, unbranched, looping right intestinal caecum; distal end ascendant; Mehlis' glands bilateral to oötype. Vagina muscular. Vaginal pore sinistroventral at level of common genital pore; distal portion expanded; seminal receptacle spherical. Oötype well developed. Vitellarium coextensive with gut, absent in regions of reproductive organs. Egg ovate, 130 (125–135; $n = 2$) long, 61 (48–75; $n = 2$) wide, with elongate filament; distal end of filament reticulate.

Holotype, type host and type-locality. MZUSP no. 6837; *Potamotrygon* cf. *scobina,* Rio Tocantins, Municipality of Ipueiras, Tocantins, Brazil (11°18′36″S, 48°27′36″W), 13 June 2005 (Host no. MZUSP TO05–22).

Other hosts and localities. 16 paratypes: 9 MZUSP no. 6838a–i, 3 CHIOC no. 37405 a–c, 2 INPA no. 549a–b, 2 USNPC no. 103651, P. cf. *scobina*, Rio Tocantins, Municipality of Ipueiras, Tocantins, Brazil (11°15′48.52″S, 48°26′56.79″W), 13 June 2005 (Host no. MZUSP TO05–22); 33 vouchers: 4 MZUSP no. 6847a–d, 1 INPA no. 551, 2 CHIOC nos 37410-37411, *Potamotrygon scobina*, Rio Urariquera, Municipality of Boa Vista, Roraima, Brazil (3°22′51.9594″N, 60°35′44.1594″W), 21 February 2007 (Host nos MZUSP AM07–40, AM07–45 and AM07–47); 13 MZUSP nos 6839–6846, 5 INPA no. 550a–e, 4 CHIOC nos 37406–37409, 4 USNPC nos 103655–103657, *Potamotrygon* sp. 'jam', Rio Jamari, Municipality of Itapoã do Oeste, Rondônia, Brazil (9°4′33.96″S, 63°18′17.64″W),

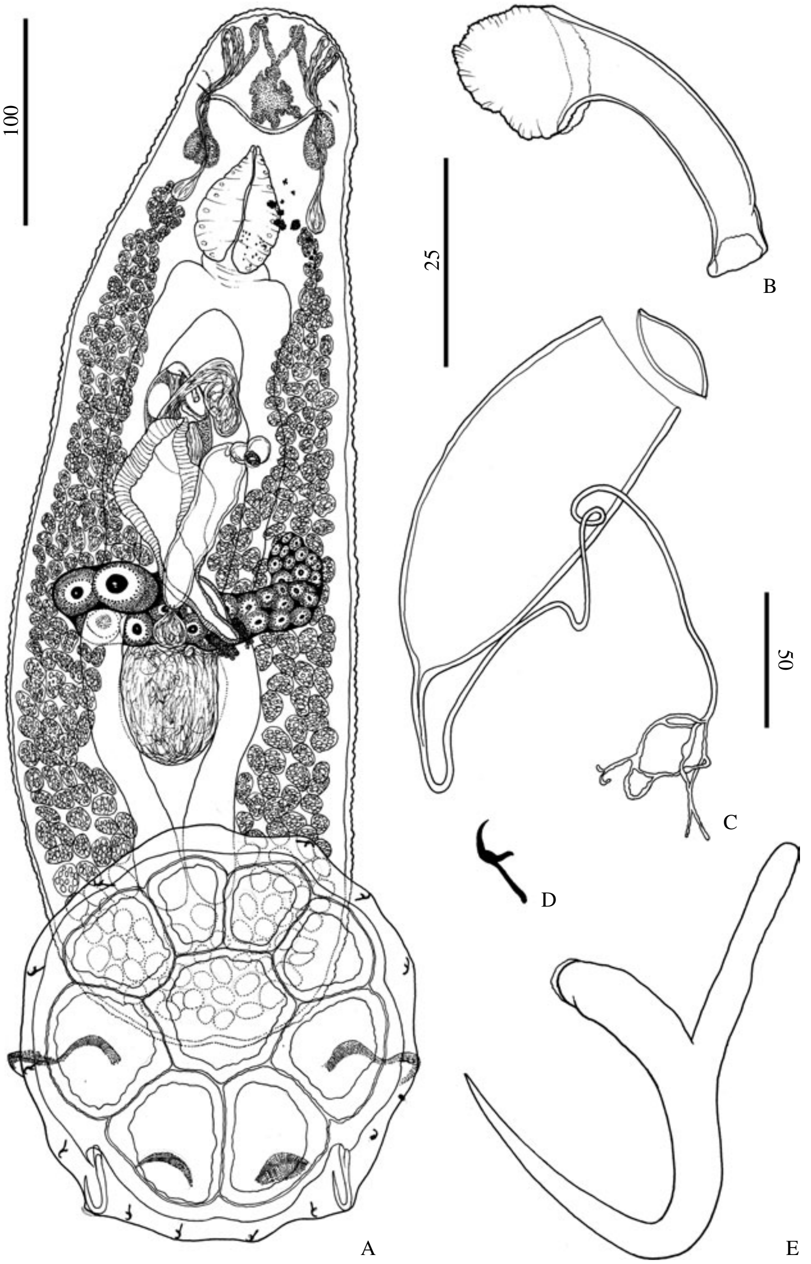

Fig. 5. *Potamotrygonocotyle tocantinsense* sp. nov. (A) Whole mount (holotype); (B) male copulatory organ; (C) egg; (D) hook; (E) anchor. Scale bars in μm.

