

Article

Three New Species of *Jainus* (Monogenea: Dactylogyridae) Parasitizing Gills of Brazilian Freshwater Fishes Supported by Morphological and Molecular Data [†]

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Abstract: The present study describes three new species of monogeneans parasitizing the gills of anostomid fishes from the Upper Paraná River basin, southeastern Brazil: *Jainus beccus* n. sp. and *Jainus radixelongatus* n. sp. on *Leporinus friderici*, *Leporinus octofasciatus*, *Leporinus striatus*, and *Megaleporinus elongatus*; and *Jainus ornatus* n. sp. on *L. friderici*. The new species differ from other congeners by the morphology of the accessory piece. There is a semicircular distal portion resembling a “bird’s beak” in *Jainus beccus* n. sp. It composed of two subunits—one ventral and more sclerotized, sickle-shaped, and another dorsal with three projections—in *Jainus radixelongatus* n. sp. There are two elongated and sclerotized subunits, both of which have a sickle-shaped distal portion, in *Jainus ornatus* n. sp. Supplementary observations not reported in the original descriptions of the type-species *Jainus piava* Karling, Bellay, Takemoto & Pavaneli, 2011 are proposed, as follows: the presence of a thin and delicate ventral bar, which can vary greatly in shape; an accessory piece not articulated with the MCO’s base. This paper provides the first phylogenetic study based on LSU rDNA and COI mtDNA gene sequences for *Jainus*, improving and clarifying the understanding of host–parasite relationships in neotropical characiforms.

Keywords: *Jainus*; new species; Ancyrocephalinae; ectoparasites; taxonomy; neotropical region; phylogeny; COI mtDNA; LSU rDNA



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1. Introduction

In Brazil until 2011, 1034 species of parasites were registered in 451 species of freshwater fishes [1]. However, these data show that only 10% of known fish species had their parasitic fauna investigated. Among the taxonomic groups of fish parasites, monogeneans can be considered one of the most diverse [2,3]. After that, Cohen et al. [4] in 2013 registered 651 species of monogeneans that parasitized fishes, amphibians, and reptiles in South America, and 67% parasitized only fishes from Brazil. The majority of these occurrences were concentrated in freshwater fishes from the basins of the Amazon and Paraná Rivers.

In most of the taxonomic studies on monogeneans, descriptions are based on morphological data and do not provide the molecular characterization of the species described [5]. Considering molecular data as a key tool for the delimitation of new species, recent studies have used molecular techniques to support morphological and taxonomic descriptions of monogenean species, providing ways of performing phylogenetic analysis based on different markers [5–9], improving the knowledge about this diverse group and elucidating the relationship between species and their hosts [5,10,11].

Jainus Mizelle, Kritsky & Crane, 1968 comprises gill parasites of neotropical characiforms. Currently, the genus includes seven species, and six of them have been described in fishes from Brazilian ecosystems: *Jainus jainus* Mizelle, Kritsky & Crane, 1968 (type species) parasitizing *Chalceus macrolepidotus* Cuvier, 1818 (Chalceidae); *Jainus robustus* Mizelle, Kritsky & Crane, 1968 from *Bryconops affinis* (Günther, 1864) (*Cretochanes affinis*) (Iguanodectidae); *Jainus iocensis* Cohen, Kohn & Boeger, 2012 from *Salminus brasiliensis* (Cuvier, 1816) (Bryconidae); *Jainus piava* Karling, Bellay, Takemoto & Pavanelli, 2011 from *Schizodon borellii* (Boulenger, 1900) (Anostomidae); *Jainus leporini* Abdallah, Azevedo & Luque, 2012 from *Hypomasticus copelandii* (Steindachner, 1875) (*Leporinus copelandii*) (Anostomidae); and *Jainus amazonensis* Kritsky, Thatcher & Kayton, 1980 from *Brycon melanopterus* (Cope, 1872) (Bryconidae) [12–16].

As part of our ongoing studies about the biodiversity of helminth parasites of freshwater fishes from tributaries of the Upper Paraná River basin, southeastern Brazil, three new species of *Jainus* are described from the gills of four anostomid fishes (*Leporinus friderici* (Block, 1794), *Leporinus striatus* Kner, 1858, *Leporinus octofasciatus* Steindachner, 1915, and *Megaleporinus elongatus* (Valenciennes, 1850)) supported by morphological and molecular data. We also present new morphological features that are added to the diagnosis of *J. piava* and its relationship with congeners and other dactylogyrids parasites of neotropical characiforms, using sequences of the partial LSU rDNA gene.

2. Materials and Methods

2.1. Host Sampling and Parasitological Procedures

Fish specimens were collected in two localities from the Upper Paraná River basin, São Paulo state, Brazil, from 2011 to 2013: Jurumirim Reservoir (23°12'17" S, 49°13'19" W) and Sapucaí-Mirim River (20°29'38.38" S, 47°51'33.11" W). Data on host species; locality; number of sampled hosts; and mean standard length (SL) in centimeters (cm) ± standard deviation (SD), followed by the range (minimum–maximum values), are in Table 1.

Table 1. List of host species sampled in the present study in the Upper Paraná River basin, São Paulo, Brazil (SL—mean standard length; SD—standard deviation).

Host Species	Locality	Sampled Hosts	SL (cm) ± SD (Range)
<i>Leporinus friderici</i>	Jurumirim Reservoir	116	16.67 ± 5.79 (8.90–27)
	Sapucaí-Mirim River	40	23.12 ± 4.59 (12.70–31.80)
<i>Leporinus octofasciatus</i>	Jurumirim Reservoir	15	14.65 ± 3.77 (9.80–20.90)
	Sapucaí-Mirim River	17	18.50 ± 3.09 (13.50–22.20)
<i>Leporinus striatus</i>	Jurumirim Reservoir	26	10.12 ± 0.85 (8.70–11.70)
<i>Megaleporinus elongatus</i>	Jurumirim Reservoir	30	13.79 ± 3.97 (9.50–28.20)
	Sapucaí-Mirim River	22	25.05 ± 4.26 (12–33.50)
<i>Schizodon nasutus</i>	Jurumirim Reservoir	30	16.18 ± 4.23 (9.60–24)
	Sapucaí-Mirim River	40	20.11 ± 0.73 (9.50–28.80)

Hosts were collected under the license number SP/538/88 DEFOP (Department of the Development of Fishery and Inspection) for the Jurumirim Reservoir, and SISBio 13794-1 (Permanent License for the Collection of Zoological Material) for the Sapucaí-Mirim River. All animal procedures were performed in full compliance with the Ethics Committee for Animal Experimentation (CEUA/protocols n. 120 and 942) of the Universidade Estadual Paulista (São Paulo State University—UNESP). Fishes were collected using a nylon monofilament gillnet. After capture, some specimens were individually stored in plastic bags and frozen for later necropsy at the laboratory. Some freshly killed hosts were examined in situ, and some specimens of monogeneans were placed into 96% molecular-grade ethanol for molecular analyses. In the laboratory, gills were removed, placed in Petri dishes with water, and checked for monogeneans under a stereomicroscope. Monogeneans were mounted on slides with Hoyer's and Gray and Wess's medium [17].

The morphology of the parasites was analyzed using V3 Leica Application Suite (LAS) computerized system for image analysis adapted in a microscope with differential interference contrast. Illustrations were performed with the aid of a drawing tube (camera lucida) mounted on a Leica DMLS microscope equipped with phase-contrast optics. Measurements of the sclerotized structures of the haptor (bar, anchors, and hooks) and copulatory complex were performed according to the scheme shown in Figure 1a–l. For the copulatory complex comprising a sclerotized accessory piece and a male copulatory organ (MCO), or cirrus, the direction of the MCO's rings (counterclockwise vs. clockwise) follows Kritsky et al. [18]. The numbering and distribution of hook pairs and the specific terminology of the genus *Jainus* follow Mizelle [19] and Mizelle et al. [12], respectively. All measurements are represented in micrometers (μm) and expressed as the means followed by the respective ranges and the numbers of specimens measured in parentheses. The prevalence and mean intensity of infection were calculated following Bush et al. [20].

