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Monogenoidean parasites of *Acestrorhynchus falcatus* (Characiformes: Acestrorhynchidae) from Pará, Brazil: species of *Diaphorocleidus* and *Rhinoxenoides* n. gen. (Monogenoidea: Dactylogyridae)

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Abstract

Two new species of *Diaphorocleidus* and one new species of *Rhinoxenoides* n. gen. are described from the gills of *Acestrorhynchus falcatus* (Bloch) from rivers of north-eastern Pará, Brazil. *Diaphorocleidus jaymedeloyolai* n. sp. is characterized by a male copulatory organ (MCO) possessing three counterclockwise coils; similar anchors with subtriangular superficial roots; a ventral bar with posteromedial projection; and hooks of pairs 1, 4 and 7 approximately three times longer than hook pair 5. *Diaphorocleidus sclerocolpus* n. sp. differs from its congeners by a dual-branched accessory piece articulated with the MCO and a sclerotized tubular vagina with a bottle-shaped vestibule. *Rhinoxenoides* n. gen. is proposed and is characterized by possessing: MCO sclerotized with clockwise coils; an accessory piece articulated to the base of MCO; a sinistroventral vaginal aperture; ventral anchor with conspicuous roots; dorsal anchor with superficial root five times longer than deep root; and absence of dorsal bar. The proposal of *Rhinoxenoides* n. gen. is also supported by its phylogenetic relationship with *Protorhinoxenus prochilodi* and species of *Rhinoxenus*, using 16 morphological characters, which resulted in the following hypothesis of sister-group relationships: *Rhinoxenoides* n. gen. [*Protorhinoxenus* (*Rhinoxenus curimatae* (*R. nyttus* (*R. bulbovaginatus* (*R. guianensis, R. piranhus, R. euryxenus* (*R. arietinus, R. anaclaudiae*)))))].

Introduction

Members of the Acestrorhynchidae (Characiformes) are endemic and widely distributed in South American rivers. The greatest diversity of acestrorhynchids occurs in the Amazon and Orinoco basins (Menezes, 2003). The family was proposed by Lucena & Menezes (1998) to accommodate *Acestrorhynchus* Eigenmann & Kennedy, 1903. Oliveira *et al.* (2011) also proposed that the family should be amplified and divided into three subfamilies: Acestrorhynchinae, Roestinae and Heterocharacinae (*sensu* Mirande, 2009) in order to accommodate six other genera of characiforms. Under this revised classification Acestrorhynchidae comprises 26 species from seven genera (Oliveira *et al.*, 2011; Nelson *et al.*, 2016), where *Acestrorhynchus* is the most diverse group, containing more than 19 nominal species of which 14 are considered valid (Menezes, 2003; Toledo-Piza, 2007). Historically, only three species of *Acestrorhynchus* have been investigated for metazoan parasites, namely *Acestrorhynchus falcatus* (Bloch), *A. falcirostris* (Cuvier) and *A. microlepis* (Jardine). Together, these species are host to 20 metazoan parasite species, including a single acanthocephalan, isopod and myxozoan, 2 monogenoid, 3 copepod, 4 trematode and 8 nematode species (table 1). Although unidentified specimens belonging to *Ameloblastella* and *Diaphorocleidus* have been reported from acestrorhynchids, these reports are from fish from rivers in south-east Brazil (Camargo *et al.*, 2015), and no monogenoids have previously been reported from acestrorhynchids in rivers of the Amazon Basin.

We investigated the monogenoidean parasites infecting *A. falcatus* that inhabit the streams and rivers of the coastal drainage ecosystem of the Amazon Basin in the State of Pará, Brazil. Two new species of *Diaphorocleidus* (Dactylogyridae) and one new species of a new genus of Dactylogyridae are described herein.

Table 1. List of species of parasites from *Acestrorhynchus* spp.

Host	Parasite	Country	Reference
A. falcatus	Trematoda		
	Bellumcorpus major	BR	Thatcher (2006)
	Acanthocephala		
	Palliolisentis polyonca	CO	Thatcher (2006)
	Nematoda		
	Paracapillaria piscicola	BR	Travassos *et al.* (1928)
	Procamallanus (*Spirocamallanus*) *inopinatus*	BR	Travassos *et al.* (1928)
	Copepoda		
	Ergasilus turucuyus	BR	Malta & Varella (1996)
	Myxozoa		
	Henneguya adherens	BR	Azevedo & Matos (1995)
A. falcirostris	Copepoda		
	Ergasilus turucuyus	BR	Malta & Varella (1996)
A. microleps	Isopoda		
	Braga amapaensis	BR	Thatcher (1996)
A. lacustres	Nematoda		
	Contracaecum sp.	BR	Camargo *et al.* (2015)
	Philometroides caudata	BR	Camargo *et al.* (2015)
	Procamallanus (*S.*) *inopinatus*	BR	Camargo *et al.* (2015)
	Procamallanus (*S.*) *saofranciscensis*	BR	Camargo *et al.* (2015)
	Procamallanus (*S.*) *paraensis*	BR	Camargo *et al.* (2015)
	Travassosnema travassosi	BR	Costa *et al.* (1991)
	Monogenoidea		
	Ameloblastella sp.	BR	Camargo *et al.* (2015)
	Diaphorocleidus sp.	BR	Camargo *et al.* (2015)
	Digenea		
	Ascocotyle sp.	BR	Camargo *et al.* (2015)
	Diplostomidae gen. sp.	BR	Camargo *et al.* (2015)
	Sphincterodiplostomum musculosum	BR	Camargo *et al.* (2015)
	Copepoda		
	Rhinergasilus piranhus	BR	Luque *et al.* (2013)

Countries: BR, Brazil, CO, Colombia.

Materials and methods

Three specimens of *A. falcatus* were captured with the aid of a trammel net; two specimens along the Açaiteua River (North/North-east Atlantic Basin; Gurupi, Turiaçu Sub-basin), Vila Fátima, Municipality of Tracuateua, Pará, Brasil (1°07′45.00″S, 47°00′26.9″W) in August of 2014; and one specimen from the Cururutuia stream, Caeté River (North/North-east Atlantic Basin; Gurupi, Turiaçu Sub-basin), Municipality of Bragança, Pará, Brazil (1°4′44.55″S, 46°44′18.54″W) in December of 2014.

Gill arches were removed and placed in vials containing heated water (~65°C). Each vial was shaken vigorously and formalin was added to obtain a 5% solution. In the laboratory, the content of each vial was examined using a dissecting microscope (Leica S6D; Leica Microsystems, Wetzlar, Germany), and helminths were removed from the gills or sediment using dissection needles. Some specimens were stained with Gomori's trichrome (Humason, 1979; Boeger & Vianna, 2006) and mounted in Dammar gum to determine internal soft structures, and others were mounted in Hoyer's medium or Gray & Wess for the study of sclerotized structures (Humason, 1979; Boeger & Vianna, 2006).

The measurements, all given in micrometres, were obtained according to the procedures of Mizelle & Klucka (1953). Dimensions of organs and other structures represent the greatest measurement in dorso-ventral view; lengths of curved or bent structures (anchors, bars and accessory piece) represent the straight-line distances between extreme ends; length of the male copulatory organ (MCO) was measured on drawing-tube images; total lengths were taken using FIJI/ImageJ 1.51d image analysis software (Rasband, 1997–2016), together with the plug-in WormBox (Vellutini & Marques, 2011–2014). Each average measurement is followed by the range and the number (*n*) of specimens measured, in parentheses. Illustrations were prepared with the aid of a drawing tube on a Leica DM 2500 microscope with differential interference contrast and phase-contrast optics. Calculations of prevalence and mean intensity followed Bush *et al.* (1997). Type specimens and vouchers were deposited in the following collections: Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, Brazil; Invertebrate Collection of the Instituto de Pesquisas da Amazônia (INPA), Manaus, AM, Brazil; Invertebrate Collection of the Museu Paraense Emílio Goeldi (MPEG), Belém, PA, Brazil.