11–13 July 2006 (Host nos MZUSP AC06–027, AC06–029–030, AC06–032–033, AC06–069).

Site of infection. Gills.

Etymology. The specific name refers to the Rio Tocantins, from where the type-series was collected.

Comparative measurements. Table 7.

Remarks. *Potamotrygonocotyle tocantinsense* sp. nov. is sister to *P. aramasae* (node I, fig. 2) based on the presence of an ejaculatory bulb with distinct internal chambers (character 3, table 1 and fig. 2). However, *P. tocantinsense* sp. nov. differs from *P. aramasae* by having two autapomorphies: male copulatory organ as a short tube (character 1, table 1 and fig. 2) and egg with elongate filament (character 11, table 1 and fig. 2).

Potamotrygonocotyle septemcotyle sp. nov

Description. Based on 17 specimens (figs 6, 7). Body fusiform, total length – excluding haptor – 350 (250–520; $n = 8$), 175 (130–200; $n = 8$) wide at level of germarium.

Table 7. Comparative measurements (in μm) of *Potamotrygonocotyle tocantinsense* sp. nov. from *Potamotrygon* cf. *scobina* (type host), *Potamotrygon scobina* and *Potamotrygon* sp. 'jam'. *N*, Number of specimens measured.

	P. cf. *scobina*	*N*	*P. scobina*	*N*	*Potamtrygon* sp. 'jam'	*N*
Body						
Length	670 (580–800)	7	–	–	–	–
Width	193 (140–270)	7	–	–	–	–
Pharynx						
Length	76 (70–88)	7	–	–	–	–
Width	47 (43–50)	4	–	–	–	–
Haptor						
Length	247 (235–265)	7	–	–	–	–
Width	236 (225–250)	7	–	–	–	–
Anchor						
Length	56 (53–58)	4	56 (51–58)	8	55 (50–59)	20
Width base	25 (23–27)	7	23 (22–25)	5	25 (23–26)	8
Hooks						
Length	12	25	11 (10–12)	23	11 (10–12)	85
MCO						
Length	42 (28–51)	11	46 (44–50)	7	41 (34–45)	22
Testis						
Length	118 (83–148)	7	–	–	–	–
Width	81 (63–117)	7	–	–	–	–
Egg						
Length	135	1	–	–	–	–
Width	48	1	–	–	–	–

Tegument smooth. Cephalic lobes poorly developed or absent; three pairs of head organs converging to unique pore; cephalic glands unicellular, posterolateral to pharynx, with rod-shaped secretion. Anteromedial gland present. Dispersed pigment granules dorsal to pharynx, infrequently absent. Mouth surrounded by sclerotized ridges, medial sclerotization of the mouth discontinuous. Pharynx elongate ovate, 53 (50–60; $n = 7$) long, 41 (38–50; $n = 7$) wide; oesophagus short; two intestinal caeca, non-confluent, lacking diverticula; distal ending close to testis. Haptor circular, 193 (180–215; $n = 6$) long, 184 (170–200; $n = 6$) wide, with one central and seven peripheral loculi (two anterior, four lateral, one posterior) (fig. 7); septa surrounded by slightly sinuous sclerotized ridge. Dorsal surface of haptor with two 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 associated with posterolateral loculi bilobate, with well-separated lobes, each lobe semicircular; posterior pair of dorsal haptoral accessory structures associated with posterior loculus semicircular (see fig. 9C). Anchors 43 (40–48; $n = 9$) long, base 20 (18–22; $n = 5$) wide, with heavy diverging roots; evenly curved shaft and point. Hooks similar, 12 (11–12; $n = 36$) long, distributed on marginal membrane of haptor, with slightly depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ sclerotized, long, straight, slightly tapered tube, 70 (60–80; $n = 8$) long, distal aperture terminal. Accessory piece absent. Testis cordiform, 62 (45–88; $n = 8$) long, 88 (75–105; $n = 7$) wide; vas deferens and seminal vesicle not observed. 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 heavily muscular. Vaginal pore sinistroventral at level of proximal portion of germarium; seminal receptacle not observed. Oötype well developed. Vitellarium coextensive with gut, absent in regions of reproductive organs. Egg ovate, 128 (125–130; $n = 2$) long, 56 (43–70; $n = 2$) wide, with long filament; distal end of filament reticulate.

Holotype, type-host and type-locality. MZUSP no. 6823; *Potamotrygon scobina*, Rio Tocantins, Baía de Marajó, Municipality of Colares, Pará, Brazil (0°55′45″S, 48°17′29″W) 16 August 2007 (Host no. MZUSP PA04–07).

Other specimens. 15 paratypes: 10 MZUSP no. 6824a–j, 2 CHIOC no. 37397a–b, 1 INPA no. 543, 2 USNPC no. 103649, *P. scobina*, Rio Tocantins, Baía de Marajó, Municipality of Colares, Pará, Brazil (0°55′34.68″S, 48°17′25.4394″W), 16 August 2007 (Host no. MZUSP PA07–07); 4 vouchers: 1 MZUSP no. 6826, 1 CHIOC no. 37398, *P. scobina*, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (0° 59′ 58.2″S 48°57′52.5594″W), 29 August 2007 (Host no. MZUSP PA07–83); 1 MZUSP no. 6825, 1 INPA no. 544, *Potamotrygon* sp. 'mar_1', Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (0°59′58.2″S 48°57′52.5594″W), 28 August 2007 (Host nos MZUSP PA07–63–64).

Site of infection. Gills.

Etymology. The specific name refers to the presence of seven peripheral loculi on the haptor.

