



A new genus and two new species of dactylogyrid monogeneans from gills of Neotropical catfishes (Siluriformes: Doradidae and Loricariidae)

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ABSTRACT

A new genus of dactylogyrid monogeneans (Ancyrocephalinae), *Paracosmetocleithrum* n. gen., is erected to accommodate *P. trachydorasi* n. sp. from *Trachydoras paraguayensis* (Siluriformes: Doradidae) in the Upper Paraná River basin, Brazil. The new genus differs from Neotropical dactylogyrids in the presence of a well-developed ornamentation in the middle portion of the ventral bar, and a sclerotized patch on the surface of the dorsal bar with an inconspicuous medial process that possesses two submedial projections arising from the tapered ends of this patch. In addition, *Demidospermus rhinelepsi* n. sp. is described from *Rhinelepis aspera* (Siluriformes: Loricariidae). The new species, which is the fifth species of the genus described from loricariids, can be differentiated from congeners by the possession of a sclerotized patch attached to the middle portion of the ventral bar, and by morphology of the accessory piece, which presents broad ends, tapering in the centre, rounded proximal end, distal end folding on both sides with folds extending to approximately $\frac{3}{4}$ of the accessory piece length. Molecular data on both new species are also provided and species composition of *Demidospermus*, recently revealed as polyphyletic by molecular studies including the present one, is discussed.

1. Introduction

Fishes of the order Siluriformes, known as catfishes, have a worldwide distribution with > 3000 species recognized as valid [1]. Approximately 1700 species (61%) are found in America, mainly in the Neotropical region [2,3]. Even though catfishes are highly diverse, abundant and of economic importance as food and ornamental fishes, the knowledge of their helminth parasites is still fragmentary and inadequate [4,5]. Neotropical catfishes host a rich fauna of gill parasites, especially monogeneans (Dactylogyridae: Ancyrocephalinae) [6,7]. However, the true diversity of these ectoparasites is still poorly known and very little is known about their interrelations [4,8].

Members of the Doradidae are popularly known as thorny or talking catfishes, have exclusively Neotropical distribution and comprise 93 species allocated in 31 genera [9]. Species of only two dactylogyrid genera, *Vancleaveus* Kritsky, Thatcher et Boeger, 1986 and *Cosmetocleithrum* Kritsky, Thatcher et Boeger, 1986, are known to parasitize doradids [10,11]. Members of the Loricariidae, known as suckermouth catfishes, are the most species-rich (approximately 900 known species)

and widespread group of the Siluriformes, distributed throughout South America, reaching north up to Costa Rica [1,2,12]. Species of four dactylogyrid genera have been reported from loricariids in the Neotropical region, namely *Demidospermus* Suriano, 1983, *Heteropriapulus* Kritsky, 2007, *Trinigyrus* Hanek, Molnar et Fernando, 1974, and *Unilatus* Mizelle et Kritsky, 1967 (see [13] and references therein).

Demidospermus was erected by Suriano [14] to accommodate dactylogyrids found on the gills of *Loricariichthys anus* (Valenciennes) (Siluriformes: Loricariidae). Suriano [14] considered the presence of encapsulated sperm within the testis to be the main diagnosis feature of the genus. Kritsky and Gutiérrez [15] added other genus-specific characteristics and described additional species. In addition, these authors considered *Omothecium* Kritsky, Thatcher et Boeger, 1987 and *Paramphocleithrum* Suriano et Incorvaia, 1995 as junior synonyms of *Demidospermus*. At present, the genus includes 29 species found on fishes of the families Auchenipteridae, Loricariidae, and Pimelodidae [see [16] and references therein], which is uncommonly broad host specificity compared to other genera of Neotropical gill monogeneans, whose species are usually specific to one fish family [6,7].

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Table 1
List of monogeneans (Dactylogyridae) included in the phylogenetic analyses.

Parasite species	Host	Host family	Locality	Genbank ID	Reference
<i>Actinocleidus recurvatus</i> *	<i>Lepomis gibbosus</i>	Centrarchidae	River Danube, Slovak Republic	AJ969951	[42]
<i>Aphanoblastella aurorae</i>	<i>Goeldiella eques</i>	Heptapteridae	Santa Clara, Peru	KP056239	[8]
<i>Aphanoblastella</i> sp. 3	<i>Goeldiella eques</i>	Heptapteridae	Nanay River, Peru	KP056238	[8]
<i>Cosmetocleithrum bifurcum</i>	<i>Hassar orestis</i>	Doradidae	Aquarium Momón, Iquitos, Peru	KP056216	[8]
<i>Cosmetocleithrum bulbocirrus</i>	<i>Pterodoras granulosus</i>	Doradidae	Upper Paraná River basin, Brazil	MG001326	Present study
<i>Demidospermus anus</i>	<i>Loricariichthys platymetopon</i>	Loricariidae	Upper Paraná River basin, Brazil	KY766957	[16]
<i>Demidospermus mortenthaleri</i>	<i>Brachyplatystoma juruense</i>	Pimelodidae	Santa Clara, Peru	KP056245	[8]
<i>Demidospermus prolixus</i>	<i>Loricaria prolixa</i>	Loricariidae	Upper Paraná River basin, Brazil	KY766955	[16]
<i>Demidospermus rhinelepsi</i> n. sp.	<i>Rhinelepis aspera</i>	Loricariidae	Upper Paraná River, basin, Brazil	MG001324	Present study
<i>Demidospermus spirophallus</i>	<i>Loricaria prolixa</i>	Loricariidae	Upper Paraná River, basin, Brazil	KY766954	[16]
<i>Demidospermus</i> sp. 11	<i>Brachyplatystoma vaillantii</i>	Pimelodidae	Nanay River, Peru	KP056235	[8]
<i>Demidospermus</i> sp. 23	<i>Brachyplatystoma vaillantii</i>	Pimelodidae	Nanay River, Peru	KP056236	[8]
<i>Ligictaluridus pricei</i> *	<i>Ameiurus nebulosus</i>	Ictaluridae	River Moldau, Czech Republic	AJ969939	[42]
<i>Onchocleidus similis</i> *	<i>Lepomis gibbosus</i>	Centrarchidae	River Danube, Slovak Republic	AJ969938	[42]
<i>Paracosmetocleithrum trachydorasi</i> n. gen., n. sp.	<i>Trachydoras paraguayensis</i>	Doradidae	Upper Paraná river basin, Brazil	MG001323	Present study
Dactylogyridae gen. sp. 1	<i>Hemisorubim platyrhynchos</i>	Pimelodidae	Upper Paraná River basin, Brazil	MG001325	Present study
Dactylogyridae gen. sp. 2	<i>Hemisorubim platyrhynchos</i>	Pimelodidae	Upper Paraná River basin, Brazil	MG001328	Present study
Dactylogyridae gen. sp. 3	<i>Sorubim lima</i>	Pimelodidae	Upper Paraná River basin, Brazil	MG001327	Present study
Dactylogyridae gen. sp. 9	<i>Platynematchthys notatus</i>	Pimelodidae	Santa Clara, Peru	KP056224	[8]
Dactylogyridae gen. sp. 10	<i>Platynematchthys notatus</i>	Pimelodidae	Santa Clara, Peru	KP056227	[8]
Dactylogyridae gen. sp. 12	<i>Sorubim lima</i>	Pimelodidae	Iquitos-Belém, Peru	KP056228	[8]
Dactylogyridae gen. sp. 13	<i>Hypophthalmus edentatus</i>	Pimelodidae	Nanay River, Peru	KP056230	[8]
Dactylogyridae gen. sp. 18	<i>Pseudoplatystoma fasciatum</i>	Pimelodidae	Santa Clara, Peru	KP056231	[8]
Dactylogyridae gen. sp. 23	<i>Platysilurus mucosus</i>	Pimelodidae	Santa Clara, Peru	KP056232	[8]
Dactylogyridae gen. sp. 26	<i>Platynematchthys notatus</i>	Pimelodidae	Santa Clara, Peru	KP056234	[8]