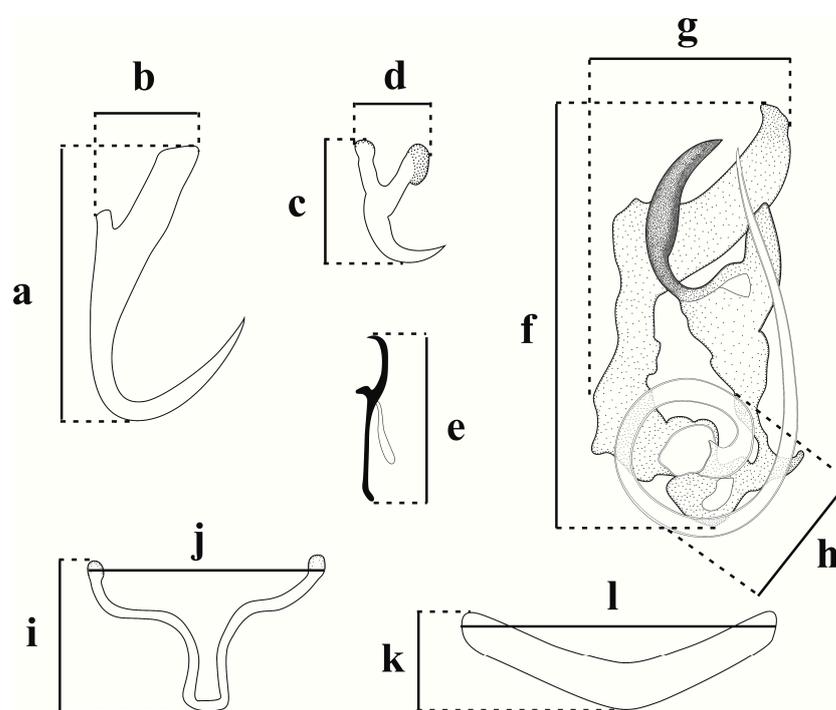


Figure 1. Scheme of measurements of the sclerotized structures of the haptor and copulatory complex of *Jainus* spp. Dorsal anchor: (a) total length, (b) width; ventral anchor: (c) total length, (d) width; hook: (e) total length; accessory piece: (f) total length, (g) total width; MCO: (h) diameter of the ring; ventral bar: (i) total length, (j) total width; dorsal bar: (k) total length, (l) total width.

Type and voucher specimens of the monogeneans were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro (state), Brazil. Other vouchers were deposited in the Helminthological Collection of the Institute of Biosciences (CHIBB), Botucatu, São Paulo (state), Brazil. According to Brazilian laws, species registration for scientific research purposes was carried out at SisGen (A8F53FE). Paratypes and vouchers examined for comparative purposes: *J. leporini* (paratypes 535a–c) and *J. amazonensis* (paratypes MZUSP, USNM, UNSM) deposited in the zoological collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Amazonas, Brazil; *J. iocensis* (paratypes 37635, 37700, 37712) and *J. piava* (paratypes 37236, 37235B, 37235C, and photomicrographs of holotype 37235A) deposited in CHIOC.

2.2. Molecular and Phylogenetic Analyses

Monogenean specimens were placed into 96% molecular-grade ethanol and subsequently used for molecular characterization. Conspecific specimens (paragenophores; see

Pleijel et al. [21]) were mounted in Hoyer's or Gray and Wess's medium and deposited in the CHIBB. The total genomic DNA of the parasites was extracted using 200 µL of a 5% suspension of Chelex in deionized water and 2 µL 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, producing a final volume of 20 µL [8]. Partial fragments of the LSU rDNA and COI mtDNA genes were amplified by conventional polymerase chain reaction (PCR) performed with a total volume of 20 µL for each reaction, containing 3 µL of extracted DNA, 10 µL of 2xMyFiTM Mix (Bioline, Taunton, MA, USA), 3.8 µL of pure water, and 1.6 µL of each PCR primer. For the LSU rDNA gene and COI mtDNA gene, the primers used and the thermocycling profile are expressed in Table 2.

Table 2. Primers used in this study. New primers obtained for the present study are in bold.

Gene/Region	Primers and Sequences 5'-3'	Cycling Conditions	Product (bp)	Source
COI	COI_Mono_5 5'-TAA TWG GTG GKT TTG GTAA-3'	Initial denaturation of 3 min at 94 °C; 40 cycles of: 94 °C for 30 s, 47 °C for 30 s, 72 °C for 60 s; final extension of 7 min at 72 °C.	~650	[22]
	COI_Mono_3 5'-TAA TGC ATM GGA AAA AAA CA-3'			
LSU	U178 5'-GCA CCC GCT GAA YTT AAG-3'	Initial denaturation of 5 min at 95 °C; 40 cycles of: 95 °C for 30 s, 56 °C for 30 s, 72 °C for 2 min; final extension of 10 min at 72 °C.	~1800	[23]
	L1642 5'-CCA GCG CCA TCC ATT TTC A-3'			Present study
	6F 5'-CGG CGA GTG AAC GGA GAT TA-3'			
	382F 5'-AGC TGG TGG AGT CAA GCT TC-3'			
	1289R 5'-TGC TCA CGT TTG ACG ATC GA-3'			

PCR products (2 µL) were run on an agarose gel (1%) using GelRed™ fluorescent nucleic acid dye and loading buffer to confirm amplicon size and yield. Amplicons were purified with the QIAquick PCR Purification Kit (Qiagen) and sequenced using the BigDye v.3.1 Terminator Cycle Sequencing kit (Applied Biosystems). Sequences were run on an Applied Biosystems ABI 3500 DNA genetic analyzer. Forward and reverse sequences were assembled and edited using Sequencher version 5.2.4 (Gene Codes, Ann Arbor, MI, USA). Datasets were separately aligned using the default parameters of the MUSCLE algorithm implemented on Geneious version 7.1.3 [24].

In total, five newly sequences for three species were submitted to the GenBank database: LSU rDNA gene—two partial sequences, (GenBank accession numbers OQ843018, OQ843019); and COI—three partial sequences, (accession numbers OQ833545, OQ833544, OQ833543) (see Table 3).

Phylogenetic relationships were reconstructed for each alignment under Bayesian inference (BI) and maximum likelihood (ML), applying the model GTR+I+G, which was selected as the best-fitting model of nucleotide evolution by the JModel Test v.2.1 using the Akaike information criterion [25] for all data sets. The BI analyses were run using MrBayes [26]. The Markov chain Monte Carlo (MCMC) chains were run with 10⁶ generations and sampling tree topologies every 100th generation. The first 25% of trees were discarded as burn-in, and the remaining trees were used for calculating the Bayesian posterior probabilities. The ML analyses were estimated using RaxML [27], with 1000 bootstrap replications. Both ML and BI analyses were performed on the computational resource CIPRES [28]. Genetic divergence was calculated with the uncorrected p-distances model in MEGA7 [29,30]. Phylogenetic trees were edited in the software FigTree v1.4 [31].

Table 3. List of monogeneans included in the phylogenetic analyses, with details of the hosts, localities, and GenBank accession numbers of sequences from the LSU rDNA and COI mtDNA genes. New sequences obtained in the present study are in bold.

Monogenea Species	Host Species	Locality	Accession Numbers		References
			LSU rDNA	COI	
Dactylogyridae					
<i>Ameloblastella chavarriai</i> (Price, 1938)	<i>Rhamdia quelen</i>	Mexico	KP056251		[6]
<i>Ameloblastella edentensis</i> Mendoza-Franco, Mendoza-Palmero & Scholz, 2020	<i>Hypophthalmus edentatus</i>	Peru	KP056255		[6]
<i>Ameloblastella unapioides</i> Mendoza-Franco, Mendoza-Palmero & Scholz, 2020	<i>Sorubim lima</i>	Peru	KP056254		[6]
<i>Aphanoblastella aurorae</i> Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012	<i>Goeldiella eques</i>	Peru	KP056239		[6]
<i>Aphanoblastella magna</i> Yamada, Acosta, Yamada, Scholz & Silva, 2018	<i>Pimelodella avanhandavae</i>	Brazil	MH688484		[32]
<i>Aphanoblastella travassosi</i> (Price, 1938)	<i>Rhamdia guatemalensis</i>	Mexico	MK358458		[9]
<i>Cacatuocotyle papilionis</i> Zago, Franceschini, Müller & Silva, 2018	<i>Astyanax lacustris</i>	Brazil	MG832889		[33]
<i>Characithecium paranapanemense</i> Zago, Franceschini, Abdallah, Müller, Azevedo & Silva, 2021	<i>Psalidodon paranae</i>	Brazil	MZ408907		[34]
<i>Cosmetocleithrum bifurcum</i> Mendoza-Franco, Mendoza-Palmero & Scholz, 2000	<i>Hassar</i> sp.	Peru	KP056217		[6]
<i>Cosmetocleithrum bulbocirrus</i> Kritsky, Thatcher & Boeger, 1986	<i>Pterodoras granulosus</i>	Brazil	MG001326		[9]
<i>Demidospermus prolixus</i> Franceschini, Zago, Müller, Francisco, Takemoto & Silva, 2017	<i>Loricaria prolixia</i>	Brazil	KY766955		[5]
<i>Demidospermus anus</i> Suriano, 1983	<i>Loricariichthys platymetopon</i>	Brazil	KY766957		[5]
<i>Demidospermus mortenthaleri</i> Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012	<i>Brachyplatystoma juruense</i>	Peru	KP056245		[6]
<i>Demidospermus rhinelepsi</i> Acosta, Scholz, Blasco-Costa, Alves & Silva, 2019	<i>Rhinelepis aspera</i>	Brazil	MG001324		[9]
<i>Demidospermus spirophallus</i> Franceschini, Zago, Müller, Francisco, Takemoto & Silva, 2017	<i>Loricaria prolixia</i>	Brazil	KY766954		[5]
<i>Diaphorocleidus magnus</i> Zago, Franceschini, Abdallah, Müller, Azevedo & Silva, 2021	<i>Astyanax lacustris</i>	Brazil	MZ408903	MZ408253	[34]
<i>Diaphorocleidus neotropicalis</i> Zago, Franceschini, Abdallah, Müller, Azevedo & Silva, 2021	<i>Astyanax lacustris</i>	Brazil	MZ408906	MZ408254	[34]
<i>Diaphorocleidus petrosusi</i> Mendoza-Franco, Aguirre-Macedo & Vidal-Martínez, 2007	-	Panama		MF939878	[35]
<i>Heteropriapulius anchoradiatus</i> Acosta, Franceschini, Zago, Scholz & Silva, 2017	<i>Pterygoplichthys ambrosettii</i>	Brazil	MF116371		[7]
<i>Heteropriapulius heterotylus</i> Acosta, Franceschini, Zago, Scholz & Silva, 2017	<i>Pterygoplichthys ambrosettii</i>	Brazil	MF116370		[7]
<i>Heteropriapulius simplex</i> Acosta, Franceschini, Zago, Scholz & Silva, 2017	<i>Pterygoplichthys ambrosettii</i>	Brazil	MF116372		[7]
<i>Heteropriapulius</i> sp.	<i>Pterygoplichthys ambrosettii</i>	Brazil	MF116373		[7]