Comparative measurements. Table 8.

Remarks. *Potamotrygonocotyle septemcotyle* sp. nov. nested as sister to the clade at node G (fig. 2), which is represented by *P. chisholmae* and its junior synonym

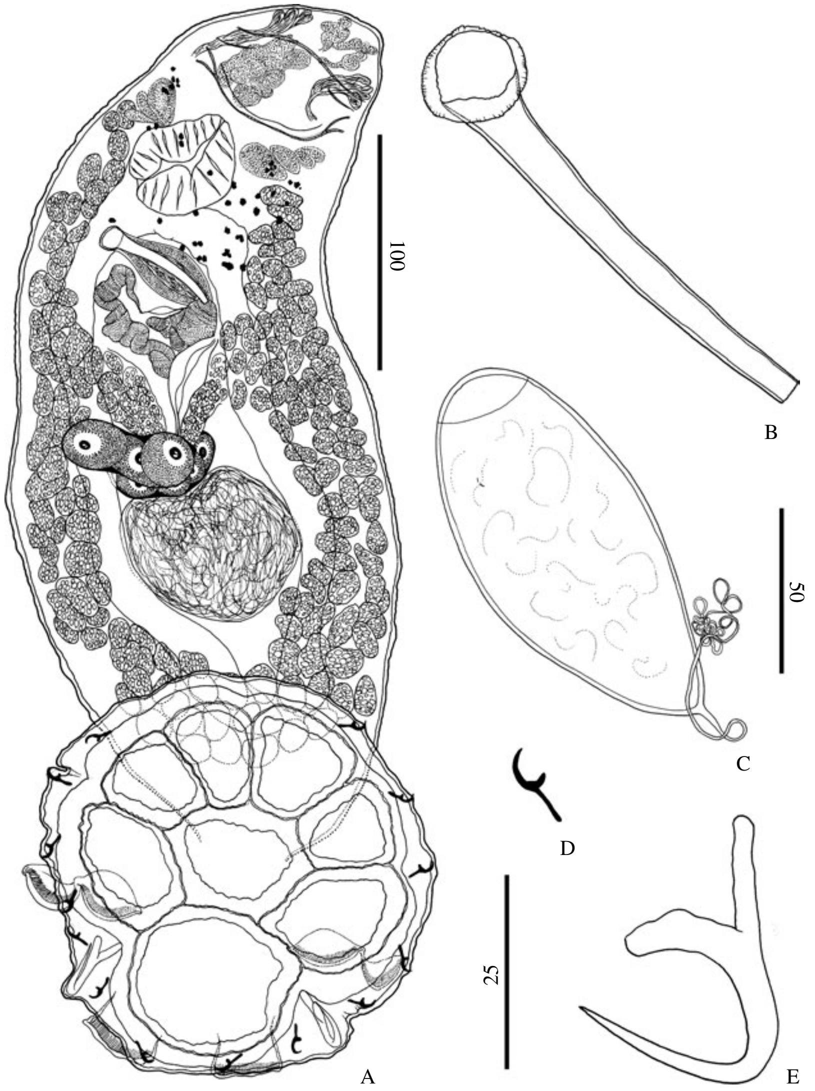

Fig. 6. *Potamotrygonocotyle septemcotyle* sp. nov. (A) Whole mount (holotype); (B) male copulatory organ; (C) egg; (D) hook; (E) anchor. Scale bars in μm.

P. uruguayense, based on the presence of a long egg filament (character 11, table 1 and fig. 2) – a homoplastic character also found in *P. tocantinsense*. *Potamotrygonocotyle chisholmae* can be distinguished from *P. septemcotyle* by possessing continuous medial sclerotization on the mouth, an autapomorphy for the former (character 9, table 1, and fig. 1B). *Potamotrygonocotyle septemcotyle* sp. nov. differs from all other species of *Potamotrygonocotyle* by possessing seven peripherical loculi (character 4, table 1 and fig. 2).

Potamotrygonocotyle auriculocotyle sp. nov

Description. Based on 11 specimens (fig. 8). Body fusiform, total length – excluding haptor – 262 (220–280; $n = 5$), 123 (120–130; $n = 4$) wide at level of germarium. Tegument smooth. Cephalic lobes poorly developed or absent; three pairs of head organs converging to unique pore; cephalic glands unicellular, posterolateral to pharynx, with rod-shaped secretion. Anteromedial gland present. Dispersed pigment granules dorsal to pharynx, infrequently absent. Mouth surrounded by sclerotized ridges, medial sclerotization of the mouth discontinuous. Pharynx elongate ovate, 43 (38–50; $n = 3$) long, 31 (28–35; $n = 3$) wide; oesophagus short; two intestinal caeca, non-confluent, lacking diverticula; distal ending close to testis. Haptor circular, haptoral disc approximately 2/3 of body length, 170 (150–185; $n = 5$) long, 173 (150–185; $n = 5$) wide, with one central and eight peripheral loculi (two anterior, four lateral, two posterior); septa ventrally surrounded by slightly sinuous sclerotized ridge. Dorsal surface of haptor with two pairs of haptoral accessory structures associated with lateral and posterior peripheral loculi; each dorsal