New sequences obtained for the present study are in bold.

* Sequences used as outgroups.

Molecular phylogenetic analyses of dactylogyrids from Neotropical siluriforms revealed that *Demidospermus* is polyphyletic, with species from loricariids and pimelodids forming two clades that are not closely related [8,16]. The type species of the genus, *D. anus* Suriano, 1983, forms a monophyletic clade with another two species from loricariids [16]. In the present study, a new species of *Demidospermus* from a loricariid host, *Rhinelepis aspera* Spix et Agassiz, in Brazil is described. In addition, a new genus is proposed to accommodate a new species found on the gills of a doradid catfish, *Trachydoras paraguayensis* (Eigenmann et Ward), and new molecular data on both species are provided. Species composition of *Demidospermus* and its broad spectrum of fish hosts are briefly discussed.

2. Material and methods

2.1. Sampling

Fish were captured using gill nets and multifilament cast nets in the Aguapeí River (21°3'36.20"S, 51°45'38.58"W), a tributary of the Paraná River, in the municipality of Castilho, between August 2013 and November 2014. The hosts were collected under the license number 577/2015 IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis). After capture, fish were frozen and later examined in the laboratory. Monogeneans were isolated from the gills, preserved in 70% ethanol, mounted on slides with Hoyer's medium to study sclerotized structures or stained with Gomori's trichrome to study internal organs [17]. The parasites were studied using V3 Leica Application Suite computerized system for image analysis with differential interference contrast and an Olympus BX51 microscope equipped with phase contrast optics. Illustrations were made with the aid of a drawing tube (camera lucida) mounted on a Leica DMLS microscope equipped with phase contrast optics.

2.2. Morphological analyses

Measurements were taken according to Mizelle and Klucka [18] and

Kritsky et al. [19]; they represent the measurement of a straight line between two points expressed in micrometers as the mean followed by range and number of measurements in parentheses. Measurements of haptor bars of a new species of *Demidospermus* follow Franceschini et al. [16]. The terminology for the components of the haptor follows Mizelle and Kritsky [20] and Kritsky and Mizelle [21]; hooks are numbered according to Mizelle [22]. Type and voucher specimens are deposited in the Helminthological Collection of the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC), the Helminthological Collection of the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil (CHIBB), and the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice (IPCAS).

For comparative purposes, the following type and voucher specimens deposited at CHIOC, CHIBB, and Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA) were studied: *Demidospermus paranaensis* Ferrari-Hoeinghaus, Bellay, Takemoto et Pavanelli, 2010 (4 paratypes – CHIOC 37255); *D. spirophallus* Franceschini, Zago, Müller, Francisco, Takemoto et Silva, 2017 (6 paratypes – CHIBB 226L–232L); *D. prolixus* Franceschini, Zago, Müller, Francisco, Takemoto et Silva, 2017 (3 paratypes – CHIBB 233L–236L); *D. anus* Suriano, 1983 (6 vouchers CHIBB 237L–243L); *Cosmetocleithrum gussevi* Kritsky, Thatcher et Boeger, 1986 (paratype – INPA PA266–2); *C. rarum* Kritsky, Thatcher et Boeger, 1986 (holotype – INPA PA270–1); *C. striatuli* Abdallah, Azevedo et Luque, 2012 (3 vouchers – CHIBB 310–312L), and *C. laciniatum* Yamada, Yamada, Silva et Anjos, 2017 (4 paratypes – CHIBB 306–309L).

2.3. DNA extraction, amplification, and sequencing

Specimens of two new species used for molecular analyses, together with three unidentified dactylogyrids morphologically corresponding to *Demidospermus* from two pimelodid catfishes and *Cosmetocleithrum bulbocirrus* Kritsky, Thatcher et Boeger, 1986 from *Pterodoras granulosus* (Valenciennes) (see Table 1), were placed on a slide with a drop of water, covered with a coverslip, identified under the microscope based

on their morphology and then placed into 96% molecular-grade ethanol. Conspecific specimens (paragenophores – see [23] for terminology) were fixed with glycerin ammonium picrate (GAP), mounted as permanent preparations with Canada balsam following Ergens [24] and deposited in IPCAS. Genomic DNA was extracted using 200 μ l of a 5% suspension of Chelex in deionized water and 2 μ l of proteinase K, followed by overnight incubation at 56 °C, boiling at 90 °C for 8 min and centrifugation at 14,000 rpm for 10 min.