Table 3. Cont.

Monogenea Species	Host Species	Locality	Accession Numbers LSU rDNA	COI	References
<i>Jainus beccus</i> n. sp.	<i>Leporinus friderici</i>	Brazil		OQ833545	Present study
<i>Jainus piava</i> Karling, Bellay, Takemoto & Pavanelli, 2011	<i>Schizodon nasutus</i>	Brazil	OQ843019	OQ833543	Present study
<i>Jainus radixelongatus</i> n. sp.	<i>Leporinus striatus</i>	Brazil	OQ843018	OQ833544	Present study
<i>Nanayella aculeatrium</i> Acosta, Mendoza-Palmero, Silva & Scholz, 2019	<i>Sorubim lima</i>	Peru	KP056228		[6]
<i>Nanayella amplofalcis</i> Acosta, Mendoza-Palmero, Silva & Scholz, 2019	<i>Hemisorubim platyrhynchos</i>	Brazil	MG001325		[9]
<i>Nanayella fluctuatrium</i> Acosta, Mendoza-Palmero, Silva & Scholz, 2019	<i>Sorubim lima</i>	Brazil	MG001327		[9]
<i>Nanayella megorchis</i> Acosta, Mendoza-Palmero, Silva & Scholz, 2019	<i>Sorubim lima</i>	Peru	MK367407		[9]
<i>Nanayella processusclavis</i> Acosta, Mendoza-Palmero, Silva & Scholz, 2019	<i>Hemisorubim platyrhynchos</i>	Brazil	MG001328		[9]
<i>Parasciadicleithrum octofasciatum</i> Mendoza-Palmero, Blasco-Costa, Hernández-Mena & Pérez-Ponce de León, 2017	<i>Rocio octofasciata</i>	Mexico	KY305885		[36]
<i>Sciadicleithrum meekii</i> Mendoza-Palmero, Blasco-Costa, Hernández-Mena & Pérez-Ponce de León, 2017	<i>Thorichthys meeki</i>	Mexico	KY305889		[36]
<i>Sciadicleithrum mexicanum</i> Mendoza-Palmero, Blasco-Costa, Hernández-Mena & Pérez-Ponce de León, 2017	<i>Rocio octofasciata</i>	Mexico	KY305886		[36]
<i>Sciadicleithrum panamensis</i> Mendoza-Franco, Aguirre-Macedo & Vidal-Martínez, 2007	-	Panama		MF939864	[35]
<i>Sciadicleithrum splendidae</i> Kritsky, Vidal-Martínez & Rodríguez-Canul, 1994	<i>Parachromis friedrichsthalii</i>	Mexico	KY305890		[36]
<i>Trinigyryus anthus</i> Franceschini, Acosta, Zago, Müller & Silva, 2020	<i>Hypostomus regani</i>	Brazil	MN947622	MN916719	[37]
<i>Trinigyryus carvalhoi</i> Franceschini, Acosta, Zago, Müller & Silva, 2020	<i>Hypostomus ancistroides</i>	Brazil	MN947608	MN922321	[37]
<i>Trinigyryus peregrinus</i> Nitta & Nagasawa, 2016	<i>Pterygoplichthys ambrosettii</i>	Brazil	MN944890	MN913212	[37]
<i>Trinigyryus peregrinus</i> Nitta & Nagasawa, 2016	<i>Pterygoplichthys disjunctivus</i>	Japan	LC104308		[38]
<i>Unibarra paranoplatensis</i> Suriano & Incorvaia, 1995	<i>Aguarunichthys torosus</i>	Peru	KP056219		[6]
<i>Unilatus unilatus</i> Mizelle & Kritsky, 1967	<i>Pterygoplichthys ambrosettii</i>	Brazil	MF102106		[7]
<i>Urocleidoides cultellus</i> Mendoza-Franco & Reina, 2008	-	Panama		MF939848	[35]
<i>Urocleidoides digitabulum</i> Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020	<i>Megaleporinus elongatus</i>	Brazil	MT556796		[39]
<i>Urocleidoides indianensis</i> Oliveira, Silva, Vieira & Acosta, 2020	<i>Parodon nasus</i>	Brazil	OK482868		[40]
<i>Urocleidoides paradoni</i> Oliveira, Silva, Vieira & Acosta, 2020	<i>Parodon nasus</i>	Brazil	OK482867		[40]
<i>Urocleidoides paradoxus</i> Kritsky, Thatcher & Boeger, 1986	<i>Leporinus friderici</i>	Brazil	MT556795		[39]

Table 3. Cont.

Monogenea Species	Host Species	Locality	Accession Numbers LSU rDNA	COI	References
<i>Urocleidoides sinus</i> Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020	<i>Schizodon nasutus</i>	Brazil	MT556799		[39]
<i>Urocleidoides tenuis</i> Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020	<i>Parodon nasus</i>	Brazil	OK465455		[40]
<i>Urocleidoides uncinus</i> Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020	<i>Gymnotus inaequilabiatus</i>	Brazil	MT556798	MT594473	[39]
<i>Vanclaveveus janauacaensis</i> Kritsky, Thatcher & Boeger, 1986	<i>Pterodoras granulosus</i>	Peru	KP056247		[6]
<i>Walteriella conica</i> Mendoza-Palmero, Mendoza-Franco, Acosta & Scholz, 2019	<i>Brachyplatystoma juruense</i>	Peru	MK834513		[41]
<i>Walteriella ophiocirrus</i> Mendoza-Palmero, Mendoza-Franco, Acosta & Scholz, 2019	<i>Platystomatichthys sturio</i>	Peru	MK834515		[41]
Diplectanidae					
<i>Murraytrema pricei</i> Caballero, Bravo & Grocott, 1955	<i>Nibea albiflora</i>	China	DQ157672		[42]
<i>Pseudorhabdosynochus epinepheli</i> (Yamaguti, 1938)	<i>Epinephelus brunneus</i>	China	AY553622		[43]
<i>Pseudorhabdosynochus lantauensis</i> (Beverley-Burton & Suriano, 1981)	<i>Epinephelus brunneus</i>	China	AY553624		[43]

3. Results

3.1. Description of *Jainus beccus* n. sp.

(Figure 2).

(urn:lsid:zoobank.org:act:A6A834C5-E70E-45B3-8B05-08C3AC3332CD).

3.1.1. Taxonomic Summary

Type host: *Leporinus friderici* (Bloch, 1794) (Characiformes, Anostomidae).

Type locality: Sapucaí-Mirim River (20°29'38.38" S, 47°51'33.11" W), Upper Paraná River basin, São Paulo, Brazil.

Other hosts: *Leporinus octofasciatus* Steindachner, 1915; *Leporinus striatus* Kner, 1858 and *Megaleporinus elongatus* (Valenciennes, 1850) (Characiformes: Anostomidae).