Sixteen characters were used for the phylogenetic analysis of ten terminals, which included *Protorhinoxenus prochilodi* Domingues & Boeger, 2002, *Rhinoxenoides horacioschneideri* n. gen. n. sp., and species of *Rhinoxenus* Kritsky, Thatcher & Boeger, 1988. *Urocleidoides* sensu stricto (*s.s.*) Mizelle & Price, 1964 and *Diaphorocleidus* Jogunoori, Kritsky & Venkatanarasaiah, 2004 were used as outgroups to root the cladogram. Characters in which the respective derived character represents an autapomorphy of a single ingroup taxon were included for definition of the generic diagnosis. The data matrix was constructed using the program Winclada (version 1.00.08; Nixon, 1999–2002). The phylogenetic analysis was performed with the program TNT (Goloboff *et al.*, 2008), using implicit enumeration, as the tree search algorithm, and collapsing unsupported branches after search. Rooting and character optimization were verified using Winclada (version 1.00.08; Nixon, 1999–2002). Bremer support for the respective nodes was determined using the program TNT, using suboptimal trees with five additional steps (SUB 1, SUB2 , …, SUB5), increasing the number of suboptimal trees in each additional step (HOLD 1000, HOLD 2000, …, HOLD 5000). All characters were considered unordered and equally weighted.

Results

Systematics

Class: Monogenoidea Bychoswky, 1937
Subclass: Polyonchoinea Bychoswky, 1937
Order: Dactylogyroidea Bychoswky, 1937
Dactylogyridae Bychowsky, 1933
Diaphorocleidus Jogunoori, Kritsky & Venkatanarasaiah, 2004

Diaphorocleidus jaymedeloyolai *n. sp.*

Description

Based on 14 specimens, nine stained with Gomori's trichrome and five mounted in Hoyer's medium. Body 181 (116–331; *n* = 8) long, 117 (84–238; *n* = 8) wide at level of vagina, elongate, foliform, comprising cephalic region, trunk and haptor (fig. 1a). Tegument smooth. Anterior region with two lateral and one terminal cephalic lobes, moderately developed; three pairs of head organs; cephalic glands not observed. Two pairs of eyespots, equidistant; anterior pair longer than posterior pair; accessory granules present, ovate, dispersed in the cephalic region (fig. 1a). Mouth subterminal, midventral; pharynx muscular, ovate or subspherical 21 (11–25; *n* = 8) long, 14 (12–18; *n* = 8) wide; oesophagus short (fig. 1a). Two intestinal caeca, confluent posteriorly to gonads, lacking diverticula (fig. 1a). Common genital-pore opening mid-ventral, near level of caecal bifurcation (fig. 1a). Genital atrium muscular. Intercaecal gonads, overlapping; germarium ventral to testis (fig. 1a). Vas deferens looping left intestinal caecum; seminal vesicle sigmoid, short. Single prostatic reservoir, saccate, posterior to copulatory complex (fig. 1a). Copulatory complex comprising male copulatory organ (MCO), accessory piece (fig. 1b). MCO sclerotized, tubular, coiled counterclockwise, with approximately three coils, 202 (189–221; *n* = 6) long; base with sclerotized cap; circular sclerotized tandem brim associated with the base of the MCO (fig. 1b). Accessory piece sclerotized, non-articulated with the MCO, subtriangular, sheath-shaped 31 (25–44; *n* = 9) long (fig. 1b). Germarium pyriform, 27 (15–31; *n* = 6) long, 18 (16–20; *n* = 6) wide. Vagina opening ventrally at the left body margin, near body mid-length, comprising vaginal vestibule with slight sclerotization at proximal portion, heavily sclerotized at distal portion, cup-shape, vaginal canal sclerotized, elongated, straight (fig. 1a). Seminal receptacle spherical, at level of anterior margin of germarium, ventral (fig. 1a). Mehlis' glands, ootype, egg not observed. Vitellarium dense throughout trunk, except in region of other reproductive organs (fig. 1a). Peduncle short. Haptor subhexagonal, 59 (48–125; *n* = 8) long, 122 (85–288; *n* = 8) wide (fig. 1a). Anchors similar with well-developed superficial root, subtriangular; poorly developed deep root; evenly curved shaft and point; point extending well past level of tip of inner base; anchor filament extending from the base to middle of shaft. Ventral anchor with angle of approximately 115°, outer 48 (40–52; *n* = 6) long, inner 48 (37–55; *n* = 6) long, base 16 (15–18; *n* = 5) (fig. 1j). Dorsal anchor with angle of approximately 95°, outer 40 (37–42; *n* = 5) long, inner 38 (36–41; *n* = 5) long, base 12 (10–15; *n* = 4) (fig. 1k). Ventral bar 46 (43–48; *n* = 8) long, broadly V-shaped with terminal enlargements, small postero-medial projection (fig. 1i). Dorsal bar 45 (36–56; *n* = 6) long, narrow, broadly U-shaped, with terminal enlargements (fig. 1h). Hooks similar in shape, shank divided into two subunits, proximal third of shank inflated; filamentous hook loop extending to near beginning of shank dilation, each with slightly erect thumb, lightly curved shaft, delicate point. Hook pairs 1, 4 and 7, 31 (25–36; *n* = 8) (fig. 1d); pair 2, 21 (16–27; *n* = 7) (fig. 1f); pair 6, 29 (24–33; *n* = 6) (fig. 1e); pair 3, 26 (21–29; *n* = 7) (fig. 1g); hook pair 5 smaller than other hooks, 11 (10–15; *n* = 5) long (fig. 1c).

Taxonomic summary

Type host. Acestrorhynchus falcatus (Bloch, 1794).

Site of infection. Gills.

Type locality. Caeté River Basin, Municipality of Bragança, Pará, Brazil (1°4′44.55″S, 46°44′18.54″W) collected in December of 2014.

Other localities. Açaiteua River, Vila Fátima, Municipality of Tracuateua, Pará, Brazil (1°07′45.00″S, 47°00′26.9″W) collected in August of 2014.

Prevalence. 100% of the three hosts examined.

Mean intensity. 10.5 parasites per infected host.

Specimens deposited. Holotype: CHIOC 39021a. Twelve paratypes: CHIOC 39021b–g, INPA 754, MPEG 0127–0129. Four vouchers: CHIOC 39022–39023, INPA 755, MPEG 0130.

Etymology. The species is named in honour of the late Prof. Jayme de Loyola-Silva (1927–2017) in recognition and admiration