Polymerase chain reactions (PCR) were made in 20 μ l of the reaction mixture with 3 μ l of extraction supernatant, 10 μ l of 2 \times MyFi™ Mix (Bioline, Taunton, Massachusetts, USA), and 1.6 μ l of each PCR primer. Partial 28S rDNA (D1–D3 region) was amplified using the primers U178 (5'-GCA CCC GCT GAA YTT AAG-3') and L1642 (5'-CCA GCG CCA TCC ATT TTC A-3') [25]. The thermocycling profile used was: denaturation of DNA (95 °C for 3 min); 35 cycles of amplification (94 °C for 30 s, 56 °C for 30 s and 72 °C for 1.5 min), and 4 min extension hold at 72 °C. PCR products were run on 1% agarose gel using gel red and loading buffer. PCR products were purified prior to sequencing using exonuclease I and shrimp alkaline phosphatase enzymes [26]. Amplicons were Sanger sequenced at GATC Biotech (Konstanz, Germany) using the PCR primers and the internal primers 900F (5'-CCGTCCTG-AAACACGGACCAAG-3') [27], 300R (5'-CAACTTCCCTCACGGTACT-TG-3') [25]; L1200R (5'-GCATAGTTCACCATCTTTCGG-3') and ECD2 (5'-CCTTGGTCCGTGTTTCAAGACGGG) [28]. Contiguous sequences were assembled using Geneious version R8 (<http://www.geneious.com/>; [29]).

com/; [29]).

2.4. Alignment and phylogenetic analyses

Six new sequences of the 28S rDNA fragment were generated (length of 1525–1560 bp) and aligned with 19 published sequences from Genbank of dactylogyrid species belonging to the Ancyrocephalinae (Table 1). Since there are no sequences of any mitochondrial gene for dactylogyrids of Neotropical siluriforms available in GenBank to include into the phylogenetic analyses, this study dealt only with a ribosomal marker. Sequences were aligned using default parameters of MAFFT implemented in Guidance [30] with the extremes of the alignment trimmed. Two datasets of partial 28S rDNA sequences were obtained for phylogenetic analyses: dataset 1 – all the sequences and nucleotide positions (827 bp); dataset 2 – a stringent alignment (747 bp) excluding nucleotide positions with alignment score below 0.5 [31].

Phylogenetic analyses were run under Maximum likelihood (ML) and Bayesian inference (BI) criteria, applying the model of nucleotide evolution GTR + Γ + I for the two datasets. The model was selected using jModelTest 2.1.1 [32,33]. ML analyses were carried out using the program RAxML v. 8 [24]. The model parameters and bootstrap support values (1000 repetitions) were estimated using RAxML. BI trees were created using MrBayes v. 3.2 [34] running two independent MCMC runs of four chains for 10^7 generations and sampling tree topologies

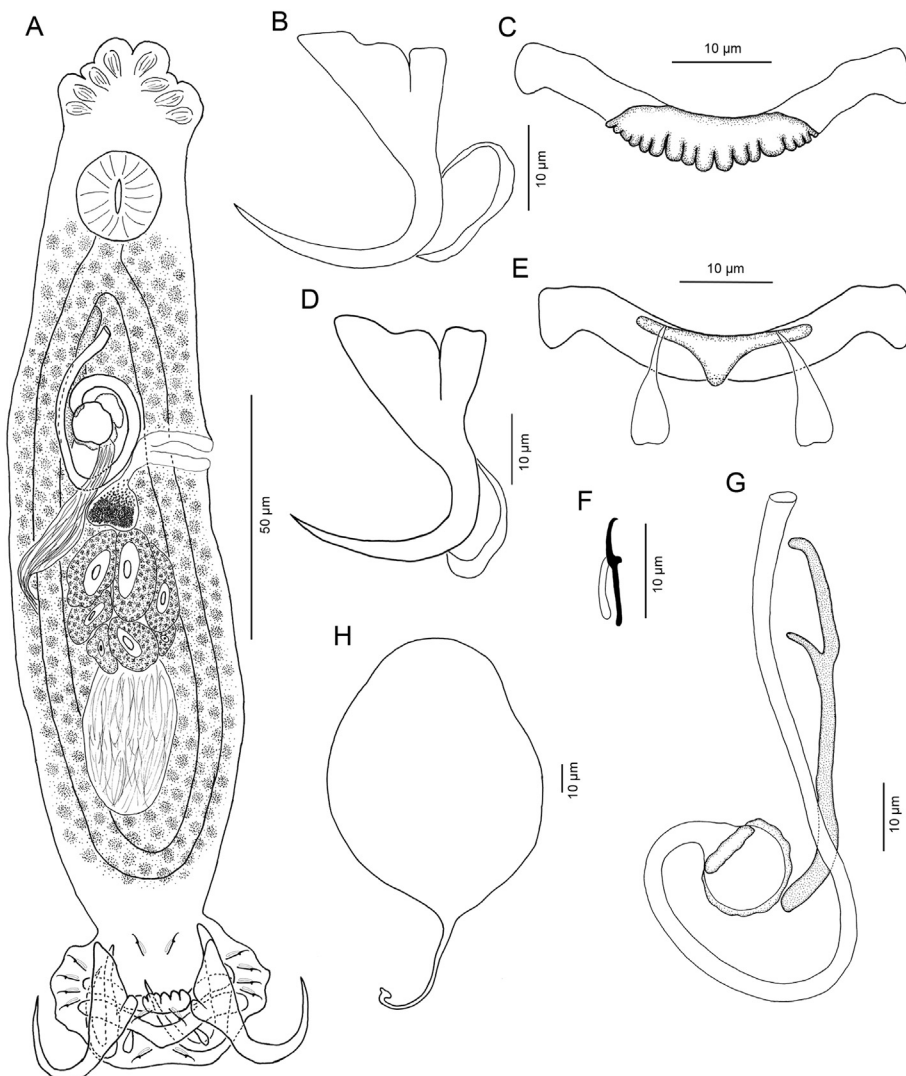


Fig. 1. *Paracosmetocleithrum trachydorasi* n. gen. and n. sp. from the gills of *Trachydoras paraguayensis*, Brazil. (A) Whole mount (dorsal view) (holotype – CHIOC 38881a); (B) ventral anchor, (C) ventral bar, (D) dorsal anchor, (E) dorsal bar, (F) hook (paratype – CHIBB 314L); (G) male copulatory complex (ventral view) (paratype – CHIBB 315L); (H) egg (paratype – CHIBB 316L).