Other locality: Jurumirim Reservoir, Parapanema River (23°12'17" S, 49°13'19" W), Upper Paraná River basin, São Paulo, Brazil.

Site of infection: Gill filaments.

Prevalence, mean intensity of infection. and range of intensity: Sapucaí-Mirim River: *L. friderici*: 31 infected fish of 40 analyzed (77.5%), 17.38 ± 3.07 (1–55); *L. octofasciatus*: 1 infected fish of 17 analyzed (5.88%), 1. Jurumirim Reservoir: *L. friderici*: 34 infected fish of 116 analyzed (29.31%), 8.06 ± 1.97 (1–51); *L. octofasciatus*: 8 infected fish of 15 analyzed (53.3%), 3.2 ± 0.7 (1–6); *L. striatus*: 1 infected fish of 25 analyzed (4%); *M. elongatus*: 12 infected fish of 30 analyzed (40%), 2.7 ± 0.5 (1–7).

Type-material: Holotype CHIOC (39959a); paratypes CHIOC (39959b, 39960–39964, 39965a, 39965b, 39966, 39967), vouchers CHIBB (703L–710L).

Molecular sequence data: A COI mtDNA (903 bp) partial sequence obtained from one monogenean specimen from *L. friderici* was deposited in GenBank under accession number OQ833545.

Etymology: The specific epithet is derived from Latin (*beccus* = bird's beak) and refers to the shape of the distal portion of the accessory piece.

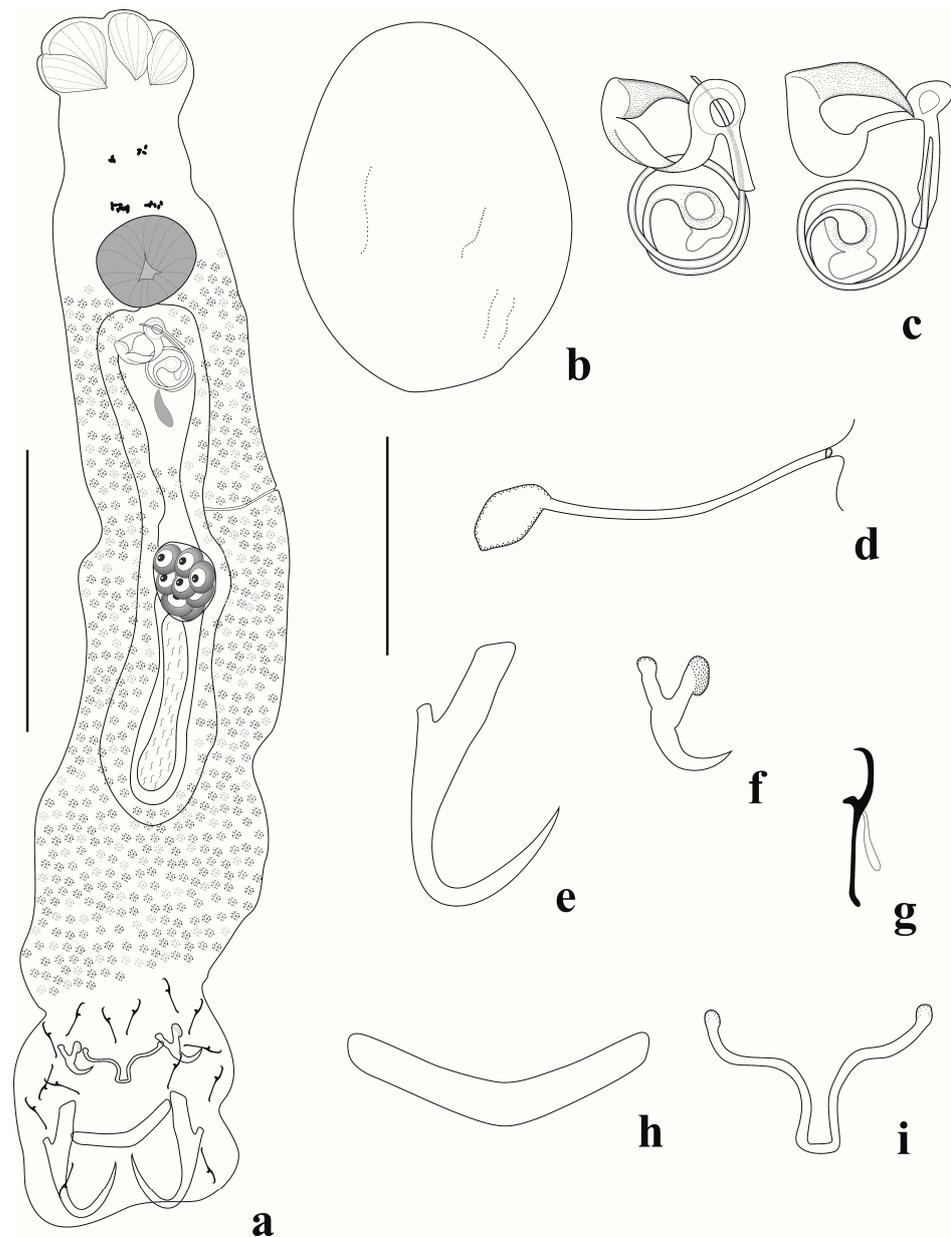


Figure 2. *Jainus beccus* n. sp. from *Leporinus friderici* (Characiformes, Anostomidae). (a) Whole mount (composite, ventral view); (b) egg; (c) copulatory complex (ventral view); (d) vagina; (e) dorsal anchor; (f) ventral anchor; (g) hook; (h) dorsal bar; (i) ventral bar (Scale bars: (a) = 50 μ m; (b–i) = 20 μ m).

3.1.2. Description (Based on 30 Specimens: 15 Mounted in Gray and Wess's Medium, and 15 Mounted in Hoyer's Medium)

Body elongate, fusiform 279 (207–400, $n = 28$) long; 58 (27–105, $n = 23$) wide near midlength. Cephalic lobes moderately developed, two pairs of head organs lying in cephalic lobes. Cephalic glands not visible. Four eyespots present; anterior pair smaller than posterior one; distance between eyespots of anterior pair smaller than that between posterior one; one member of each pair sometimes absent. Accessory granules are small, generally elongate, and frequently in the anterior trunk. Pharynx subspherical 16 (12–21, $n = 13$) wide; esophagus short. Peduncle short. Haptor sub-square, 41 (30–54, $n = 22$) long, 48 (36–55, $n = 24$) wide. Anchors dissimilar in shape and size. Ventral anchor 10 (8–11, $n = 25$) long, 6 (5–7, $n = 25$) wide, curved point and shaft, superficial root dilatated in distal portion and with grooves and elongated deep root. Dorsal anchor 27 (22–33, $n = 27$) long, 9 (7–11, $n = 27$) wide, with elongated curved point, straight and short shaft, deep root

poorly developed, superficial root elongated and rectangular. Ventral bar 10 (7–14, $n = 22$) long, 22 (17–30, $n = 24$) wide, delicate and filamentous. Dorsal bar 8 (5–11, $n = 24$) long, 25 (20–31, $n = 24$) wide, robust, V-shaped. Seven pairs of similar and marginal hooks, 12 (10–15, $n = 34$) long, with erect thumb, curved point, erect shank; filament hook (FH) loop half of shank length. Copulatory complex comprising a male copulatory organ (MCO) with two counterclockwise rings 10 (9–14, $n = 20$) in diameter, a sclerotized expanded and ornamented base, and an accessory piece 16 (13–19, $n = 18$) wide, well developed, with proximal portion formed by a concave rod that guides the MCO and a semicircular distal portion resembling a “bird’s beak”, not articulated to the MCO. Vagina simple, sinistral, comprising a delicate tube. Gonads intercaecal, in tandem. Germarium 17 (14–21, $n = 4$) long, 13 (9–17, $n = 4$) wide. Testis 42 (30–63, $n = 4$) long, 10 (6–14, $n = 4$) wide, elongated and posterior to germarium. Seminal vesicle a dilation of vas deferens. Seminal receptacle anterior to germarium. Prostatic reservoir present. Oviduct, ootype, and uterus not observed. Vitellaria densely dispersed throughout trunk, absent in reproductive organ regions. Egg 77 ($n = 1$) long, 47 ($n = 1$) wide, without filament.