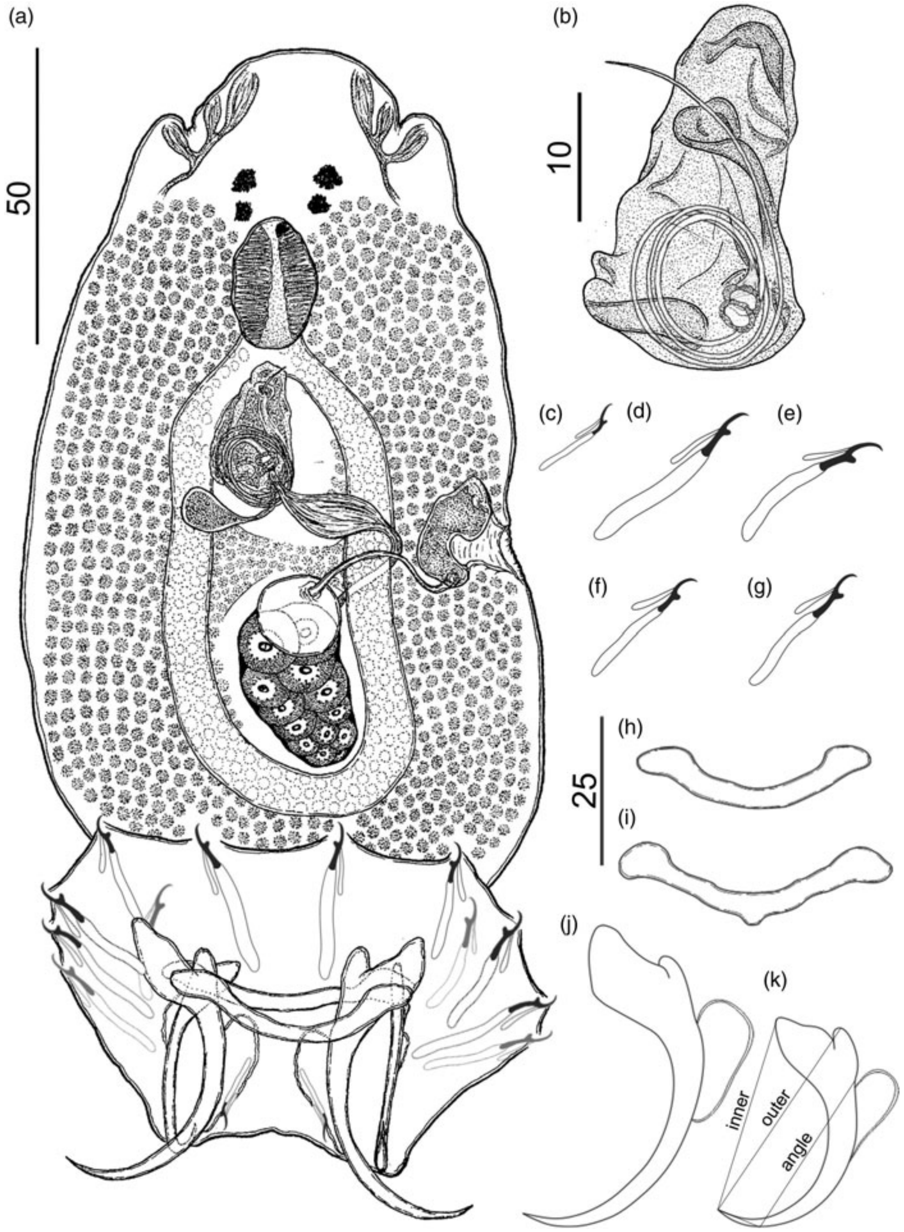

Fig. 1. *Diaphorocleidus jaymedeloyolai* n. sp. (a) Holotype, whole-mount (ventral view); (b) copulatory complex (ventral view); (c) hook pair 5; (d) hook pairs 1, 4 and 7; (e) hook pair 6; (f) hook pair 2; (g) hook pair 3; (h) dorsal bar; (i) ventral bar; (j) ventral anchor; (k) dorsal anchor. Scale bars: (a) 50 µm; (b) 10 µm; (c–k) 25 µm.

of his dedication to research and the teaching of zoology to many generations of Brazilian zoologists.

Comparative measurements. See table 2.

Remarks

Diaphorocleidus jaymedeloyolai n. sp. differs from its congeners by possessing an MCO with three counterclockwise coils, hook pairs 1, 4 and 7 approximately three times longer than hook pair 5; similar anchors with subtriangular superficial roots, and ventral bar with posteromedial projection.

Diaphorocleidus sclerocolpus *n. sp.*

Description

Based on 11 specimens, three mounted in Gomori's trichrome, three mounted in Hoyer's medium, five mounted in Gray & Wess. Body 160 (133–186; $n = 10$) long, 99 (70–120; $n = 10$) wide at anterior portion of germarium, elongate, foliform, comprising cephalic region, trunk and haptor (fig. 2a). Tegument smooth. Cephalic region broad; cephalic lobes inconspicuous; three pairs of head organs; cephalic glands not observed. Two pairs of eyespots, equidistant; anterior and posterior pairs similar in size; accessory granules present, ovate, slightly scattered in the cephalic region (fig. 2a). Mouth subterminal, midventral; pharynx muscular, ovate or subspherical 16 (13–19; $n = 10$) long, 14 (11–15; $n = 10$) wide; oesophagus short (fig. 2a). Two intestinal caeca, confluent posteriorly to gonads, lacking diverticula (fig. 2a). Common genital pore opening mid-ventral near level of caecal bifurcation. Genital atrium muscular. Intercaecal gonads overlapping; germarium ventral to testis (fig. 2a). Vas deferens looping left intestinal caecum; seminal vesicle sigmoid, short; distal portion looping anteriorly before entering base of MCO. Single prostatic reservoir, saccate, posterior to copulatory complex. Copulatory complex comprising MCO, accessory piece. MCO

Table 2. Comparative measurements (in µm) of specimens of *Diaphorocleidus jaymedeloyolai* n. sp. from two localities.

	Cururutuia stream*	*N*	Açaiteua River	*N*
Body				
Length	181 (116–331)	8	160 (135–174)	5
Width	117 (84–238)	8	64 (48–82)	5
Pharynx				
Length	21 (11–25)	8	–	–
Width	14 (12–18)	8	–	–
Haptor				
Length	59 (48–125)	8	44 (30–54)	5
Width	122 (85–288)	8	78 (44–100)	5
Ventral anchor				
Outer	48 (40–52)	6	45 (35–51)	5
Inner	48 (37–55)	6	44 (30–53)	5
Base	16 (15–18)	5	14 (11–15)	5
Dorsal anchor				
Outer	40 (37–42)	5	38 (36–40)	4
Inner	38 (36–41)	5	34 (33–35)	4
Base	12 (10–15)	4	11 (11–13)	4
Ventral bar				
Length	46 (43–48)	8	47 (41–56)	5
Width	4 (3–5)	8	4 (3–5)	5
Dorsal bar				
Length	45 (36–56)	6	42 (36–49)	4
Width	4 (3–5)	6	3 (3–4)	4
Hook pair 1, 4, 7	31 (25–36)	8	28 (26–30)	4
Hook pair 2	21 (16–27)	7	19 (17–21)	4
Hook pair 6	29 (24–33)	6	26 (22–30)	4
Hook pair 3	26 (21–29)	7	23 (19–26)	4
Hook pair 5	11 (10–15)	5	13 (10–16)	3
Germarium				
Length	27 (15–31)	6	–	–
Width	18 (16–20)	6	–	–
MCO	202 (189–221)	6	211 (196–220)	4
Accessory piece				
Length	31 (25–44)	9	34 (27–40)	4

*Type locality. MCO, male copulatory organ.

sclerotized, tubular, coiled counterclockwise, with approximately three coils, 158 (135–193; $n = 8$) long, base with sclerotized cap; circular sclerotized tandem brim associated with the base of the MCO (fig. 2b). Accessory piece sclerotized, non-articulated with the MCO, bifurcate, pincer-shaped, 47 (36–55; $n = 8$) long (fig. 2b). Germarium ovate to pyriform, 30 (28–34; $n = 4$) long, 21 (16–28; $n = 4$) wide. Vagina opening ventrally at the left body margin, near body mid-length, comprising vaginal vestibule heavily sclerotized, bottle-shape, vaginal canal sclerotized, elongate with proximal portion sigmoid, distal portion looping posteriorly before entering seminal receptacle (fig. 2a). Seminal receptacle subspherical, at level of anterior margin of germarium, ventral (fig. 2a). Mehlis' glands, ootype, egg not observed. Vitellarium dense throughout trunk, except in region of other reproductive organs (fig. 2a). Peduncle short. Haptor hexagonal 14 (11–15; $n = 10$) long, 61 (58–67; $n = 10$) wide (fig. 2a). Anchors similar with well-developed superficial root, subtriangular; poorly developed deep root. Ventral anchor with curved shaft, elongate point extending past level of tip of superficial root, forming angle of approximately 98°, outer 28 (27–29; $n = 8$) long, inner 27 (26–29; $n = 8$) long, base 12 (10–14; $n = 8$) (fig. 2g). Dorsal anchor with arched shaft, point extending slightly past level of tip of superficial root, forming angle of approximately 65°, outer 26 (24–28; $n = 6$) long, inner 27 (24–29; $n = 6$) long, base 9 (8–11; $n = 4$) (fig. 2h). Ventral bar, 32 (29–35; $n = 9$) long, broadly V-shaped, with terminal enlargements (fig. 2c). Dorsal bar 31 (36–27; $n = 9$) long, U-shaped, with terminal enlargements (fig. 2d). Hooks similar in shape with shank divided into two subunits, delicate point, blade gently curved, thumb slightly erect; filamentous hook loop extending to near beginning of shank dilation. Hook pairs 1–4, 6–7, 18 (16–20; $n = 17$) long, with proximal half of shank inflated (fig. 2e); hook pair 5 smaller than other hooks, 10 (9–11; $n = 2$) long with proximal three-quarters of shank inflated (fig. 2f).