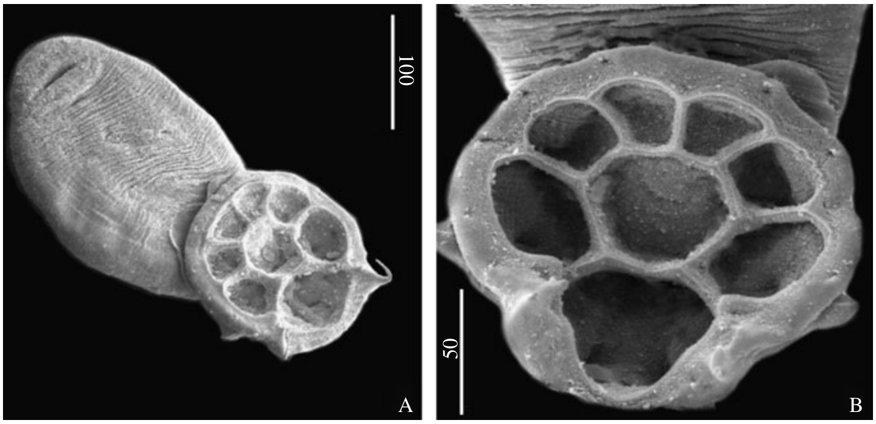

Fig. 7. Morphology of structures of *Potamotrygonocotyle septemcotyle* sp. nov., scanning electron micrographs. (A) Whole specimen, ventral view; (B) ventral view of haptor showing loculi. Scale bars in μm.

haptoral accessory structure with sclerotized margins. Anterior pair of dorsal haptoral accessory structures associated with posterolateral loculi bilobate, each dorsal haptoral accessory structure with lobe with slightly concave separation between lobes, each lobe semicircular; posterior pair of dorsal haptoral accessory structures associated with posterior loculi semicircular, each lobe laterally expanded, ear-like (fig. 9D). Anchors 41 (40–42; $n = 6$) long, base 17 (16–17; $n = 2$) wide, with heavy diverging roots; deep root twice as large as superficial root; evenly curved shaft and point. Hooks similar, 10 (9–11; $n = 32$) long, distributed on marginal membrane of haptor, with slightly depressed thumb, shaft with proximal portion dilated and curved point. Male copulatory organ sclerotized, long arcuate tapered tube, inverted ‘J’ shape, 64 (59–76; $n = 11$) long, aperture terminal. Accessory piece absent. Testis cordiform, 44 (40–50; $n = 3$) long, 51 (50–53; $n = 3$) wide; vas deferens and seminal vesicle not observed. 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 heavily muscular. Vaginal pore sinistroventral at level of genital pore; seminal receptacle not observed. Oötype well developed. Vitellarium coextensive with gut, absent in regions of reproductive organs. Egg not observed.

Table 8. Comparative measurements (in μm) of *Potamotrygonocotyle septemcotyle* sp. nov. from *Potamotrygon scobina* (type host) and *Potamotrygon* sp. ‘mar_1’. *N*, Number of specimens measured.

	P. scobina	*N*	*Potamotrygon* sp. ‘mar_1’	*N*
Body				
Length	350 (250–520)	8	180	–
Width	175 (130–200)	8	110	–
Pharynx				
Length	53 (50–60)	7	20	–
Width	41 (38–50)	7	25	–
Haptor				
Length	193 (180–215)	6	120	–
Width	236 (225–250)	7	125	–
Anchor				
Length	43 (40–48)	9	30	20
Width base	20 (18–22)	5	16	8
Hooks				
Length	12 (11–12)	36	12 (11–12)	6
MCO				
Length	70 (60–80)	8	41 (34–45)	43
Testis				
Length	62 (45–87)	8	30	1
Width	87 (75–105)	7	50	1
Egg				
Length	128 (125–130)	2	–	–
Width	56 (43–70)	2	–	–