3.1.3. Remarks

Jainus beccus n. sp. can be distinguished from other congeners by the morphology of the accessory piece (well developed with a proximal portion formed by a concave rod that guides the MCO, and the semicircular distal portion resembling a “bird’s beak”) and the ventral anchor (a superficial root dilatated in the distal portion, with grooves). The new species closely resembles *J. piava* and *J. leporini* by having a delicate ventral bar and dorsal bar curved or V-shaped. However, *Jainus beccus* n. sp. differs from these congeners by having a simple vagina (vagina associated with a plate-like sclerotized structure in *J. leporini*; vagina with terminal bulbous expansion in *J. piava*). Analyses of the paratypes of *J. leporini* described by Abdallah et al. [16] and holotypes and paratypes of *J. piava* described by Karling et al. [14] confirmed these resemblances and differences reported in the current study.

3.2. Description of *Jainus ornatus* n. sp.

(Figure 3).

(urn:lsid:zoobank.org:act:AE22AE7F-7474-43C0-8292-C8DB1B943081).

3.2.1. Taxonomic Summary

Type host: *Leporinus friderici* (Bloch, 1794) (Characiformes, Anostomidae).

Type locality: Jurumirim Reservoir, Paranapanema River (23°12'17" S, 49°13'19" W), Upper Paraná River basin, São Paulo, Brazil.

Site of infection: Gill filaments.

Prevalence, mean intensity of infection, and range of intensity: 8 infected fish of 116 analyzed (6.89%), 3.2 ± 0.79 (1–8).

Type-material: Holotype CHIOC (39968a); paratypes CHIOC (39968b–39968d, 39969); vouchers CHIBB (711L–719L).

Etymology: The specific epithet is derived from Latin (*ornatus* = adorned) and refers to the presence of an adorned root of the ventral anchor.

3.2.2. Description (Based on 23 Specimens Mounted in Hoyer’s Medium)

Body robust, fusiform, 1041 (774–1586, $n = 15$) long, and 429 (325–620, $n = 14$) wide near midlength. Cephalic lobes poorly developed; head organs and cephalic glands not observed. Four eyespots. Anterior pair is smaller than posterior one; one member of each pair sometimes absent. Accessory granules small, generally elongate, and frequently in anterior trunk. Pharynx subspherical 54 (41–64, $n = 16$) wide. Peduncle short.

Haptor sub-square, 76 (61–93, $n = 9$) long, 136 (118–157, $n = 9$) wide. Anchors dissimilar in shape and size. Ventral anchor 25 (23–27, $n = 8$) long, 19 (16–22, $n = 8$) wide, with deep and superficial roots variable in shape and bearing irregular sclerotization (like an ornament),

blade-like point and shaft. Dorsal anchor 23 (14–25, $n = 12$) long, 13 (11–14, $n = 12$) wide, with curved point and shaft, well-developed deep and superficial roots. Ventral bar 12 (7–19, $n = 8$) long, 42 (26–56, $n = 8$) wide, delicate, curved, and with enlarged ends. Dorsal bar 7 (6–7, $n = 5$) long, 36 (31–42, $n = 12$) wide, V-shaped. Seven pairs of similar and marginal hooks, 15 (14–16, $n = 26$) long, with erect thumb, curved point, arcuated shank; FH loop $\frac{3}{4}$ shank length. Copulatory complex comprising a MCO with one counterclockwise ring 15 (15–16, $n = 7$) in diameter, and accessory piece 59 (54–63, $n = 5$) long, 21 (20–24, $n = 6$) wide, robust, comprising two elongated and sclerotized subunits, both with sickle-shaped distal portions, articulated to the MCO. Gonads intercaecal, overlapping. Germarium 225 (136–313, $n = 3$) long, 59 (42–71, $n = 3$) wide. Testis 205 (155–315, $n = 5$) long, 65 (52–91, $n = 5$) wide, elongated, and dorsal to germarium. Vitellaria densely dispersed throughout trunk, absent in reproductive organ regions. Vagina, seminal vesicle, seminal receptacle, prostatic reservoir, oviduct, ootype, and uterus not observed. Egg 116 (74–154, $n = 7$) long, 89 (69–104, $n = 5$) wide, with filament.

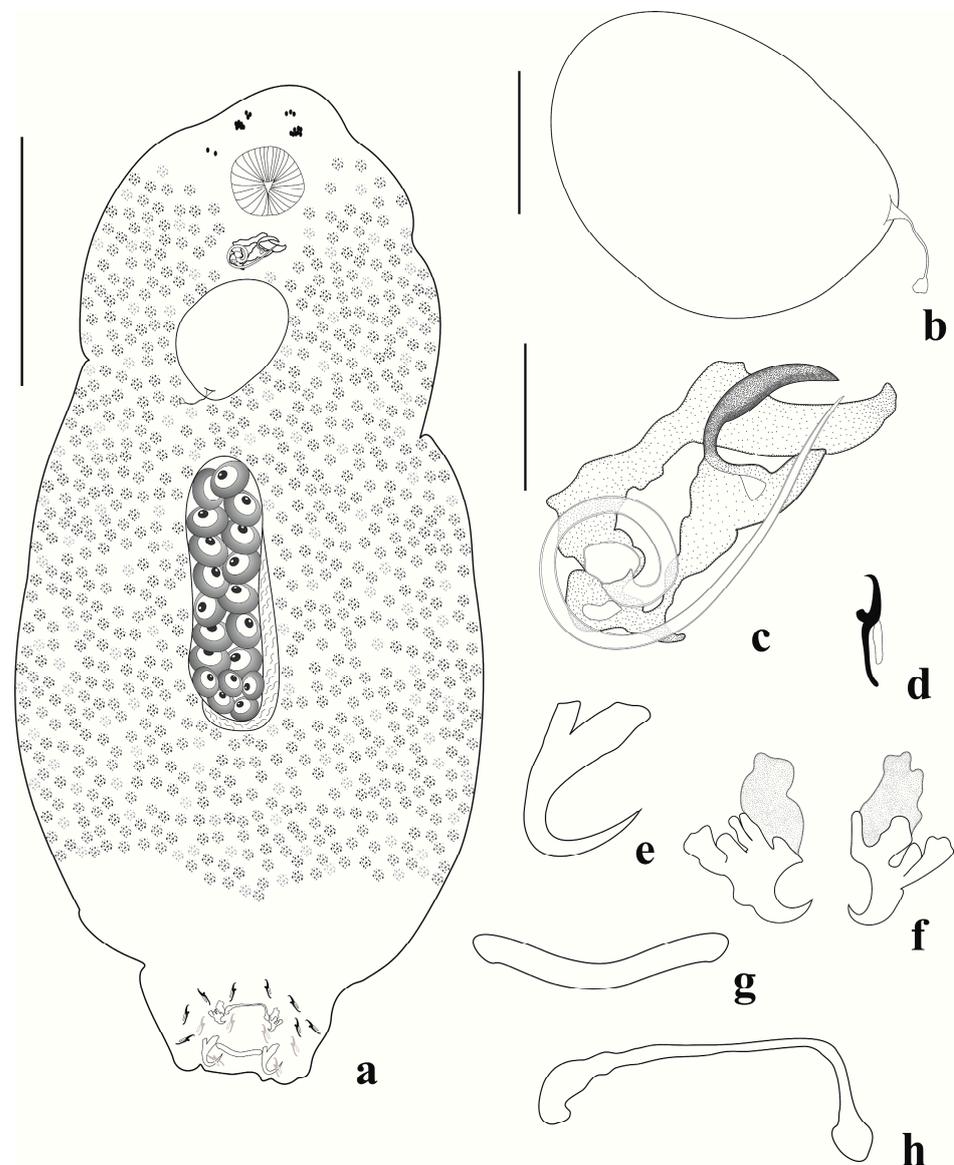


Figure 3. *Jainus ornatus* n. sp. from *Leporinus friderici* (Characiformes, Anostomidae). (a) Whole mount (composite, ventral view); (b) egg; (c) copulatory complex (ventral view); (d) hook; (e) dorsal anchor; (f) ventral anchors; (g) dorsal bar; (h) ventral bar (scale bars: (a) = 200 μm ; (b) = 50 μm ; (c–h) = 20 μm).

3.2.3. Remarks

Jainus ornatus n. sp. can be distinguished from most of its congeners by the morphology of the accessory piece (comprising two elongated and sclerotized subunits, both with sickle-shaped distal portions) and ventral anchor (with deep and superficial roots variable in shape and bearing irregular sclerotization, blade-like point, and shaft). Based on the shape of the ventral anchor, *Jainus ornatus* n. sp. closely resembles *J. iocensis* by possessing roots with irregular sclerotization. However, the new species differs from *J. iocensis* by having a delicate ventral bar with enlarged ends (V-shaped and with a posteromedian projection in *J. iocensis*), and an accessory piece with two elongated and sclerotized subunits, both with a sickle-shaped distal portion (hook-shaped in *J. iocensis*). Examination of paratypes of *J. iocensis* described by Cohen et al. [15] confirmed these resemblances and differences reported in the current study.