Taxonomic summary

Type host. *Acestrorhynchus falcatus* (Bloch, 1794).

Type locality. Cururutuia stream, Caeté River, Municipality of Bragança, Pará, Brazil (1°4′44.55″S, 46°44′18.54″W) collected in December 2014.

Specimens deposited. Holotype: CHIOC 39024a. Ten paratypes: CHIOC 39024b–f, INPA 756, MPEG 0131–0132.

Etymology. The specific name reflects the sclerotized nature of the vagina.

Remarks

Diaphorocleidus sclerocolpus n. sp. is morphologically similar in the structure of the copulatory complex to *Diaphorocleidus petrosusi* Mendoza-Franco, Aquirre-Macedo & Vidal-Martínez, 2007, but the new species differs by having an MCO with approximately three coils, whereas *D. petrosusi* has only one coil. The new species also differs from *D. petrosusi* by possessing both ventral and dorsal bars with blunt ends, while those of *D. petrosusi* have rounded ends.

Rhinoxenoides *n. gen.*

Diagnosis

Body comprising cephalic region, trunk and haptor. Tegument thin, smooth. Cephalic region with terminal cephalic lobe poorly developed. Bilateral pairs of head organs opening subterminal to tip of cephalic lobes; cephalic glands lateral or postero-lateral to pharynx. Eyes present (two pairs); accessory granules present. Mouth subterminal, midventral; pharynx muscular, glandular; oesophagus short. Two intestinal caeca, confluent posteriorly to gonads, lacking diverticula. Genital pore mid-ventral near level of caecal bifurcation. Genital atrium muscular. Gonads tandem,

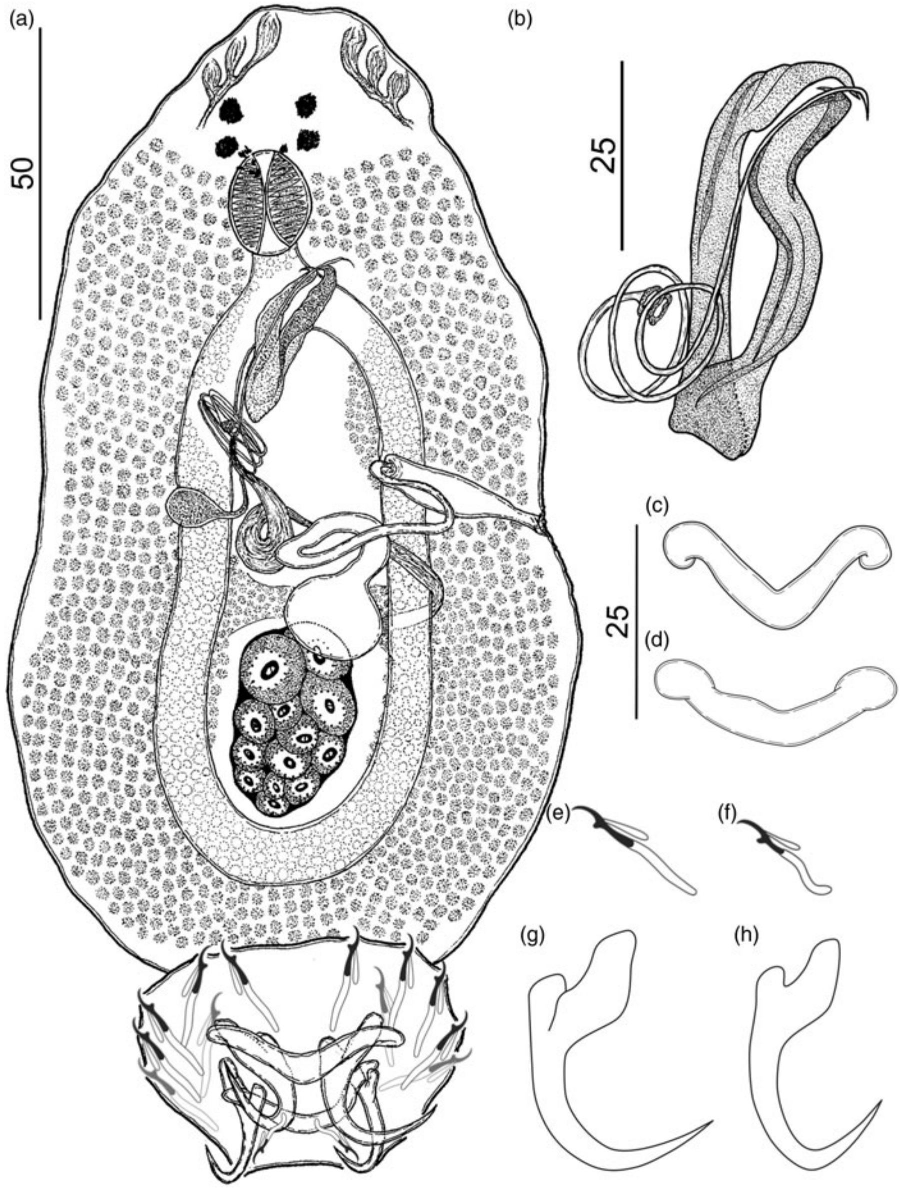

Fig. 2. *Diaphorocleidus sclerocolpus* n. sp. (a) Holotype, whole-mount (ventral view); (b) copulatory complex (ventral view); (c) ventral bar; (d) dorsal bar; (e) hook pairs 1, 2, 3, 4, 6 and 7; (f) hook pair 5; (g) ventral anchor; (h) dorsal anchor. Scale bars: (a) 50 µm; (b) 25 µm; (c–h) 25 µm.

testis dorsal to germarium. Vas deferens looping left intestinal caecum before entering the male copulatory organ; seminal vesicle sigmoid, representing a dilation in the vas deferens. Copulatory complex comprising male copulatory organ, accessory piece; MCO sclerotized, spiral, clockwise; accessory piece sclerotized, articulated with the male copulatory organ. One prostatic reservoir, saccate. Germarium elongate. Vagina single; vaginal aperture sinistro-ventral, marginal opening at level of vitelline commissure; vaginal vestibule slightly sclerotized; vaginal canal sclerotized, sigmoid. Seminal receptacle present, anterior to germarium. Vitellaria well developed, coextensive with intestinal caeca. Haptor armed with 14 hooks (seven pairs) with ancyrocephaline distribution (Mizelle, 1936). Pair of ventral and dorsal anchors; ventral anchors with well-defined roots. Dorsal anchor with superficial root twice as big as deep root. Ventral bar present; dorsal bar absent. Parasites of gills of Neotropical characiform fish.

Taxonomic summary

Type species. *Rhinoxenoides horacioschneideri* n. sp.

Etymology. The generic name reflects similarity of the new genus to *Rhinoxenus* Kritsky, Boeger & Thatcher, 1988.

Remarks

Rhinoxenoides n. gen. is characterized by the following: (1) MCO a coiled tube with clockwise rings articulated to the accessory piece by copulatory ligament; (2) dorsal anchor with superficial root twice as long as the deep root; straight shaft, curved point; and (3) dorsal bar absent. *Rhinoxenoides* n. gen. is similar to *Rhinoxenus* Kritsky, Boeger & Thatcher, 1988 and *Protorhinoxenus* Domingues & Boeger, 2002, mainly by sharing the general morphology of the copulatory complex and the shape of dorsal anchors. However, *Rhinoxenoides* n. gen. is different from members of both genera by the following: (1) anchors with conspicuous roots, without sclerotized cap in the base (inconspicuous roots and base with sclerotized cap in *Protorhinoxenus* and *Rhinoxenus*); (2) MCO with clockwise rings (counterclockwise rings in *Protorhinoxenus* and *Rhinoxenus*); and (3) base of the

MCO conical surrounded by simple sclerotized ring (two circular sclerotized rings in tandem in *Protorhinoxenus* and *Rhinoxenus*).