every 10^3 generations. Burn-in periods were set to the first 25,000 generations. MrBayes and RAxML analyses were carried out on the computational resource CIPRES [35]. Genetic divergences were calculated as uncorrected p-distances using MEGA v.6 [36].

3. Results

3.1. Morphological characterization of the new genus

3.1.1. *Paracosmetocleithrum* n. gen. (Fig. 1A–H)

Diagnosis: Dactylogyridae, Ancyrocephalinae. Body divided into cephalic region, trunk, peduncle, and haptor. Tegument thin, surface smooth. Head organs, cephalic lobes present; cephalic glands unicellular, comprising two bilateral groups, posterolateral to pharynx. Eyes absent. Mouth subterminal, midventral; pharynx muscular, glandular; oesophagus present; intestinal caeca 2, confluent posterior to testis, lacking diverticula. Gonads in tandem, intercaecal; testis posterior to ovary; ovary spherical. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens; prostatic reservoir present. Copulatory complex comprising non-articulated copulatory organ and accessory piece; copulatory organ counterclockwise coiled; accessory piece rod-shaped with bifurcated distal end. Common genital pore midventral, at level of intestinal bifurcation. Seminal receptacle at level of anterior margin of ovary; vaginal pore dextral-lateral, non-sclerotized. Vitellarium scattered throughout trunk in form of numerous follicles, except in regions of the reproductive organs. Haptor armed with two pairs of anchors (dorsal and ventral), 14 hooks with ancyrocephalinae distribution [22], ventral and dorsal bars. Ventral bar presents heavily sclerotized ornamentation in its middle portion; dorsal bar presents more sclerotized patch on surface of bar with medial process and with two submedial projections arising from tapered ends of this patch. Parasite of the gills of Neotropical catfishes (Doradidae). Type and only species *Paracosmetocleithrum trachydorasi* n. sp.

Etiology: The generic name is a combination of the Greek suffix *para* (= resembling, similar to) and *Cosmetocleithrum* because of the morphological resemblance of the new genus with that genus.

3.1.2. Differential diagnosis

Paracosmetocleithrum n. gen. differs from all but one Neotropical dactylogyrid genera by the presence of two submedial projections on the dorsal bar (Fig. 1E). The new genus can be distinguished from *Cosmetocleithrum*, the only genus with two submedial projections on the dorsal bar, by the presence of a well-developed ornamentation in the middle portion of the ventral bar, and a sclerotized patch on the surface of the dorsal bar with an inconspicuous medial process that possesses two submedial projections arising from the tapered ends of this patch. *Paracosmetocleithrum* n. gen. possesses a dextral vagina, whereas the vagina of all but one species of *Cosmetocleithrum* is sinistral [17,37]. The only species of *Cosmetocleithrum* with dextral vagina, *C. tortum* Mendoza-Franco, Mendoza-Palmero et Scholz, 2016 from the doradid *Nemadoras hemipeltis* (Eigenmann) in Peru, differs from the single species of the new genus by the characteristic ornamentation of the ventral bar and a sclerotized patch on the dorsal bar.

3.1.3. *Paracosmetocleithrum trachydorasi* n. sp. (Fig. 1A–H)

Diagnosis based on 12 specimens fixed in Hoyer's medium and 8 stained with Gomori's trichrome: Body fusiform 400 (209–484; 10) long, greatest width 90 (44–116; 11) at mid-length. Cephalic lobes poorly developed; 4 bilateral head organs; eyes absent. Pharynx spherical, 23 (19–26; 5) long, 22 (17–26; 5) wide; oesophagus short; intestinal caeca confluent posterior to testis. Peduncle inconspicuous; haptor subhexagonal, 45 (29–63; 4) long, 56 (44–65; 4) wide (Fig. 1A). Anchors similar in shape, shaft and point evenly curved extending beyond anchor base, base with groove, short superficial and deep roots; ventral anchor 27 (24–29; 12) long, base 18 (17–20; 12) wide (Fig. 1B); dorsal anchor 27 (25–29; 12) long, base 18 (16–19; 12) wide (Fig. 1D).

Ventral bar bowed, broad at ends, with heavily sclerotized ornamentation in its middle portion, 45 (39–49; 11) long (Fig. 1C). Dorsal bar bowed, broad at ends, with more sclerotized patch on surface of bar with medial process, and two submedial projections arising from tapered ends of this patch (Fig. 1E), 47 (41–52; 12) long. Hooks similar, 12 (11–12; 12) long, recurved point, erect thumb, slender shank, FH loop about less than $\frac{3}{4}$ of shank length (Fig. 1F). Male copulatory organ (MCO) slender, forming 1 incomplete counterclockwise ring, broad base, 65 (40–76; 12) long, proximal ring diameter 28 (20–34; 10) (Fig. 1G). Accessory piece 59 (52–65; 11) long, rod shaped with bifurcated distal end (Fig. 1G). Gonads in tandem; ovary pretesticular. Testis 32 ($n = 1$) long, 18 ($n = 1$) wide; seminal vesicle dilation of vas deferens; prostatic reservoir present. Vas deferens looping left intestinal caecum. Ovary 41 (39–43; 3) long, 26 (23–27; 1) wide, spherical; oviduct, ootype, and uterus not observed. Seminal receptacle at level of anterior margin of ovary; vagina dextral-lateral, non-sclerotized (Fig. 1A). Vitellarium scattered throughout trunk, except in regions of reproductive organs. Egg oval, with filament opposite to opercular end, 110 (109–110; 2) long, 60 (57–63; 2) wide (Fig. 1H).