3.3. Description of *Jainus radixelongatus* n. sp.

(Figure 4).

(urn:lsid:zoobank.org:act:7B431077-6265-4DA9-9D04-20CA031BD673).

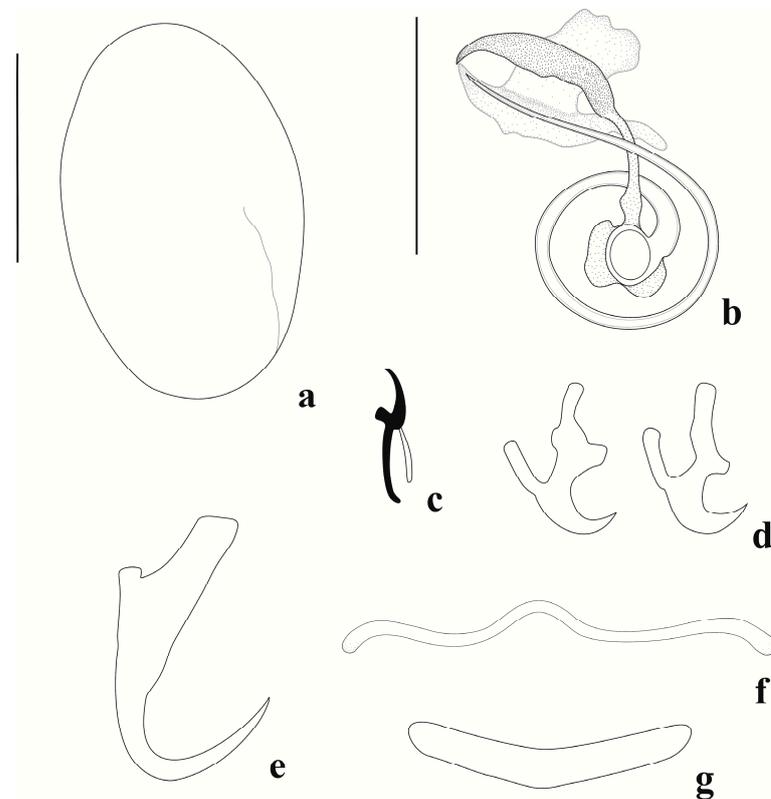


Figure 4. *Jainus radixelongatus* n. sp. from *Leporinus striatus* (Characiformes, Anostomidae). (a) Egg; (b) copulatory complex (ventral view); (c) hook; (d) ventral anchor; (e) dorsal anchor; (f) ventral bar; (g) dorsal bar (scale bars: (a) = 50 μ m; (b–g) = 20 μ m).

3.3.1. Taxonomic Summary

Type host: *Leporinus striatus* Kner, 1858 (Characiformes: Anostomidae).

Type locality: Jurumirim Reservoir, Paranapanema River (23°12'17" S, 49°13'19" W), Upper Paraná River basin, São Paulo, Brazil.

Other hosts: *Leporinus friderici* (Bloch, 1794), *Leporinus octofasciatus* Steindachner, 1915, and *Megaleporinus elongatus* (Valenciennes, 1850) (Characiformes: Anostomidae).

Other locality: Sapucaí-Mirim River (20°29'38.38" S, 47°51'33.11" W), Upper Paraná River basin, São Paulo, Brazil.

Site of infection: Gill filaments.

Prevalence, mean intensity of infection, and range of intensity: Jurumirim Reservoir: *L. striatus*: 6 infected fish of 25 analyzed (24%), 2 ± 0.5 (1–4); *L. octofasciatus*: 6 infected fish of 15 analyzed (40%), 2.1 ± 0.4 (1–4). Sapucaí-Mirim River: *L. friderici*: 27 infected fish of 40 analyzed (67.5%), 5.53 ± 1.31 (1–30); *L. octofasciatus*: 7 infected fish of 17 analyzed (41.18%), 2 ± 0.54 (1–4); *M. elongatus*: 1 infected fish of 22 analyzed (4.55%), 4.

Type-material: Holotype CHIOC (39970a); paratypes CHIOC (39970b, 39971, 39972); vouchers CHIBB (720L, 721L).

Molecular sequence data: LSU rDNA (1369 bp) and COI mtDNA (895 bp) partial sequences obtained of the same monogenean specimen from *L. striatus* were deposited in GenBank under accession numbers OQ843018 and OQ833544, respectively.

Etymology: The specific name derives from Latin (*radix*= root) and refers to the large size of the superficial and deep roots of the ventral anchor.

3.3.2. Description (Based on Nine Specimens Mounted in Gray and Wess's Medium)

Body elongate, fusiform, 547 (415–651, $n = 4$) long, and 73 (34–100, $n = 3$) wide near midlength. Cephalic lobes poorly developed. Cephalic glands not visible. Four eyespots present. Anterior pair is smaller than posterior pair; distance between eyespots of anterior pair smaller than posterior one; one member of each pair sometimes absent. Accessory granules elongate, frequently in anterior trunk. Pharynx subspherical 19 (13–24, $n = 7$) wide. Peduncle short. Haptor sub-square, 50 (34–69, $n = 4$) long, 48 (40–54, $n = 4$) wide. Anchors dissimilar in shape and size. Ventral anchor 14 (13–14, $n = 8$) long, 8 (6–9, $n = 8$) wide, short point and shaft, superficial and deep root elongated, with small protuberance on the inner surface of base. Dorsal anchor 24 (23–25, $n = 7$) long, 9 (7–12, $n = 7$) wide, with elongate curved point, short and curved shaft, poorly developed deep root, elongated and square-like superficial root. Ventral bar 10 (6–12, $n = 4$) long, 25 (18–32, $n = 6$) wide, delicate, filamentous, and enlarged ends. Dorsal bar 7 (5–11, $n = 7$) long, 24 (17–27, $n = 7$) wide, broadly V-shaped. Seven pairs of marginal and similar hooks, 12 (10–13, $n = 23$) long, with erect thumb, slightly curved point, arcuated shank, and FH loop half of shank length. Copulatory complex comprising a MCO with $1\frac{1}{2}$ counterclockwise rings 12 (11–13, $n = 9$) in diameter; and an accessory piece 29 (26–32, $n = 9$) long, 12 (9–16, $n = 9$) wide, robust, composed of two subunits: one ventral and more sclerotized, sickle-shaped, and another dorsal with three projections (proximal projection elongated, middle projection enlarged, and lateral, distal projection, spine-shaped), articulated with base of the MCO. Vagina simple, sinistral. Prostatic reservoir present. Oviduct, ootype, and uterus not observed. Vitellaria densely dispersed throughout trunk. Egg 95 ($n = 1$) long, 61 ($n = 1$) wide, without filament.

3.3.3. Remarks

Jainus radixelongatus n. sp. can be distinguished from its congeners mainly by the morphology of the accessory piece, which is composed of two subunits: one ventral and more sclerotized and sickle-shaped, and another dorsal with three projections (proximal projection, elongated; middle projection, enlarged and lateral; distal projection, spine-shaped). The morphologies of these subunits of the accessory piece are markedly different from those observed in *Jainus ornatus* n. sp. The new species resembles *J. amazonensis* by possessing a ventral anchor with superficial and deep roots with elongated termination; however, these species differ by the morphology of the dorsal bar (V-shaped in *Jainus radixelongatus* n. sp. and simple and arcuate in *J. amazonensis*) and the delicate ventral bar, being filamentous with enlarged ends in the new species and broadly V-shaped with small medial projection in *J. amazonensis*). Examination of paratypes of *J. amazonensis* described by Kritsky et al. [13] and deposited in the zoological collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Amazonas, Brazil, confirmed these resemblances and differences reported in the current study. There were not appropriate specimens for a whole-mount illustration.