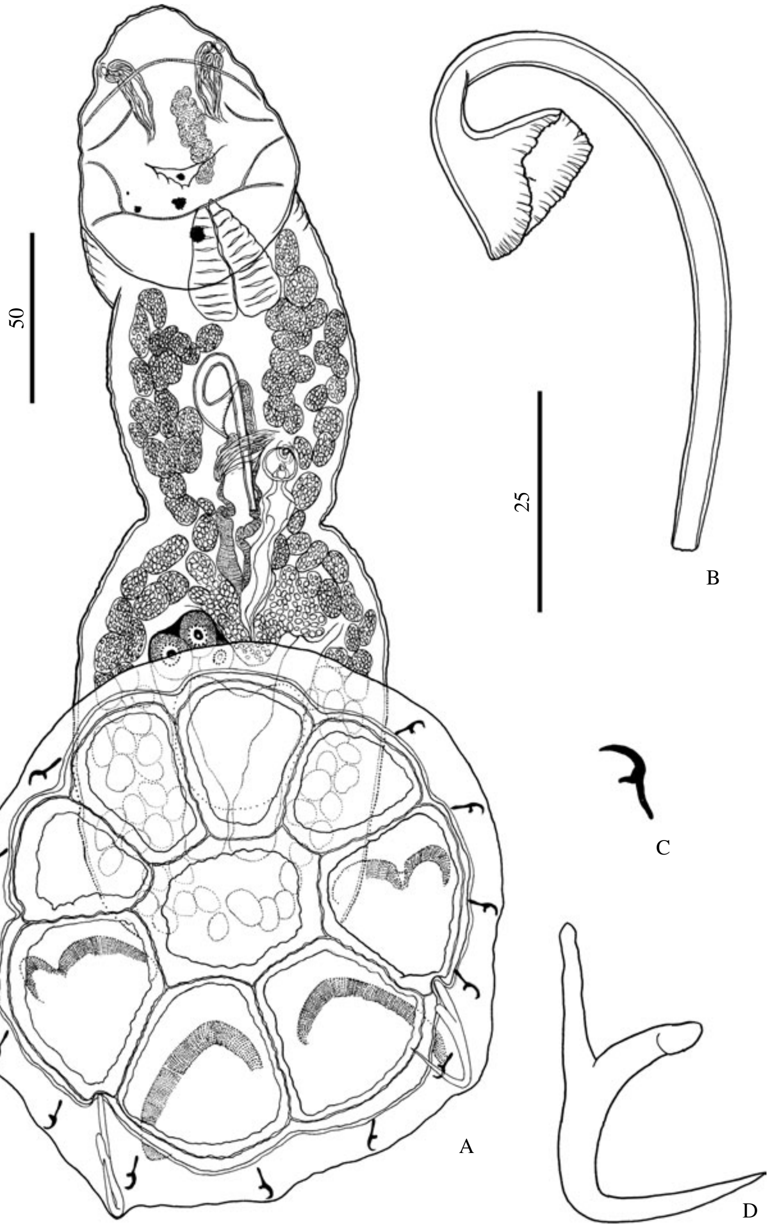

Fig. 8. *Potamotrygonocotyle auriculocotyle* sp. nov. (A) Whole mount (composite); (B) male copulatory organ; (C) hook; (D) anchor. Scale bars in μm.

Holotype, type-host and type-locality. MZUSP no. 6827; *P. motoro*, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (1°00′36″S 48°57′36″W), 28 August 2007 (Host no. MZUSP PA07−50).

Other specimens. 9 paratypes: 7 MZUSP no. 6828a−h, 1 CHIOC no. 37399, 1 INPA no. 545, *P. motoro*, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (0°59′58.2″S 48°57′52.5594″W), 28 August 2007 (Host nos MZUSP PA07−50); 26 vouchers: 1 CHIOC no. 37403, *P. motoro*, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (0°59′58.2″S 48°57′52.5594″W), 29 August 2007 (Host no. MZUSP PA07−82); 8 MZUSP nos 6829−6834, 4 CHIOC no. 37400-37402, 5 INPA no. 547a−e, 3 USNPC nos 103652−103654, 3 HWML no. 49136, *Potamotrygon* sp. ‘mar_1’, Rio Arari, Igarapé do Urubu, Municipality of Cachoeira do Arari, Pará, Brazil (0°59′58.2″S 48°57′52.5594″W), 28 August 2007 (Host nos MZUSP PA07−62−64, PA07−84−85); 1 INPA no. 546, 1 HWML no. 49317, *P. scobina*, Baía de Marajó, Rio Tocantins, Municipality of Colares, Pará, Brazil (0°55′34.68″ S, 48°17′25.4394″W), 22 August 2007 (Host no. MZUSP PA07−46).

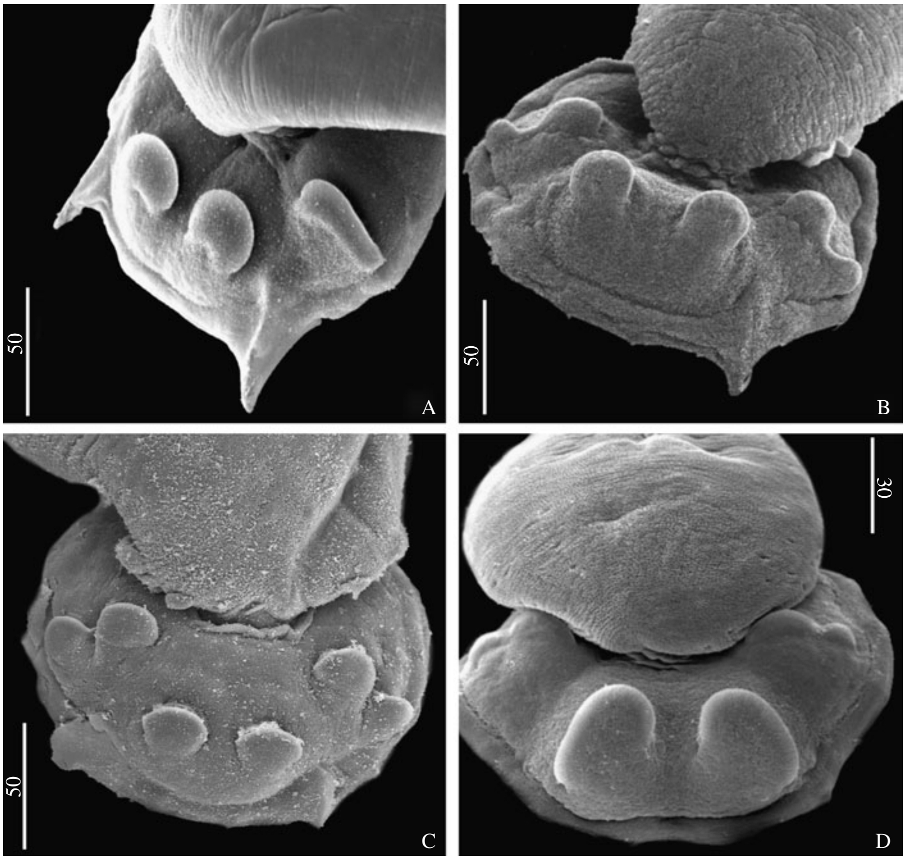

Fig. 9. Morphology of dorsal haptoral accessory structures of *Potamotrygonocotyle* spp., scanning electron micrographs. (A) *Potamotrygonocotyle tatianae* sp. nov.; (B) *Potamotrygonocotyle tocantinsense* sp. nov.; (C) *Potamotrygonocotyle septemcotyle* sp. nov.; (D) *Potamotrygonocotyle auricolacotyle* sp. nov. Scale bars in μm.