Rhinoxenoides horacioschneideri *n. sp.*

Description

Based on four specimens, two mounted in Gomori's trichrome and two mounted in Gray & Wess. Body 209 (180–245; $n = 3$) long, 64 (45–85; $n = 4$) wide at level of germarium, elongate, fusiform. Anterior region with four cephalic lobes moderately developed, two terminals, two bilateral; three pair of head organs; cephalic glands not observed (fig. 3a). Two pairs of eyespots, equidistant, posterior pair longer than anterior pair; accessory granules present, elongated, slightly scattered in the cephalic region (fig. 3a). Pharynx subspherical 14 (13–15; $n = 4$) long, 15 (12–18; $n = 4$) wide. MCO with 2½ coils, 98 (96–100; $n = 2$) long; accessory piece comprising variable distal sheath with articulation process extending within coils to the base of MCO (fig. 3b). Prostatic reservoir pyriform, anterior to MCO (fig. 3a). Oviduct, Mehlis' glands, uterus, eggs not observed. Germarium pyriform, 22 ($n = 1$) long, 11 ($n = 1$) wide. Vaginal vestibule cup-shaped, vaginal canal sigmoid; seminal receptacle spherical 12 ($n = 1$) long, 10 ($n = 1$) wide (fig. 3a). Haptor subrectangular to trapezoidal, 47 (44–50; $n = 3$) long, 64 (50–74; $n = 3$) wide (fig. 3a). Ventral anchor truncated with superficial root well developed, root deep, short, rounded; evenly curved shaft, point; forming angle

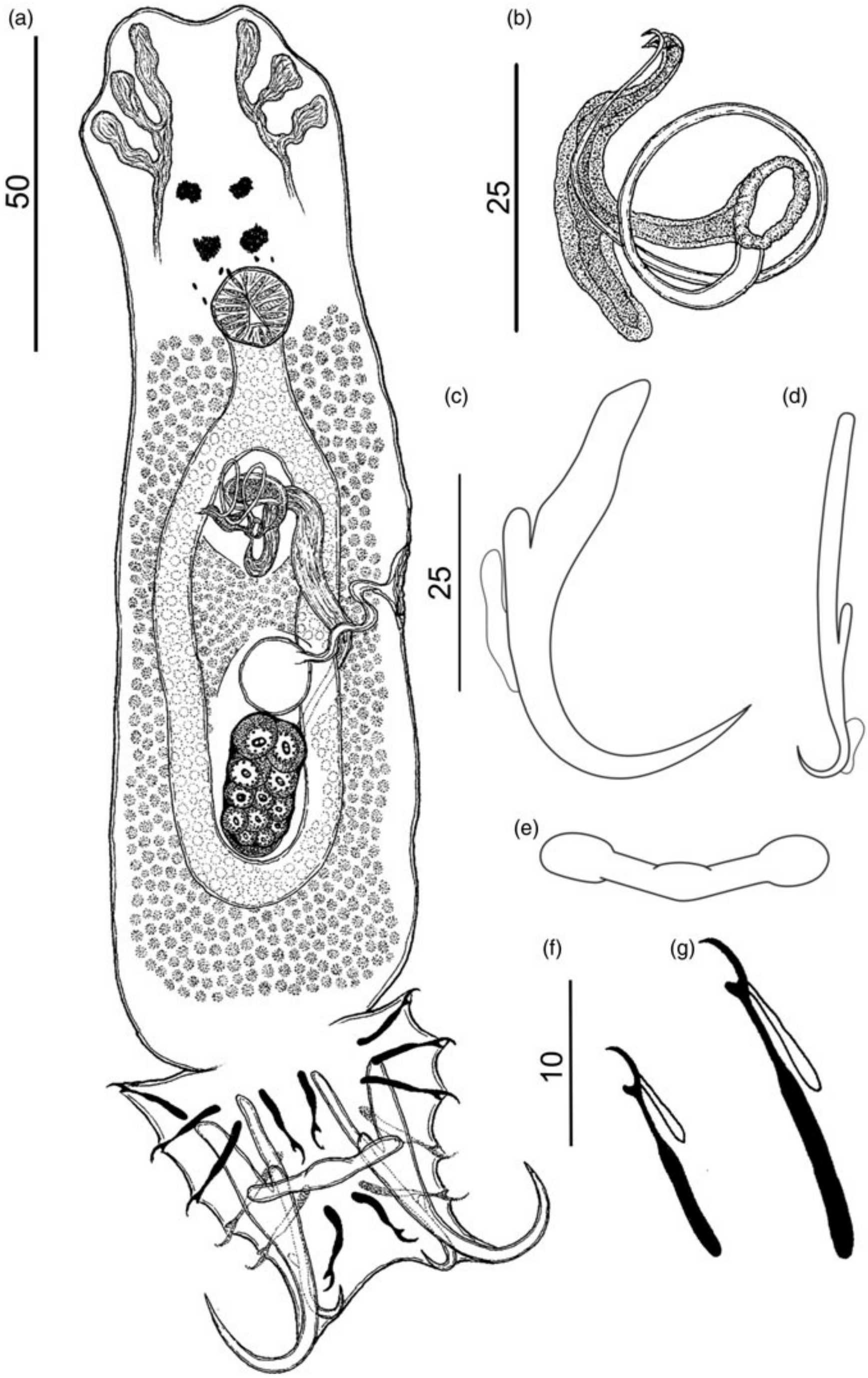

Fig. 3. *Rhinoxenoides horacioschneideri* n. sp. (a) Holotype, whole-mount (ventral view); (b) copulatory complex (ventral view); (c) ventral anchor; (d) dorsal anchor; (e) ventral bar; (f) hook pairs 1, 2, 3 and 5; (g) hook pairs 4, 6 and 7. Scale bars: (a) 50 µm; (b–e) 25 µm; (f, g) 10 µm.

of approximately 90°; point acute, extending well past level of tip of inner base; anchor filament extending from the base to middle of shaft, outer 36 (35–36; $n = 3$) long, inner 37 (34–38; $n = 3$) long, base 12 (11–12; $n = 2$) (fig. 3c). Dorsal anchor with superficial root twice as long as deep root; straight shaft; point short, forming an angle of approximately 60°; point hook-shaped; outer 19 (18–21; $n = 2$) long, inner 37 (35–39; $n = 2$) long, base 4 (3–4; $n = 2$) (fig. 3d). Ventral bar 29 (25–34; $n = 4$) long, V-shaped with terminal enlargements (fig. 3e). Hooks similar in shape, shank divided into two subunits, proximal one-third of shank inflated; filamentous hook loop extending to near beginning of shank dilation, each with erect thumb, curved long shaft, delicate point. Hook pairs 1–3 and 5, 13 (13–14; $n = 3$) long (fig. 3f); hook pairs 4, 6 and 7, 18 (17–18; $n = 3$) long (fig. 3g).

Taxonomic summary

Type host. *Acestrorhynchus falcatus* (Bloch, 1794).

Site of infestation. Gills.

Type locality. Açaiteua River, Municipality of Tracuateua, Pará, Brazil (1°07′45.00″S, 47°00′26.9″W), collected in August 2014.

Additional locality. Cururutuia stream, Caeté River), Municipality of Bragança, Pará, Brazil (1°4′44.55″S, 46° 44′18.54″W), collected in December 2014.

Prevalence. 67% of three hosts examined.

Mean intensity. 6.5 parasites per infested host.

Specimens deposited. Holotype: CHIOC 39025a. Three paratypes: CHIOC 39025b–c, 39026. Nine vouchers: CHIOC 39027a–d, INPA 757, MPEG 0133–0134.

Etymology. The specific name is in honour of Dr Horácio Schneider of the Federal University of Pará, Brazil, in recognition of his valuable work on Amazonian biodiversity and also for being responsible for stimulating research in the eastern Amazon over the past three decades.

Comparative measurements. See table 3.