3.4. Supplementary Observations of *Jainus piava* Karling, Bellay, Takemoto & Pavanelli, 2011 (Figure 5).

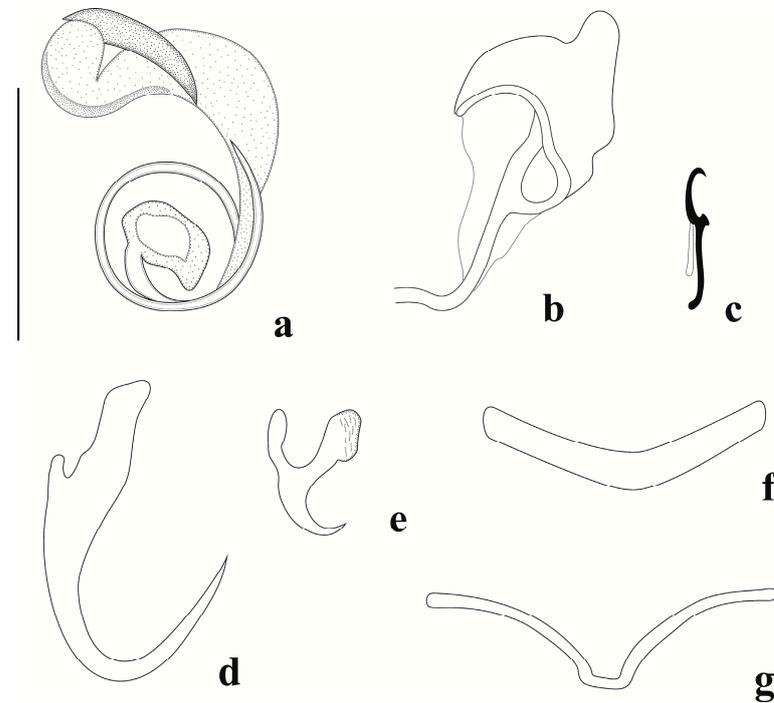


Figure 5. *Jainus piava* from *Schizodon nasutus* (Characiformes, Anostomidae). (a) Copulatory complex (ventral view); (b) vagina; (c) hook; (d) dorsal anchor; (e) ventral anchor; (f) dorsal bar; (g) ventral bar (Scale bars: (a–g) = 20 μ m).

3.4.1. Taxonomic Summary

Type host: *Schizodon borellii* (Boulenger, 1900) (Characiformes, Anostomidae).

Type-locality: Upper Paran river floodplain, Brazil (2250'–2270' S, 5315'–5340' W).

Current records: *Schizodon nasutus* Kner, 1858 (Characiformes, Anostomidae) from Jurumirim Reservoir (2312'17" S, 4913'19" W) and Sapuca-Mirim River (2029'38.38" S, 4751'33.11" W), Upper Paran river basin, So Paulo, Brazil.

Site of infection: Gill filaments.

Prevalence, mean intensity of infection, and range of intensity: Jurumirim Reservoir: 10 infected fish of 30 analyzed (33.3%), 3.4 ± 0.9 (1–9). Sapuca-Mirim River: 12 infected fish of 40 analyzed (30%), 2.86 ± 0.63 (1–7).

Specimens deposited: vouchers CHIOC (39973a, 39973b, 39974) and CHIBB (722L–724L).

Molecular sequence data: LSU rDNA (1377 bp) and COI mtDNA (651 bp) partial sequences, obtained from the same specimen, were deposited in GenBank under accession numbers OQ843019 and OQ833543, respectively.

3.4.2. Supplementary Observations (Based on 30 Specimens Mounted in Gray and Wess's Medium)

Body elongate, fusiform 390 (221–642, $n = 29$) long; 85 (40–170, $n = 26$) wide near midlength. Two pairs of cephalic lobes; two pairs of head organs; cephalic glands not visible. Four eyespots; anterior pair smaller than the posterior one; distance between the eyespots of the anterior pair smaller than the posterior one. Accessory granules small, generally elongate, present in some specimens in the cephalic area. Pharynx subspherical, 19 (13–27, $n = 25$) wide; esophagus short. Peduncle short. Haptor sub-square, 52 (34–68, $n = 25$) long, and 47 (34–72, $n = 25$) wide. Anchors dissimilar in shape and size. Ventral anchor 10 (8–11, $n = 28$) long and 7 (6–9, $n = 28$) wide. It had a short point and shaft, an elongate deep root, a superficial root with a striate, and expanded terminal extension.

Dorsal anchor 24 (22–26, $n = 30$) long and 9 (7–10, $n = 30$) wide, with an elongate curved point, incurved shaft, poorly developed deep root, and elongate superficial root. Ventral bar 9 (6–13, $n = 22$) long and 21 (12–28, $n = 24$) wide, delicate, and variable in shape. Dorsal bar 6 (4–8, $n = 27$) long, 23 (20–28, $n = 30$) wide, and arcuated. Seven pairs of similar and marginal hooks, 11 (11–12, $n = 24$) long, with erect thumb, curved point, and erect shank with slightly curved distal portion; FH loop $\frac{3}{4}$ shank length. Copulatory complex comprising a MCO with $1\frac{1}{2}$ counterclockwise rings 12 (9–13, $n = 26$) in diameter, a sclerotized expanded base, and an accessory piece 21 (16–24, $n = 28$) long, well developed, variable in shape, not articulated to the MCO. Vagina sinistral, marginal, sclerotized, adorned, and connected to the seminal receptacle. Gonads intercaecal and overlapping. Germarium elongate, 59 ($n = 1$) long, and 12 ($n = 1$) wide. Testis elongate, dorsal to germarium, 86 ($n = 1$) long, and 46 ($n = 1$) wide. Seminal vesicle as a dilation of the vas deferens. Seminal receptacle anterior to the germarium. Prostatic reservoir present. Oviduct, ootype, and uterus not observed. Vitellaria densely dispersed throughout trunk, absent in reproductive organ regions.

3.4.3. Remarks

Jainus piava was described by Karling et al. [14] in the gills of *S. borellii*. Since then, its occurrence in several other host species has been reported. Analyzing the holotype and paratypes (CHIOC 37235A and CHIOC 37236, 37235B–C, respectively) we can confirm the identity of the specimens found in *S. nasutus* as *J. piava*. Nonetheless, comparative analyses of the morphology of the specimens analyzed for the original description and that found in the present study revealed differences not reported in the original description, such as the presence of a thin and delicate ventral bar, with great variation in its shape; a ventral anchor presenting a striated region at the end of the superficial root; an accessory piece not articulated with MCO base; and the ends of the accessory piece being smooth and not present as many undulations, as in the drawing of the original description.

3.5. Molecular Analyses

We successfully obtained two LSU rDNA partial sequences from specimens of *Jainus radixelongatus* n. sp. (GenBank accession number OQ843018; length of 1369 bp) and of *Jainus piava* (GenBank accession number OQ843019; length of 1377 bp) and three COI mtDNA partial sequences from specimens of *Jainus radixelongatus* n. sp. (GenBank accession number OQ833544; length of 895 bp), *Jainus beccus* n. sp. (GenBank accession number OQ833545; length of 903 bp), and *J. piava* (GenBank accession number OQ833543; length of 651 bp).

For the LSU rDNA gene, a dataset was constructed including the newly generated sequences of *Jainus* spp., 46 sequences of the Dactylogyridae retrieved from GenBank and 3 sequences of the Diplectanidae (used as outgroup) (Table 3). The final alignment was 1472 bp long considering the longest newly generated sequence (*J. piava*OQ843019). The BI and ML phylogenetic analyses of the LSU rDNA produced phylograms with similar topologies to most clades supported (Figure 6). In both analyses, *Jainus* was recovered as monophyletic; *Jainus radixelongatus* n. sp. and *J. piava* were grouped together in a monophyletic clade (pp = 1; bootstrap = 100), closely related to *Trinigyryrus* spp., *Unilatus unilatus* Mizelle & Kritsky, 1967 (MF 1021106), and *Heteropriapulid* spp., which are described as parasites of fishes belonging to Loricariidae (Siluriformes).

For the COI mtDNA gene, we constructed a dataset including the newly generated sequences of *Jainus* spp. and nine sequences of the Dactylogyridae retrieved from GenBank, and *Sciadicleithrum panamensis* Mendoza-Franco, Aguirre-Macedo & Vidal-Martínez, 2007 (MF939864) was used as the outgroup (Table 3). The BI and ML analyses of the partial COI mtDNA alignment also produced phylograms with similar topologies and most supported clades (Figure 7). The newly generated sequences of *Jainus radixelongatus* n. sp., *Jainus beccus* n. sp., and *J. piava* clustered in a monophyletic clade (pp = 0.88; bootstrap = 78), with *Urocleidoides* spp. and *Diaphorocleidus* spp. as sister groups (not supported by any of the analyses). The position of *Trinigyryrus* clade appeared unresolved.