Site of infection. Gills.

Comparative measurements. Table 9.

Etymology. The specific name is from Greek and refers to the posterior dorsal haptoral accessory structure which is similar to an ear.

Remarks. *Potamotrygonocotyle auriculocotyle* sp. nov. nested in a polytomy with *P. rionegrense* and a large clade comprised of most of the species of *Potamotrygonocotyle* (node D) in a node (C) supported by the presence of the dorsal haptoral accessory structure associated with the posterolateral loculi bilobate (character 5, table 1 and fig. 2). *Potamotrygonocotyle auriculocotyle* sp. nov. differs from *P. rionegrense* by having the posterior dorsal haptoral accessory structure elongated laterally, similar to an 'ear' (character 7, table 1 and fig. 2), which is semicircular in *P. rionegrense*.

Discussion

Taxonomic status of Potamotrygonocotyle

The monophyly of *Potamotrygonocotyle* is supported by the presence of sclerotizations around the mouth. We did not observe eggs in *P. auriculocotyle* sp. nov. and *P. rarum;* however, based on our analysis, the presence of an ovate egg seems to represent another synapomorphy for the genus. We recognized 12 valid species for the genus after examining 772 specimens collected in 436 potamotrygonid host individuals from 19 localities (i.e. sub-basins) throughout South America. This is the most extensive sampling effort ever to document the monogenoid fauna of freshwater stingrays, without which we would not have been able to acknowledge the morphological variability within each nominal species and provide a more robust taxonomy for this genus.

Early attempts to advance our understanding of the diversity of freshwater monocotylids generally overlooked the importance of biogeographical and host representation on the circumscription of *Potamotrygonocotyle* species. As a result, two recently erected species (e.g. *Potamotrygonocotyle eurypotamoxenus* and *P. uruguayense*; Domingues & Marques, 2007) are now considered junior synonyms of *P. tsalickisi* and *P. chisholmae*, respectively. For each case, as more material became available to us, it became clear that certain diagnostic characters that were once thought to be discrete are, in fact, part of a continuous morphological cline. Thus, since we have examined more specimens for

Table 9. Comparative measurements (in µm) of *Potamotrygonocotyle auriculocotyle* sp. nov. from *Potamotrygon motoro* (type host), *Potamotrygon scobina* and *Potamotrygon* sp. 'mar_1'. *N*, Number of specimens measured.

	P. motoro	*N*	*P. scobina*	*N*	*Potamotrygon* sp. 'mar_1'	*N*
Body						
Length	262 (220–280)	5	320 (290–350)	2	288 (200–320)	8
Width	123 (120–130)	4	155 (140–170)	2	100 (70–140)	8
Pharynx						
Length	43 (38–50)	3	40 (38–43)	2	34 (25–38)	6
Width	31 (28–35)	3	33 (30–35)	2	28 (25–35)	6
Haptor						
Length	170 (150–185)	5	200	1	159 (140–175)	7
Width	173 (150–185)	3	195 (190–200)	2	170 (135–200)	7
Anchor						
Length	41 (40–42)	6	–	–	37 (35–42)	13
Width base	17 (16–17)	2	–	–	16 (14–18)	14
Hooks						
Length	10 (9–11)	32	–	–	10 (9–12)	48
MCO						
Length	64 (59–76)	11	63 (61–65)	3	62 (45–73)	22
Testis						
Length	44 (40–50)	3	–	–	44 (37–50)	6
Width	51 (50–53)	3	–	–	31 (17–43)	6
Egg						
Length	–	–	–	–	–	–
Width	–	–	–	–	–	–

each nominal species, understanding the boundaries of interspecific variability for each of these nominal species became possible. This observation only points out the obvious: we need large sample sizes and meaningful biogeographical and host representation to achieve our goal of a fair assessment and understanding of the diversity of freshwater monocotylids. This pattern should not be different for any other parasite group, but historically our understanding of the diversity of parasites of potamotrygonids, except for a few recent studies (e.g. Marques & Brooks, 2003; Reyda, 2007), has traditionally been based on biological data obtained from restricted biogeographical areas, low host representation, and only a few examined specimens. Our prediction is that, as we study other taxa of parasites of potamotrygonids within the framework adopted in the present study, we will better understand the diversity of this host–parasite system and consequently be able to define species boundaries based on robust and unambiguous morphological characters.

It is worth mentioning that the species recognition approach used here for *Potamotrygonocotyle* is underlined by a species concept that provided hypotheses for nominal species that are themselves open to critical test. We recognized as valid species all terminals that either possessed an autapomorphy or could be distinguished from those in the topology (fig. 2). As will be discussed below, some valid species (e.g. *P. chisholmae* and *P. tsalickisi*) recognized here have a widespread distribution through out South America and are hosted by many different species of potamotrygonids, raising the possibility that these comprise species complexes. Be that as it may, we believe that the approach undertaken in this study is sound, even though we were limited by the number of morphological characters considered informative for the systematics of this group, which was unable to resolve 30% of the nodes. The lack of resolution is attributed to the low number of informative characters compiled. We believe that the inclusion of different sources of characters (e.g. molecular) will not only improve the resolution of the sister-group relationships within *Potamotrygonocotyle* but also test our concept of valid species within the genus.