3.1.4. Taxonomic summary

Type-host: *Trachydoras paraguayensis* (Eigemann et Ward) (Siluriformes: Doradidae).

Type-locality: Aguapeí River (Paraná River basin), municipality of Castilho, São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Site on host: Gills.

Infection rate: Prevalence: 70% (49 fish infected of 70 examined); mean intensity 8 ± 3 (1–127).

Specimens deposited: Holotype (CHIOC 38881a); 3 paratypes (CHIOC 38881b–d); 4 paratypes (CHIBB 313–316L), 8 vouchers (CHIBB 317–324L); 4 paratypes (IPCAS M-675); paragenophore (IPCAS M-675).

Representative DNA sequence: 1547 bp long sequence of the D1–D3 region of the *lsrDNA* of one specimen from *T. paraguayensis* (GenBank Accession No. MG001323).

Etiology: The specific name refers to the generic name of its hosts.

3.1.5. Remarks

The new species is the type and single species of the newly erected genus. Therefore, it can be distinguished from species of other dactylogyrid genera by the characteristics listed in the differential diagnosis of the new genus, i.e., the presence of two submedial projections on the dorsal bar, a well-developed ornamentation in the middle portion of the ventral bar, and a sclerotized patch on the surface of the dorsal bar with an inconspicuous medial process with the two submedial projections arising from the tapered ends of this patch (see above).

Analyses of datasets 1 and 2 (see Section 2.4. above) yielded similar topologies and for each of the dataset the BI and ML analyses produced phylogenetic trees with mostly congruent branching topology and support values. Fig. 3 shows the phylogenetic tree obtained using dataset 2. In a preliminary phylogenetic analysis, *P. trachydorasi* did not cluster with any other sequenced genera of Neotropical siluriforms, including those of clade A in Mendoza-Palmero et al. [8] that comprises dactylogyrids from Neotropical siluriforms and Holarctic perciforms. More specific phylogenetic analyses (dataset 1 and 2) revealed *P. trachydorasi* as an independent lineage, sister to the clade composed of *Demidospermus* spp. and Dactylogyridae gen. spp. from pimelodid catfishes. In turn, this clade is sister to that including two species of *Cosmetocleithrum* from doradids and 4 species of *Demidospermus* from lorcarids. However, the relationship of the new genus with dactylogyrids from pimelodids remains unresolved due to weak support of this clade (Fig. 3). *Paracosmetocleithrum trachydorasi* n. sp. appeared genetically distant from all genera of Neotropical dactylogyrids from siluriforms included in this study (Table 2).

Table 2
Nucleotide divergence (p-distance) among selected dactylogyrids from Neotropical catfishes (747 bp alignment).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 <i>Aphanoblastella</i> sp. 3 (KP056238)																						
2 <i>Aphanoblastella aurorae</i> (KP056239)	7																					
3 <i>Demidospermus prolixus</i> (KY766955)	25	24																				
4 <i>Demidospermus spirophallus</i> (KY766954)	26	28	11																			
5 <i>Demidospermus anus</i> (KY766957)	26	27	7	10																		
6 <i>Demidospermus rhinelepisi</i> n. sp. (XXXXX)	27	27	10	12	9																	
7 <i>Cosmetocleithrum bifurcum</i> (KP056216)	34	35	26	28	28	26																
8 <i>Cosmetocleithrum bulbocirrus</i> (XXXXX)	33	32	22	23	23	23	16															
9 <i>Paracosmetocleithrum trachydorasi</i> n. gen., n. sp. (XXXXX)	30	30	24	25	25	25	31	29														
10 Dactylogyridae gen. sp. 9 (KP056224)	28	31	22	25	24	24	29	26	26													
11 Dactylogyridae gen. sp. 26 (KP056234)	29	29	23	24	23	24	29	26	26	7												
12 <i>Demidospermus</i> sp. 23 (KP056236)	27	29	22	24	23	24	28	27	27	13	14											
13 <i>Demidospermus</i> sp. 11 (KP056235)	28	29	24	25	25	30	27	26	14	15	2											
14 <i>Demidospermus mortenthaleri</i> (KP056245)	30	32	21	23	23	29	28	27	18	17	17	18										
15 Dactylogyridae gen. sp. 23 (KP056232)	30	31	22	23	24	30	29	28	17	17	17	18	10									
16 Dactylogyridae gen. sp. 18 (KP056231)	31	31	24	26	26	30	29	29	21	19	19	20	15	16								
17 Dactylogyridae gen. sp. 10 (KP056227)	29	30	23	24	25	26	28	26	26	19	18	17	12	14	16							
18 Dactylogyridae gen. sp. 13 (KP056230)	31	32	22	24	23	30	28	26	18	17	17	18	14	14	16	13						
19 Dactylogyridae gen. sp. 1 (XXXXX)	31	32	25	27	26	29	27	28	20	19	19	20	15	17	18	16	18					
20 Dactylogyridae gen. sp. 2 (XXXXX)	31	31	25	27	26	30	27	27	21	20	21	22	18	18	20	17	19	6				
21 Dactylogyridae gen. sp. 3 (XXXXX)	29	29	23	25	24	29	27	26	17	16	18	19	14	13	15	13	13	13	16			
22 Dactylogyridae gen. sp. 12 (KP056228)	28	29	23	25	24	30	28	26	18	17	19	20	14	13	17	14	14	14	17	3		

Taxa are listed as they appear in Fig. 3. All values represent percentages.