Table 3. Comparative measurements (in µm) of specimens of *Rhinoxenoides horacioschneideri* n. sp. from two localities.

	Cururutuia stream	*N*	Açaiteua River*	*N*
Body				
Length	199 (166–238)	8	209 (180–245)	3
Width	66 (47–83)	9	64 (45–85)	4
Pharynx				
Length	18 (14–20)	8	14 (13–15)	4
Width	17 (13–20)	8	15 (12–18)	4
Haptor				
Length	43 (40–49)	6	47 (44–50)	3
Width	60 (55–72)	6	64 (50–74)	3
Ventral anchor				
Outer	38 (35–41)	6	36 (35–36)	3
Inner	40 (37–43)	7	37 (34–38)	3
Base	12 (8–15)	7	12 (11–12)	3
Dorsal anchor				
Outer	20 (20–21)	5	19 (18–21)	2
Inner	39 (37–42)	6	37 (35–39)	2
Base	4 (3–4)	4	4 (3–4)	2
Ventral bar				
Length	32 (26–39)	5	29 (25-34)	4
Width	–	–	–	–
Hook pairs 1,2,3,5	13 (12–14)	3	13 (13–14)	3
Hook pairs 4,6,7	19 (19–20)	3	18 (17–18)	3
Germarium				
Length	24	1	22	1
Width	10	1	11	1
MCO	80 (74–88)	4	98 (96–100)	2
Seminal receptacle				
Length	–	–	12	1
Width	–	–	10	1

*Type locality. MCO, male copulatory organ.

Remarks

The new species is characterized by the following: (1) ventral anchor with superficial truncated root, which is well developed; (2) prostate reservoir is not divided into zones; (3) vagina sinistral–ventral, not sclerotized; (4) dorsal anchor with well-developed superficial root comprising more than 50% of total length; and (5) dorsal bar with enlarged ends.

Phylogeny

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 used for this analysis is presented in table 4. In the following, 'figs' indicates figures in the present paper and 'Figs', figures in cited references.

Character 1. MCO coil [CI 100; RI 0]. (0) Counterclockwise (figs 1b, 2b; Kritsky *et al.* (1986), Figs 3, 12; Kritsky *et al.* (1988), Figs 3, 11–12, 21; Domingues & Boeger (2002), Fig. 2; Domingues & Boeger (2005), Figs 1C, D, F, 2B, 3A; Ferreira *et al.* (2017), Figs 1b, 2a, 3a, 4a); (1) clockwise (fig. 3b). A clockwise MCO is autapomorphic for *Rhinoxenoides* n. gen.

Character 2. MCO rings [CI 33; RI 33]. (0) MCO with more than three rings (figs 1b, 2b; Kritsky *et al.* (1988), Fig. 21; Domingues & Boeger (2002), Fig. 2; Domingues & Boeger (2005), Figs 1C, 3A); (1) MCO with fewer than three rings (fig. 3b; Kritsky *et al.* (1986), Figs 3, 12; Kritsky *et al.* (1988), Figs 3, 11–12; Boeger *et al.* (1995), Fig. 2; Domingues & Boeger (2005), Figs 1D, 1F, 2B, 3G, 4C; Ferreira *et al.* (2017), Figs 1b, 2a, 3a, 4a).

Character 3. Circular, tandem, sclerotized brims of MCO [CI 100; RI 0]. (0) Present (figs 1b, 2b; Domingues & Boeger (2002), Fig. 2; Domingues & Boeger (2005), Figs 1A, C, D, F, 2B, 3 A, G, 4C; Ferreira *et al.* (2017), Figs 1b, 2a, 3a, 4a); (1) absent (fig. 3b). The absence of circular, tandem, sclerotized brims is autapomorphic for *Rhinoxenoides* n. gen.

Table 4. Character matrix to reconstruct evolutionary relationships of *Protorhinoxenus*, *Rhinoxenoides* and species of *Rhinoxenus*. For definitions of character numbers 1–16, refer to Character analysis in the Results section; numbers in square brackets refer to polymorphic characters.

	Character															
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diaphorocleidus	0	0	0	0	0	0	[02]	0	0	0	0	0	0	0	0	0
Urocleidoides	0	1	0	0	[01]	1	[02]	0	0	0	0	[01]	0	0	0	0
Protorhinoxenus prochilodi	0	0	0	1	1	1	1	1	1	1	0	1	1	0	0	0
Rhinoxenoides horacioschneideri	1	1	1	1	0	1	0	0	0	0	0	1	0	0	1	0
Rhinoxenus nyttus	0	0	0	1	0	0	1	0	1	1	0	2	1	1	1	1
Rhinoxenus curimbatae	0	0	0	1	0	0	1	0	1	1	0	2	1	0	1	1
Rhinoxenus bulbovaginatus	0	1	0	1	0	0	1	1	1	1	0	2	1	1	1	1
Rhinoxenus arietinus	0	1	0	1	0	0	2	0	1	1	1	2	1	0	1	1
Rhinoxenus guianensis	0	1	0	1	0	0	2	1	1	1	1	2	1	1	1	1
Rhinoxenus anaclaudiae	0	1	0	1	0	0	2	1	1	1	1	2	1	0	1	1
Rhinoxenus piranhus	0	1	0	1	0	0	2	1	1	1	2	2	1	1	1	1
Rhinoxenus euryxenus	0	1	0	1	0	0	2	1	1	1	2	2	1	1	1	1

Character 4. MCO/accessory piece articulation process [CI 100; RI 100]. (0) Absent (figs 1b, 2b; Ferreira *et al.* (2017), Figs 1b, 2a, 3a, 4a); (1) present (fig. 3b; Kritsky *et al.* (1988), Figs 3, 11–12, 21; Domingues & Boeger (2002), Fig. 2; Domingues & Boeger (2005), Figs 1A, C, D, F, 2B, 3A, G, 4C).

Character 5. Vagina [CI 100; RI 0]. (0) Sinistral (figs 1a, 2a, 3a; Kritsky *et al.* (1986), Fig. 1; Kritsky *et al.* (1988), Figs 1–2, 9, 18; Domingues & Boeger (2005), Figs 2F, 4A; Ferreira *et al.* (2017), Figs 2b, 3b, 4b); (1) dextral (Kritsky *et al.* (1986), Fig. 10; Domingues & Boeger (2002), Fig. 1). This character seems to be polymorphic for the *Urocleidoides*. Kritsky *et al.* (1986) observed that *Urocleidoides paradoxus* (*s.s.*) Kritsky, Thatcher & Boeger, 1986 presents a dextral vaginal opening different from those observed in the other species of the genus. A dextral vaginal opening in the ingroup represents an automorphic feature for *Protorhinoxenus*.

Character 6. Vaginal vestibule [CI 50; RI 50]. (0) Heavily sclerotized (figs 1a, 2a; Kritsky *et al.* (1988), Figs 1–2, 9, 18; Boeger *et al.* (1995), Fig. 3; Domingues & Boeger (2005), Figs 1B, 2E, 3B, H, 4B); (1) soft to slightly sclerotized (fig. 3a; Domingues & Boeger (2002), Fig. 3; Ferreira *et al.* (2017), Fig. 2b, 3b).

Character 7. Vaginal canal [CI 100; RI 100]. (0) Sinuous without looping (fig. 1a, 3a; Kritsky *et al.* (1986), Fig. 10); (1) sinuous with more than one loop (Kritsky *et al.* (1986), Fig. 1; Kritsky *et al.* (1988), Fig. 18; Domingues & Boeger (2002), Fig. 3; Domingues & Boeger (2005), Fig. 3B); (2) sinuous with only one loop (fig. 2a; Kritsky *et al.* (1986), Fig. 1; Kritsky *et al.* (1988), Figs 1–2, 9; Boeger *et al.* (1995), Figs 1, 3; Domingues & Boeger (2005), Figs 1B, 2E, 3H, 4B; Ferreira *et al.* (2017), Figs 2a, 3b, 4b). This character seems to be polymorphic for *Diaphorocleidus* and *Urocleidoides* (*s.s.*).