Patterns of distribution and host specificity in Potamotrygonocotyle

The pattern of distribution for species of *Potamotrygonocotyle* seems to mirror those already reported for other helminths (cestodes) inhabiting potamotrygonids (Marques & Brooks, 2003; Marques *et al.*, 2003; Bueno, 2010). For instance, some species are known to inhabit a single host species, which is in turn only found in a single biogeographical region (i.e. sub-basin), such as *P. quadracotyle* and *P. umbella* from *Potamotrygon* sp. 'cururu', all restricted to Rio Negro. On the other hand, *P. rarum* collected from *Potamotrygon schroederi* in Rio Negro and *P. tatianae* sp. nov. from *Paratrygon* sp. '1' from the upper Solimões seem to be biogeographically restricted despite the observation that their hosts are found elsewhere. *Potamotrygon schroederi* is also known from the Orinoco basin (Carvalho *et al.*, 2003) and this undescribed species of *Paratrygon* has been collected in the upper Solimões and lower Amazon (F.P.L. Marques, unpublished data). Close attention to the pattern of distribution of *Potamotrygonocotyle aramasae* reveals that this species is tightly associated with its host (*Paratrygon aiereba*) throughout its distribution. This observation suggests that we should expect to find *P. rarum* and *P. tatianae* in the Orinoco and lower Amazon, respectively, if this distributional pattern is not constrained by any particular abiotic properties of South American rivers (see below). Since we did not have access to gill samples from these particular hosts in other localities, this prediction remains to be tested by additional sampling.

An indication that some species are biogeographically restricted despite the distribution of their hosts is somewhat illustrated by the species of *Potamotrygonocotyle* found in the lower Amazon (i.e. Baía de Marajó, Pará, Brazil). We reported two species for this area, *P. septemcotyle* sp. nov. and *P. auriculocotyle* sp. nov.; both found in *Potamotrygon scobina* and an undescribed species of *Potamotrygon* (herein referred to as 'mar_1'), in addition to the report of *P. auriculocotyle* sp. nov. in *Potamotrygon motoro*. Although this undescribed species of *Potamotrygon* is only known for Baía de Marajó and we have not obtained samples of *Potamotrygon scobina* from elsewhere, we examined many specimens of *Potamotrygon motoro* from other localities without finding *P. auriculocotyle* sp. nov.

In contrast to the patterns described above, four species of *Potamotrygonocotyle* (i.e. *P. tsalickisi*, *P. chisholmae*, *P. dromedarius* and *P. rionegrense*) are widely distributed. In all cases, they can be found in both major basins of South America: Amazon and La Plata. Among those, some parasite species are restricted to as few as 4–5 host species (i.e. *P. dromedarius* and *P. rionegrense*, respectively) or to as many as 10 (i.e. *P. tsalickisi* and *P. chisholmae*). This pattern of host specificity and distribution is unusual among monogenoids, which are considered to exhibit strict host specificity (Bychowsky, 1957; Llewellyn, 1982). One could argue that some of these species may include hidden lineages within their concept (i.e. cryptic species). We do not rule out this possibility, the corollary of which would be that the pattern observed is due to taxonomic artefact, which could be tested with additional material and the assessment of different source data.

At the present state of knowledge, we provide no detailed explanation for the patterns described above. This depends primarily on additional samples from areas that have never been appropriately surveyed, better refinement on the taxonomy of the hosts, and examination of additional host species, among others. It is clear that this is a complex system of host–parasite association, nested in a biogeographical region with equally complex history that currently houses a mosaic of aquatic environments – each of these with their own abiotic properties. The underlying processes responsible for the patterns recovered thus far ought to have historical and ecological components.

Explanations for the absence of lineages of *Potamotrygonocotyle* in some hosts and localities are equally complex to provide, due to the patterns described above. Throughout this study, members of *Potamotrygonocotyle* have been collected from the gills of 12 nominal species of Potamotrygonidae from the major basins of South America. Although we have sampled more than 60% of 16–21 valid host species, in addition to 171 unidentified hosts from an unknown number of undescribed potamotrygonids, we have not collected members of *Potamotrygonocotyle* in *Plesiotrygon iwamae* Rosa, Castello & Thorson, 1987, *Potamotrygon signata* Garman, 1913, an undescribed species of *Potamotrygon* from upper Tabajós (herein referred to as *Potamotrygon* sp. 'tpj_2'), and three distinct chromatic patterns of an unidentified species of

Table 10. List of hosts for which no parasites were recorded. A, Meruu–Acará–Guama–others; B, Parnaíba; C, Amazonas–Tapajós–Juruena; D, Tocantins–Paranã–Palma; E, Rio Araguaia–Crixas Açu–Peixe; F, Solimões–Negro–Branco; G, Amazonas–Xingú–Iriri-Paru; H, Amazonas–Trombetas–others; I, Rio Araguaia–Crixas Açu–Peixe; J, Tocantins–Manoel Alves–Sono.