3.2. Description of a new *Demidospermus* species from loricariid

3.2.1. *Demidospermus rhinelepisi* n. sp. (Fig. 2A–I)

Diagnosis based on 15 specimens fixed in Hoyer's medium and 10 stained with Gomori's trichrome: Body fusiform 559 (394–814; 10) long, greatest width 121 (100–136; 10) at mid-length. Cephalic lobes poorly developed; 4 bilateral head organs; eyes absent. Pharynx spherical, 41 (37–47; 10) long, 39 (36–47; 10) wide; oesophagus short; intestinal caeca confluent posterior to testis. Peduncle broad; haptor subhexagonal, 58 (53–66; 8) long, 75 (69–80; 8) wide (Fig. 2A). Anchors similar in shape, each with poorly developed roots, presenting fissures at base, shaft, and point evenly curved at level of superficial root; ventral anchor 29 (27–32; 15) long, base 20 (18–22; 15) wide (Fig. 2B); dorsal anchor 28 (27–31; 17) long, base 20 (19–22; 15) wide (Fig. 2D). Ventral bar V-shaped, constricted in the middle with sclerotized patch attached to middle portion, rounded ends, with variable aperture of angulation, 66 (61–72; 15) total length, distance between ends 56 (42–64; 15), 19 (12–28; 15) high (Fig. 2C). Dorsal bar V-shaped, articulated in middle with broader two pieces, tapering along its extension, rounded ends, 66 (59–74; 15) total length, distance between ends 55 (40–68; 7), 19 (14–24; 15) height (Fig. 2E). Hooks similar, 14 (14–15; 15) long, recurved point, erect thumb, slender shank, with round, weakly sclerotized subunit at base, FH loop about 3/4 of

shank length (Fig. 2F). MCO slender, forming about 1½ counter-clockwise rings, 39 (33–52; 14) long, proximal ring diameter 23 (20–26; 14) (Fig. 2G). Accessory piece 50 (44–53; 15) long, with broad ends and tapering in middle, rounded in proximal end, rounded proximal end, distal end folding on both sides with folds extending to approximately 3/4 of accessory piece length (Fig. 2G). Gonads in tandem; ovary pretesticular. Testis 53 (45–61; 3) long, 33 (26–38; 1) wide; seminal vesicle a dilation of vas deferens, with two conspicuous dilations; prostatic reservoir not observed. Vas deferens looping left intestinal caecum. Ovary 115 (99–129; 4) long, 54 (44–60; 4) wide, spherical; oviduct, ootype, and uterus not observed. Vaginal aperture sinistral-lateral, spherical in shape; vaginal tube sclerotised (Fig. 2I). Vitellarium in form of follicles densely scattered throughout trunk, except around reproductive organs. Egg oval, with filament opposite to opercular end, 87 (1) long, 45 (1) wide (Fig. 2H).

3.2.2. Taxonomic summary

Type-host: *Rhinelepis aspera* Spix et Agassiz, 1829 (Siluriformes: Loricariidae).

Type-locality: Aguapeí River (Paraná River basin), municipality of Castilho, São Paulo State, Brazil (21°3'36.20"S, 51°45'38.58"W).

Site on host: Gills.