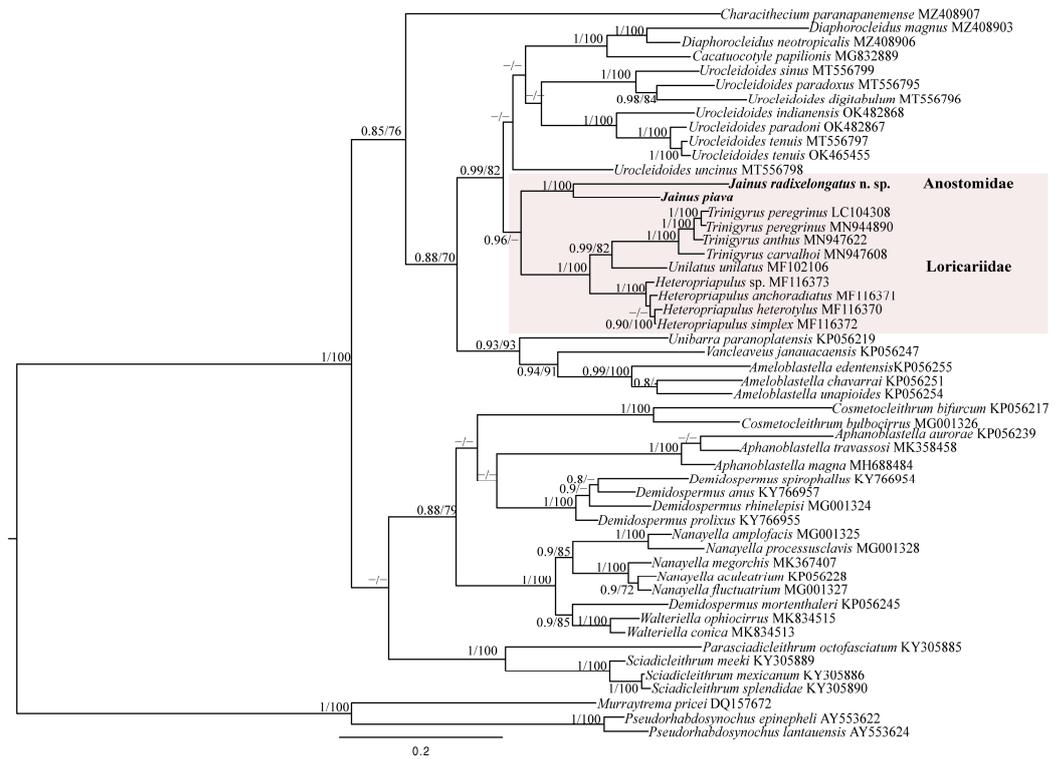


Figure 6. Bayesian topology based on partial LSU rDNA sequences of selected monogenean species. *Murraytrema pricei* and *Pseudorhabdosynochus* spp. were used as the outgroup. GenBank accession numbers are after species names. Newly sequenced species are in bold. The support values are included above the nodes as follows: posterior probabilities for BI analyses, followed by bootstrapping for the ML analyses (posterior probabilities < 0.70 and bootstrap scores < 70 are not shown and are represented by dashes). The branch-length scale bar indicates the number of substitutions per site.

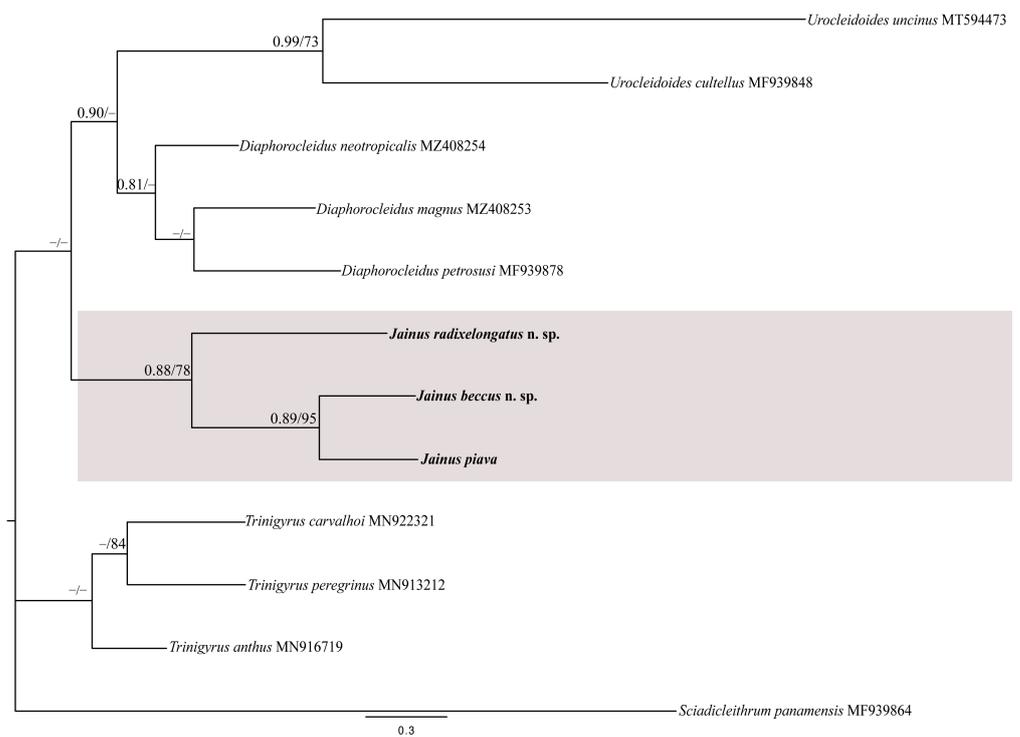


Figure 7. Bayesian topology based on partial COI mtDNA sequences of dactylogrydids from the neotropical region. *Sciadicleithrum panamensis* was used as the outgroup. GenBank accession numbers

are after species names. Newly sequenced species are in bold. The support values are included above the nodes as follows: posterior probabilities for BI, followed by bootstraps for the ML analyses (posterior probabilities < 0.70 and bootstrap scores < 70 are not shown and are represented by dashes). The branch-length scale bar indicates the number of substitutions per site.

The genetic divergence found in the LSU rDNA partial sequences between *Jainus radixelongatus* n. sp. and *J. piava* was 14.3% (213 bp), and the genetic divergence in the COI mtRNA partial sequences ranged from 14.6% (54 bp—*J. piava* versus *J. beccus* n. sp.) to 21.8% (80 bp—*J. piava* versus *J. radixelongatus* n. sp.).

4. Discussion

The new species proposed here were erected with support by a combination of the differences observed in the morphological and molecular data among *Jainus* spp. To date, nine species of *Jainus* (including the three new species here proposed) were described in Brazil. Only one, *Jainus hexops* Kritsky & Leiby, 1972, was reported to parasitize *Astyanax fasciatus* (Cuvier, 1819) (Characidae) from Puntarenas Province, Costa Rica. Species of this genus were reported to parasitize mainly characiforms belonging to Chalceidae, Anostomidae, Iguanodectidae, and Bryconidae [12–16,44].

In the present study, the monogenean species recovered from the gills of the anostomid fishes in the southeast of Brazil are allocated to *Jainus* due to their possession of a robust body with a poorly developed peduncle; one testis and one ovary in tandem or overlapping; a modified ventral anchor; a vaginal sinistral (not observed in *Jainus ornatus* n. sp.); and a male copulatory organ that is nonarticulated proximally (observed in *Jainus beccus* n. sp.) (see [12]). In addition, as emended by Karling et al. [14] they include: a male copulatory organ and accessory piece articulated proximally (observed in *Jainus ornatus* n. sp. and *Jainus radixelongatus* n. sp.); and a male copulatory organ with coils (observed in all new species described herein) or not. In general, *Jainus* spp. presents a robust accessory piece that is variable in shape; a coiled MCO; a delicate or filamentous ventral bar; a V-shaped dorsal bar; a delicate ventral anchor with modified deep and superficial roots; similar hooks; and a simple vagina. However, *J. hexops* runs outside of these morphological patterns by presenting a different haptor hook and a sinuous MCO.

To date, there is only one sequence of *Jainus* spp. available in GenBank—that of *J. hexops* (OM397908)—which was not used in the final alignment because it was too short (299 bp) for the chosen alignment criteria. Nevertheless, we included it and tested it in previous alignments (not shown here); however, we obtained inconclusive results, since this sequence was not placed together with the other sequences of *Jainus*. The sequence identified as that of *J. hexops* (OM397908) might not belong to the genus *Jainus*; therefore, we recommend a revision of this species and an improvement in the sequence length to a better fit for future alignment and analyses. According to the phylogeny of the LSU, *Jainus* spp. (recovered from fishes belonging to Anostomidae, Characidae, and Bryconidae) are sister taxa to *Trinigyryrus* spp., *Unilatus unilatus* (MF 1021106), and *Heteropriapulius* spp. (recovered from fishes belonging to Loricariidae). This relationship is not well defined, since there is no synapomorphy in the morphology. Therefore, more studies should be carried out in the future to confirm its relationship.

Our results described three new species of *Jainus* based on morphological and molecular data (partial LSU rDNA and COI mtDNA genes) and supplementary data on the morphology of *J. piava*. We provide the first phylogenetic study based on LSU rDNA and COI mtDNA gene sequences for species of this genus, improving and clarifying the understanding of host–parasite relationships in neotropical characiforms.

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