Character 8. Shape of ventral anchor [CI 33; RI 60]. (0) Shaft and point similar (fig. 1j, 2g, 3c; Kritsky *et al.* (1986), Figs 8, 17; Kritsky *et al.* (1988), Figs 17, 24; Domingues & Boeger (2005), Figs 1E, 3C); (1) shaft longer than point (Domingues & Boeger (2002), Fig. 9; Boeger *et al.* (1995), Fig. 5; Kritsky *et al.* (1988), Fig. 8; Domingues & Boeger (2005), Figs 2H, 3L, 4G).

Character 9. Sclerotized cap in the anchors with projection for articulation to ventral bar [CI 100; RI 100]. (0) Absent (figs 1j, 2g, 3c; Ferreira *et al.* (2017), Figs 1G, 2H, 3G, 4G); (1) present (Kritsky *et al.* (1988), Figs 8, 17, 24; Domingues & Boeger (2002), Fig. 3; Domingues & Boeger (2005), Figs 1E, 2H, 3C, L, 4G).

Character 10. Roots of ventral anchors [CI 100; RI 100]. (0) Present (figs 1j, 2g, 3c; Kritsky *et al.* (1988), Fig. 17; Ferreira *et al.* (2017), Figs 1g, 2h, 3g, 4g); (1) absent (Kritsky *et al.* (1988), Fig. 8; Domingues & Boeger (2002), Fig. 3; Domingues & Boeger (2005), Figs 1E, 2H, 3C, L, 4G).

Character 11. Point of ventral anchor [CI 100; RI 100]. (0) Acute (figs 1j, 2g, 3c; Kritsky *et al.* (1986), Figs 8, 17; Kritsky *et al.* (1988), Fig. 24; Boeger *et al.* (1995), Fig. 5; Domingues & Boeger (2002) Fig. 9; Domingues & Boeger (2005), Figs 1E, 3C); (1) blunt (Kritsky *et al.* (1988), Fig. 17; Domingues & Boeger (2005), Figs 3L, 2H); (2) flattened (Kritsky *et al.* (1988), Fig. 8, Domingues & Boeger (2005), Fig. 4G).

Character 12. Shape of dorsal anchors [CI 100; RI 100]. (0) Evenly curved shaft and point (figs 1k, 2h; Ferreira *et al.* (2017), Figs 1f, 2g, 3g, 4g); (1) shaft straight/slightly curved, longer than point (fig. 3d; Kritsky *et al.* (1986), Figs 9, 18; Domingues & Boeger (2002), Fig. 8); (2) spike-like (Kritsky *et al.* (1988), Figs 5, 16, 22; Boeger *et al.* (1995), Fig. 8; Domingues & Boeger (2005), Figs 2G, 3H, M, 4H). This character seems to be polymorphic for *Urocleidoides* (*s.s.*).

Character 13. Root of dorsal anchors [CI 100; RI 100]. (0) Present (fig. 1k, 2h, 3d; Ferreira *et al.* (2017), Figs 1h, 2i, 3h, 4h); (1) absent (Kritsky *et al.* (1988), Figs 5, 16, 22; Boeger *et al.* (1995), Fig. 8; Domingues & Boeger (2002), Fig. 8; Domingues & Boeger (2005), Figs 2G, 3D, M, 4H).

Character 14. Ventral bar [CI 50; RI 75]. (0) Bar with extremities lightly expanded (figs 1i, 2c, 3e; Kritsky *et al.* (1986), Figs 6, 15; Kritsky *et al.* (1988), Fig. 13; Domingues & Boeger (2002), Fig. 4; Domingues & Boeger (2005), Figs 2F, 3C, 3K; Ferreira *et al.* (2017), Figs 1c, 2d, 3e, 4c); (1) bar with projections for articulation with ventral anchor (Kritsky *et al.* (1988), Figs 4,

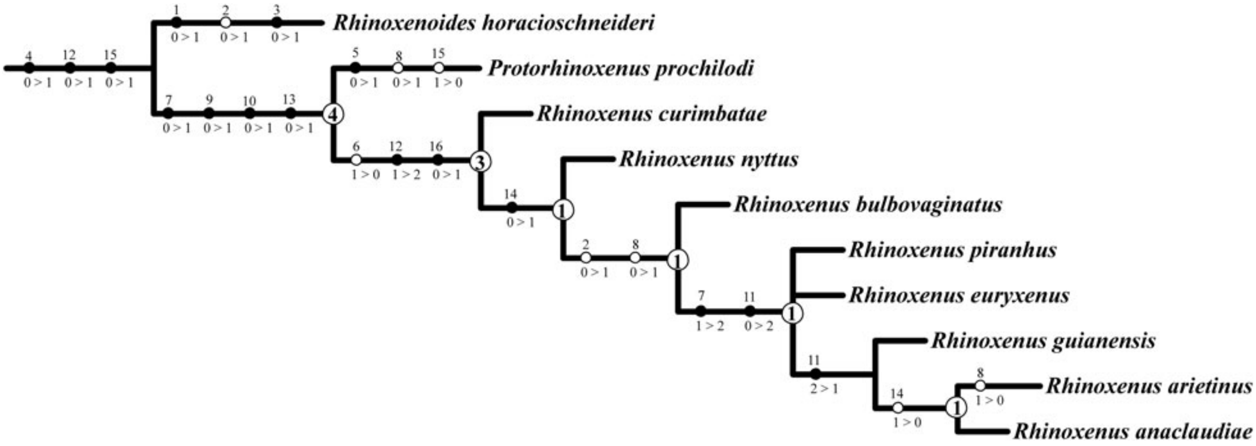

Fig. 4. Phylogenetic hypothesis for members of three genera of Dactylogyridae, including one new genus proposed, based on 16 morphological characters. Tree length = 24; consistency index = 73; retention index = 79. Numbers above the branches indicate the respective characters. Numbers below the branches refer to postulated evolutionary changes. Filled circles on the branches indicate a synapomorphic or autapomorphic character state, open circles on the branches indicate a homoplastic character state. Open circles with a number on the nodes of the ingroup indicate Bremer support.

23; Boeger *et al.* (1995), Fig. 4; Domingues & Boeger (2005), Figs 3K, 4F).

Character 15. Dorsal bar [CI 50; RI 50]. (0) Present (figs 1a, 2a; Domingues & Boeger (2002), Fig. 1); (1) absent (fig. 3a, Kritsky *et al.* (1988), Figs 1, 9, 18; Domingues & Boeger (2002), Fig. 1; Domingues & Boeger (2005), Figs 2A, 4A). This character has ambiguous distribution for the clade composed of *Protorhinoxenus*, *Rhinoxenoides* and *Rhinoxenus*. The state 'absence of dorsal bar' sometimes appears as a synapomorphy for the three taxa (ACCTRAN optimization) with a reversion in *Protorhinoxenus*, or the dorsal bar seems to have been lost independently in *Rhinoxenoides* and *Rhinoxenus* (DELTRAN optimization).

Character 16. Hook pair 2 [CI 100; RI 100]. (0) Located within haptor (fig. 1a, 2a, 3a; Kritsky *et al.* (1986), Figs 1, 10; Domingues & Boeger (2002), Fig. 1); (1) located in two bilateral lobes in trunk (Kritsky *et al.* (1988), Figs 1, 9, 18; Domingues & Boeger (2005), Figs 2A, 4A, 3B–H, 4B).

The phylogenetic hypothesis depicted in fig. 4 is one of two most parsimonious trees produced through the program TNT 1.0 using 16 morphological characters (table 4) (length = 24; CI = 73; RI = 79). According to the hypothesis, *Rhinoxenoides horacioschneideri* is sister taxon to the clade that includes all other species. *Protorhinoxenus* and species of *Rhinoxenus* are sister taxa, based on: (1) vaginal canal without loops (character 7); (2) the presence of sclerotized cap on the base of anchors (character 9); and the absence of roots on the dorsal anchors (character 13).