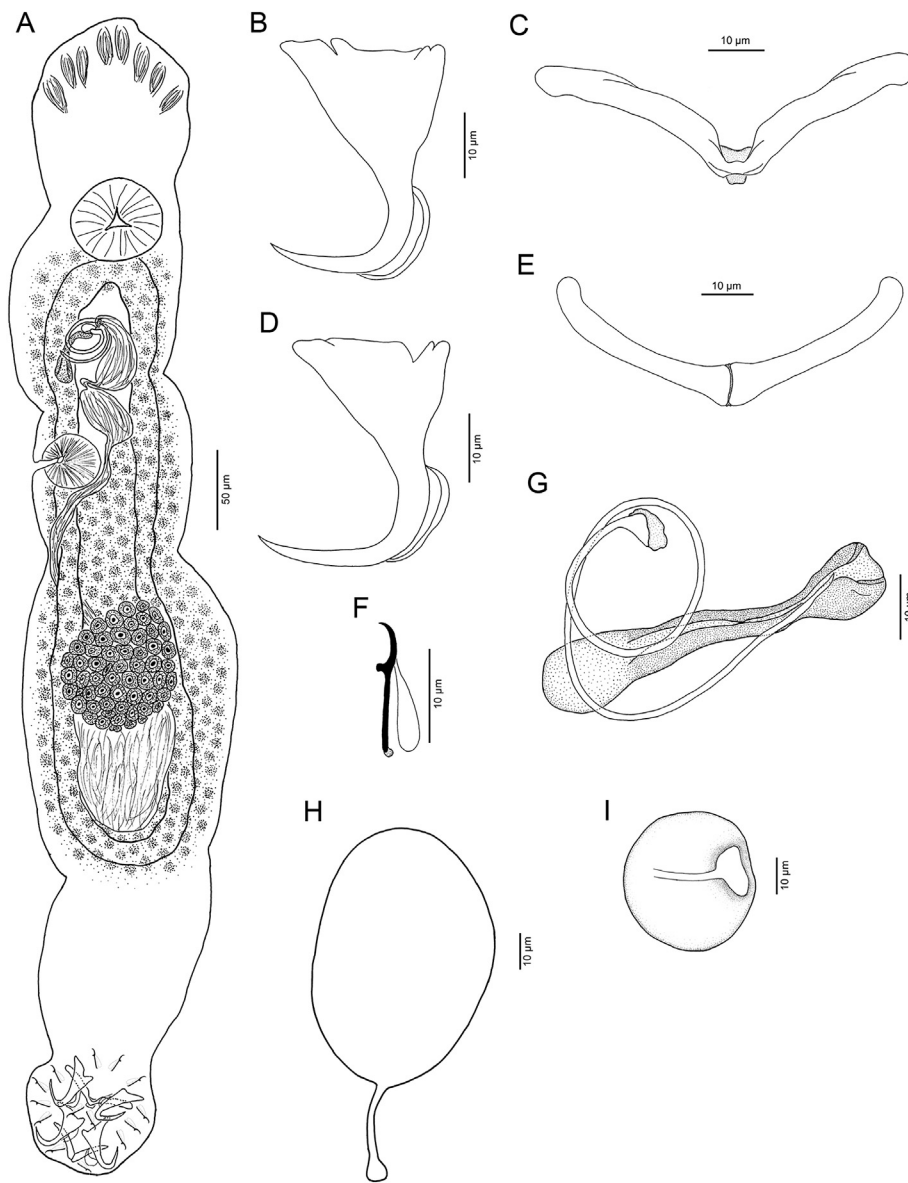


Fig. 2. *Demidospermus rhinelepsi* n. sp. from the gills of *Rhinelepis aspera*, Brazil. (A) Whole mount (dorsal view) (holotype – CHIOC 38880a); (B) ventral anchor, (C) ventral bar, (D) dorsal anchor, (E) dorsal bar, (F) hook (paratype – CHIBB 325L); (G) male copulatory complex (ventral view) (paratype – CHIBB 326L); (H) egg (paratype – CHIBB 326L); (I) vagina (paratype – CHIBB 327L).

Infection rate: Prevalence: 100% (30 fish infected of 30 examined); mean intensity 12 ± 3 (1–70).

Specimens deposited: Holotype (CHIOC 38880a); 4 paratypes (CHIOC 38880b–e); 4 paratypes (CHIBB 325–328L) 11 vouchers (CHIBB 329–340L); 5 paratypes (IPCAS M-676); paragenophore (IPCAS M-676).

Representative DNA sequence: 1542 bp long sequence of the D1–D3 region of the *lsrDNA* of one specimen from *R. aspera* (GenBank Accession No. MG001324).

Etymology: The specific name refers to the generic name of its host.

3.2.3. Remarks

The new species is considered a member of *Demidospermus* because of the presence of the characteristics presented in an amended diagnosis of the genus proposed by Kritsky and Gutiérrez [15], i.e., eyes absent, gonads tandem, MCO coiled counterclockwise, accessory piece of MCO sheath-like, vaginal aperture sinistral, and bars V-shaped. *Demidospermus rhinelepsi* n. sp. differs from its congeners by the possession of a sclerotized patch attached to the middle portion of the ventral bar (Fig. 2C), and by morphology of the accessory piece, which presents broad ends, tapering in the centre, rounded proximal end, distal end folding on both sides with folds extending to approximately $\frac{3}{4}$ of the

accessory piece length (Fig. 2G).

MCO of *D. rhinelepsi* n. sp. resembles in shape that of *D. anus*, *D. paranaensis*, *D. prolixus*, and *D. spirophallus*. The new species presents about $1\frac{1}{2}$ counterclockwise rings similarly as *D. prolixus*, but it differs in the shape of the accessory piece, which is straight in shape and smaller in *D. prolixus* (44–53 µm in *D. rhinelepsi* n. sp. versus 32–38 µm in *D. prolixus*, see Fig. 3A in [16]). The MCO of *D. anus* presents 1– $1\frac{1}{2}$ counterclockwise ring, but differs from the new species in the shape (broad distal end and slender proximal end) and smaller size of the accessory piece (30–33 µm) in *D. anus*, (see Fig. 4A in [16]). *Demidospermus paranaensis* presents MCO with 1 counterclockwise ring and differs from *D. rhinelepsi* n. sp. in the shape of the accessory piece, which is rod-like without any folds, with a broader distal end (see Fig. 1G in [39]). *Demidospermus spirophallus* presents $2\frac{1}{2}$ but differs from the new species in the shape of the accessory piece, which is tubular with a bifurcated proximal end (see Fig. 1B in [16]).

Demidospermus rhinelepsi n. sp. is the fifth species of the genus described from loriciid catfishes. Molecular data (partial sequences of 28S rDNA) revealed the new species to be sister to *D. anus*, the type species of the genus, within a well-supported clade composed exclusively of species of *Demidospermus* from loriciids (Fig. 3).