Species	*N*	Basin	Sub-basin	Locality
Plesiotrygon awamae	5	Amazon	A	Baía do Marajó, Municipality of Colares, Pará, Brazil (0°55′45″S, 48°17′29″W), August 2007
Potamotrygon signata	8	Amazon	B	Confluency of Rio Poty and Rio Parnaíba, Municipality of Terezina, Piauí, Brazil (5°2′10.6794″S, 42°50′17.8794″W), September 03 2007
Potamotrygon sp. 'tpj_2'	7	Amazon	C	Rio Teles Pires, Municipality of Alta Floresta, Mato Grosso, Brazil (8°52′48″S, 57°22′48″W), December 2005
Potamotrygon sp. 'toc_1'	9	Tocantins		Rio Tocantins, Municipality of Paranã, Tocantins, Brazil, (12°37′48″S, 52°57′36″W), July and August 2004
Potamotrygon sp. 'toc_4'	1	Tocantins	E	Rio Araguaia, Municipality of Caseara, Tocantins, Brazil (9°16′11″S, 49°57′36″W), June 2005
Potamotrygon orbignyi	17	Amazon	F	Rio Negro, Municipality of Barcelos, Amazonas, Brazil (62°55′13″W, 0°58′11″S), January–February 2005
	6	Amazon	G	Rio Xingú, Municipality of São Félix do Xingú, Pará, Brazil (6°39′36″S, 52°0′0″W), July 2005
	3	Amazon	C	Rio Tapajós, Municipality of Santarém, Pará, Brazil (2°16′47″S, 55°0′0″W), October 2005
	1	Amazon	H	Rio Urariquera, Municipality of Boa Vista, Roraima, Brazil (2°48′00″N, 60°39′00″W), February 2007
	3	Amazon	A	Baía do Marajó, Municipality of Colares, Pará, Brazil (0°55′45″S, 48°17′29″W), August 2007
	30	Tocantins	I	Rio Araguaia, Municipality of Caseara, Tocantins, Brazil (9°16′11″S, 49°57′47″W), June 2005
	1	Tocantins	J	Rio Tocantins, Municipality of Ipueiras, Tocantins, Brazil (11°18′36″S, 48°27′36″W), June 2005
Potamotrygon cf. *orbignyi*	1	Tocantins	J	Rio Tocantins, Municipality of Ipueiras, Tocantins, Brazil (11°18′36″S, 48°27′36″W), June 2005

Potamotrygon from upper Tocantins (referred to as *Potamotrygon* spp. 'toc_1', 'toc_3' and 'toc_4') (table 10). Although few specimens have been found parasitizing *Potamotrygon orbignyi* from the upper Amazon region (Rio Yavari and Rio Tarauacá), we did not find *Potamotrygonocotyle* on *P. orbignyi* from five Amazonian sub-basins (e.g. Negro, Xingú, Tapajós, Urariquera, Rio Araguaia/Tocantins; see table 10) and the mouth of the Amazon (i.e. Baía de Marajó; see table 10).

The absence of members of *Potamotrygonocotyle* in *Plesiotrygon iwamae* could have at least two explanations: one related to abiotic conditions of the locality from which the available samples came, the other historical. The samples we examined for this host came from a survey carried out in Baía de Marajó, an area of the lower Amazon which suffers tidal and salinity fluctuations throughout the year. Potamotrygonids have been known to exhibit low tolerance to brackish water (Thorson *et al.*, 1983), although the limits tolerated seem to be unknown and certainly varied among different species (Charvet-Almeida, 2001). Among the species collected at the same locality from which we obtained our samples for *Plesiotrygon iwamae*, the latter seems to be the most tolerant to different levels of diluted salt in the water (Charvet-Almeida, 2001). If members of *Potamotrygonocotyle* do not share the same level of salt tolerance as *Plesiotrygon iwamae*, we would expect that salinity is determining the distribution of these parasites in this species at this particular collecting site. If that is true, we would expect to find members of *Plesiotrygon iwamae* hosting monogenoids in the middle and upper portions of the Amazon, both of which are within the distributional range of this potamotrygonid. If upon the examination of additional hosts we fail to encounter members of *Potamotrygonocotyle*, sorting events (i.e. extinction or failure to speciate) could be contemplated as a hypothesis to explain the absence of the parasite on this host species.

Compared to most species of potamotrygonids, *Potamotrygon signata*, an endemic species from Rio Parnaíba, has been known to host very few species of cestodes (Marques & Brooks, 2003). For instance, cestode genera commonly found in potamotrygonids (i.e. *Acanthobothrium*, *Rhinebothrium* and *Potamotrygonocestus*) have never been reported after examining more than 20 host specimens (Marques & Brooks, 2003; Marques *et al.*, 2003; Reyda, 2007; Bueno, 2010). Compared to the rivers from Amazon and La Plata basins, Rio Parnaíba has a very distinct geological and palaeoclimatic history (see Rosa *et al.*, 2003; Ribeiro, 2006). Compared to adjacent and historically connected sub-basins of the Amazon, Pantanal and north-eastern Brazil, Rio Parnaíba has a much lower fish diversity, of which a little more than 90% of the lineages found in that river have also been reported elsewhere. The underlined assumption to explain the low diversity of Rio Parnaíba is the extinction of many lineages as a result of the palaeogeographical history of this basin. Thus, the absence of parasites in *Potamotrygon signata* mirrors the absence of many groups of fishes found in other rivers that have been connected historically to Rio Parnaíba (see Hubert & Renno, 2006).

The absence of members of *Potamotrygonocotyle* in *Potamotrygon orbignyi* and other species from Rio Tocantins and Tapajós (see above) remains puzzling. These species are hosts of the majority of the lineages of cestodes documented for potamotrygonids (Marques & Brooks, 2003; Marques *et al.*, 2003, Reyda, 2007; Bueno, 2010). There are no data available on the ecology of these species that would allow any ecological explanation for the absence of members of *Potamotrygonocotyle*. The same applies for any historical explanation, which would depend on evaluating the phylogenetic positions of these hosts in comparison to the phylogeny of the parasites. This and a number of other unanswered questions, which ultimately depend on refining the taxonomy and phylogeny of each component of the system under the possession of more robust data, should be addressed as we acummulate more information on this host–parasite system.

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