All species of *Rhinoxenus* form a clade, supported by the sharing the presence of (1) a heavily sclerotized vaginal vestibule; (2) spike-like dorsal anchors; and (3) hook pair 2 located in two bilateral lobes in the trunk. The sister group relationships within species of *Rhinoxenus* presented in fig. 4 differ from the relationships of the other equally parsimonious tree by the relative position of *R. guianensis* Domingues & Boeger, 2005, *R. piranhus* Kritsky Boeger & Thatcher, 1988, *R. euryxenus* Domingues & Boeger, 2005, and the clade *R. arietinus* Kritsky Boeger & Thatcher, 1988 + *R. anaclaudiae* Domingues & Boeger, 2005. This variation is apparently related to the multi-state character 'point of ventral anchor'. The tree presented in fig. 4 suggests that the point of ventral anchor evolved from the plesiomorphic character state 'acute' into 'flattened' and to 'blunt'. The last character state appears as synapomorphy uniting *R. guianensis* and the clade '*R. arietinus* + *R. anaclaudiae*'. In the other tree, the plesiomorphic character state evolved into 'blunt' and to 'flattened', where the presence of a flattened point of the ventral anchor arises as synapomorphy for *R. piranhus* and *R. euryxenus*.

Our hypothesis differs significantly from that proposed by Domingues & Boeger (2005) on the sister-group relationship within species of *Rhinoxenus*. The previous hypothesis (Domingues & Boeger, 2005) suggests that the clade composed by *Rhinoxenus nyttus* and *R. curimbatae* appears as the sister group of all other congeneric species. In the present hypothesis, *R. curimbatae* appears as the sister group of the clade D (fig. 4). Domingues & Boeger (2005) suggest that *R. arietinus* is sister group of *R. guianensis*, *R. anaclaudiae, R. piranhus* and *R. euryxenus*. However, in our hypothesis, *R. arietinus* is sister taxon of *R. anaclaudiae* in the clade H, while *R. guianensis* appears as sister taxon of clade H, *R. piranhus* and *R. euryxenus*, or in a polytomy with clade H and the clade composed by *R. piranhus* + *R. euryxenus* (see comments above).

Discussion

Diaphorocleidus Jogunoori, Kritsky & Venkatanarasaiah, 2004 was proposed by Jogunoori *et al.* (2004) to accommodate their new species, *Diaphorocleidus armillatus* Jogunoori, Kritsky & Venkatanarasaiah, 2004, and three other species previously referred as members of *Urocleidoides* sensu lato (*s.l.*): *Diaphorocleidus affinis* (Mizelle, Kritsky & Crane, 1968), *D. kabatai* (Molnar, Hanek & Fernando, 1974) and *D. microstomus* (Mizelle, Kritsky & Crane, 1968) (see Jogunoori *et al.*, 2004).

Except for *D. kabatai, D. orthodusus* Mendoza-Franco, Reina & Torchin, 2009, *D. petrosusi* Mendoza-Franco, Aguirre-Macedo & Vidal-Martínez, 2007, described from characid fish from Central America and/or south-east Mexico, and *D. armillatus* introduced to India, via the aquarium trade (Mizelle *et al.*, 1968; Molnar *et al.*, 1974; Jogunoori *et al.*, 2004; Mendoza-Franco *et al.*, 2007, 2009), only three species of this genus have been described for characiform fish from South America: *D. affinis* from *Bryconops affinis* (Günther) (Characidae), *D. altamirensis* Moreira, Scholz & Luque, 2016 from *Argonectes robertsi* Langeani (Hemiodontidae) and *D. microstomus* from *Hemigrammus* *microstomus* Durbin (Characidae). Camargo *et al.* (2015) reported some specimens of *Diaphorocleidus* parasitizing the gills of *Acestrorhynchus lacustris* (Lütken) collected from the Peixe River, São Paulo. However, since these materials were not available for comparative study, we cannot make any suggestions about the real taxonomic status of these specimens. The two new species described herein, *D. jaymedeloyolai* and *D. sclerocolpus*, represent the first described species of monogenoids from acestrorhynchids and increase our knowledge of this parasite genus for the eastern Amazon Basin, together with the species *D. altamirensis* already described from the Xingu River.

Domingues & Boeger (2002) proposed *Protorhinoxenus*, and indicated that it could be closely related to *Rhinoxenus*. Members of the two genera share the presence of a coiled MCO with counterclockwise coils, absence of superficial and deep roots in both pairs of anchors, and dorsal anchors with elongate and straight shaft. *Rhinoxenus* also resembles *Rhinoxenoides* proposed herein, mainly by sharing the absence of dorsal bar, which is present in *Protorhinoxenus*.

In our cladistic hypothesis, *Protorhinoxenus*, *Rhinoxenoides* and species of *Rhinoxenus* share the presence of a copulatory ligament and dorsal anchors with a straight long shaft. *Protorhinoxenus* and species of *Rhinoxenus* are closely related, based on the presence of a sclerotized cap on the base of ventral anchors and the absence of roots on the dorsal anchors. Also, in the present hypothesis, the presence/absence of a dorsal bar in *Rhinoxenoides, Protorhinoxenus* and *Rhinoxenus* is ambiguous and could be interpreted as an independent secondary loss in *Rhinoxenoides* and *Rhinoxenus*, or its absence could be shared by the three genera as a synapomorphy with secondary acquisition in *Protorhinoxenus*.

According to the present knowledge on the diversity of monogenoids from the Neotropics, species of *Protorhinoxenus*, *Rhinoxenoides* and *Rhinoxenus* seem to be exclusively found infecting characiform fishes from South America. Unlike *Rhinoxenoides*, members of the two other genera are not restricted to members of one host genus, as reported for other monogenoidean genera occurring in characiforms (i.e. *Anacanthoroides* Kritsky & Thatcher, 1976; *Ancistrohaptor* Agarwal & Kritsky, 1998; *Apedunculata* Cuglianna, Cordeiro & Luque, 2009; *Characithecium* Mendoza-Franco, Reina & Torchin, 2009; *Curvianchoratus* Hanek, Molnar & Fernando, 1974; *Linguadactyloides* Thatcher & Kritsky, 1983; *Monocleithrium* Price & McMahon, 1966; *Notothecioides* Kritsky, Boeger & Jégu, 1997; *Odothecium* Kritsky, Boeger & Jégu, 1997; *Palombitrema* Price & Bussing, 1968), or to one host family (*i.e. Amphithecium* Boeger & Kritsky, 1988; *Cacatuocotyle* Boeger, Domingues & Kritsky, 1997; *Calpidothecioides* Kritsky, Boeger & Jégu, 1997; *Calpidothecium* Kritsky, Boeger & Jégu, 1997; *Enallothecium* Kritsky, Boeger & Jégu, 1998; *Heterothecium* Kritsky, Boeger & Jégu, 1997; *Mymarothecium* Kritsky, Boeger & Jégu, 1996; *Notothecium* Boeger & Kritsky, 1988; *Pithanothecium* Kritsky, Boeger & Jégu, 1997). The monotypic, *Protorhinoxenus prochilodi* Domingues & Boeger, 2002 is reported from the gills of fishes from the families Erythrinidae and Prochilodontidae (Domingues & Boeger, 2002), whereas species of *Rhinoxenus* are found parasitizing the nasal cavities of Anostomidae, Characidae, Curimatidae, Prochilodontidae and Serrasalmidae (Domingues & Boeger, 2005). Furthermore, several undescribed species of *Rhinoxenus* are reported from Cynodontidae and Erythrinidae (Domingues & Boeger, 2005; Santos Neto *et al.*, 2015).

Domingues & Boeger (2005) suggested that the origins of some lineages of *Rhinoxenus* were associated with events of co-speciation with the ancestors of their respective characiform host families. However, the co-evolutionary analysis suggested that events of duplication, dispersion and extinction were also required to explain the observed host–parasite association. The occurrence of *Protorhinoxenus* into two phylogenetically distant characiform host families also supports the hypothesis that co-speciation is not the only event associated with these associations. Concerning the relevance of these host–parasite associations, we believe that a more extensive study, based on denser sampling for monogenoidean parasites of members of all characiform families and cladistics studies, is required.

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Conflict of interest. None.

Ethical standards. Specimens were collected under the license for collection of biological material (43381) granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

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