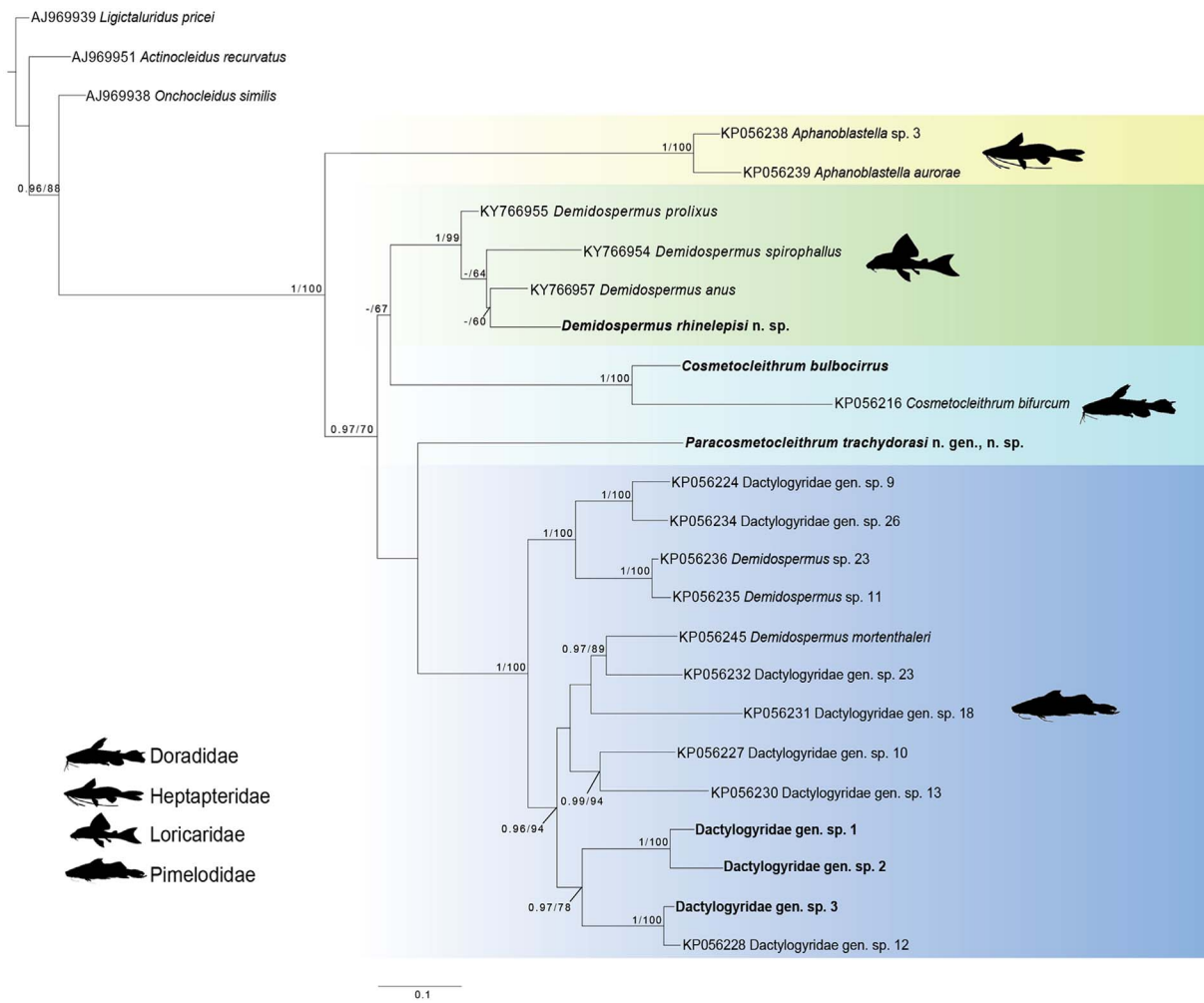


Fig. 3. Maximum likelihood phylogram based on 28S rDNA sequences. Newly sequenced species are in bold. Posterior probabilities followed by bootstrap support values are given above the branches (posterior probability < 0.90 and bootstrap values < 60 are not shown). *Ligictaluridus pricei*, *Actinocleidus recurvatus*, and *Onchocleidus similis* were used as outgroups. Branch length scale bar indicates number of substitution per site.

3.3. Phylogenetic relationships among Neotropical dactylogyrids from pimelodids

Three newly sequenced unidentified species (Dactylogyridae gen. sp. 1, 2, and 3) from pimelodid catfishes [two species from *Hemisorubim platyrhynchos* (Valenciennes) and one from *Sorubim lima* (Bloch et Schneider)] are not closely related to species of *Demidospermus* from loricariids (Fig. 3), even though their morphology resembles that of species of this genus (data not shown). In contrast, these monogeneans from pimelodids group within a big clade composed of *Demidospermus mortenthaleri*, two unidentified species of this genus (*Demidospermus* sp. 11 and sp. 23 of [8]) and 7 dactylogyrids not identified to the genus level (Dactylogyridae gen. sp. 9, 10, 12, 13, 18, 23 and 26 of [8]), all parasitising pimelodid catfishes. The newly sequenced monogeneans form a relatively well-supported clade with another unidentified species, Dactylogyridae gen. sp. 12, which parasitizes the same host, *S. lima*.

4. Discussion

In the present study, two new species of dactylogyrid monogeneans were described from Neotropical catfishes. For the new species from a doradid catfish, a new genus is erected because this species possesses unique ornamentation of connecting bars, which is not present in any other dactylogyrid genus [6,7]. The second new species is described

from a loricariid fish and it is assigned to *Demidospermus*, one of the species-richest genera of Neotropical monogeneans [16]. Molecular analyses have shown that this genus, which includes species parasitising three different families of siluriform fishes, namely Auchenipteridae, Loricariidae, and Pimelodidae, is not monophyletic [8,16]. It is apparent that the species composition of the genus and its delimitation require revision based on this new molecular evidence. The new species of *Demidospermus* described in the present paper groups with the type species of the genus, *D. anus*, and thus its generic assignment is unquestionable as is its validity considering the morphological and molecular evidence presented herein. Phylogenetic relationships of the new genus to other dactylogyrid genera from Neotropical catfishes remain unresolved due to weak support for the internal nodes of the clade. Nevertheless, nucleotide divergence of its type and only species, *Paracosmetocleidum trachydorasi* n. sp., from species of other genera is rather high (see Table 2).

Whereas circumscription of individual species of Neotropical gill monogeneans does not represent a serious problem, delimitation of genera remains problematic in many cases, especially because of the existence of very few synapomorphies that would define unequivocally individual genera. Some genera are defined by unique combination of non-unique characteristics including those of internal structures (e.g. mutual position of genital organs – see [17]). Species composition of some genera such as *Urocleidoides* Mizelle et Price, 1964 and *Demidospermus* has changed considerably, depending on the generic concept

accepted by given authors; these genera include species with a relatively broad spectrum of morphological variability [4,11]. Regarding the polyphyly of *Demidospermus*, the present study provides additional evidence that revision of the species composition and delimitation of this genus is needed. The genus currently includes 29 species from auchenipterids, loricariids and pimelodids [11,16], which is an unusually broad range of definitive hosts for a dactylogyrid genus [40]. A most recent study by Mendoza-Palmero et al. [38] showed that morphologically similar species from same fish hosts may be genetically distant, likely as a result of convergent evolution of morphological traits.

It is possible that the explosive radiation of pimelodid and other catfishes in South America, mostly caused by long and complex history of South American rivers [41], may be reflected in morphological similarity of phylogenetically distantly related dactylogyrids, including species of *Demidospermus* from different catfish families. This rapid radiation of Neotropical catfishes may not have been accompanied by corresponding phenotypic differentiation.

5. Conclusion

Based on morphological and molecular evidence, a new genus of dactylogyrid monogeneans is erected to accommodate a new species from a relatively poorly known group of Neotropical catfishes. In addition, new genetic and morphological data are provided on another new species of *Demidospermus*, a polyphyletic genus of Neotropical gill monogeneans. These data may help better circumscribe individual lineages of this artificial assemblage of monogeneans from different families of catfishes.

Conflict of interest

None

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