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Trinigyrus spp. (Monogenea: Dactylogyridae) from Brazilian catfishes: new species, molecular data and new morphological contributions to the genus

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Research Paper

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Abstract

This study describes two new species, *Trinigyrus anthus* n. sp. and *Trinigyrus carvalhoi* n. sp., from gills of *Hypostomus* spp. from the Upper Paraná River basin, Brazil. *Trinigyrus peregrinus* is redescribed based on examination of its holotype, paratypes and new material of specimens parasitizing *Pterygoplichthys ambrosettii*, also from the Upper Paraná River basin, Brazil. New morphological features were included in the diagnosis of the genus, such as the presence of a sclerotized border on the anchor base, and a weakly sclerotized fringe on the base of the male copulatory organ (MCO). *Trinigyrus anthus* n. sp. differs from other congeners by the shape of the MCO, presenting an enlarged base with sclerotized fringes resembling flower petals. *Trinigyrus carvalhoi* n. sp. and *T. peregrinus* are similar but can be differentiated from each other mainly by the sclerotization of the vagina (absent in the new species), and the morphology of the MCO (C-shaped versus one counterclockwise circle, respectively). For the first time, gene sequences of *Trinigyrus* spp. from Brazil were obtained (partial ribosomal 28S and mitochondrial cytochrome *c* oxidase I (mtCOI)). The genetic divergences among the new species and *T. peregrinus* varied from 2 to 3% (6–18 pb) based on sequences of 28S ribosomal DNA (rDNA), and 6–7% (83–92 pb) using mtCOI. Phylogenetic analyses based on partial 28S rDNA revealed that *Trinigyrus*, *Heteropriapulus* and *Unilatus* formed a monophyletic and well-supported clade of monogeneans from Neotropical freshwater loricariids, suggesting a closer relationship among these dactylogyrids and their hosts.

Introduction

Siluriformes is considered one of the world's oldest fish groups (Mo, 1991). The biogeographical evolution of this group is undetermined, but it is highly probable that the marine forms evolved from freshwater forms (Nelson, 2006) and carried with them their respective parasites. To date, Siluriformes consists of approximately 39 families and over 6700 valid living species distributed in freshwater, brackish and marine environments in every continent of the world (Eschmeyer *et al.*, 2019), except in Antarctica where they have been present in the past (Grande & Eastman, 1986).

Trinigyrus Hanek, Molnar & Fernando, 1974 comprises gill parasites of loricariid fishes from the Neotropical region (Boeger & Belmont-Jégu, 1994; Nitta & Nagasawa, 2016). Currently, the genus includes five species: *Trinigyrus hypostomatis* Hanek, Molnar & Fernando, 1974, described as a parasite of *Hypostomus robinii* Valenciennes, 1840, *Trinigyrus tentaculoides* Kritsky, Boeger & Thatcher, 1986 from *Hypoptopoma thoracatum* Günther, 1868, *Trinigyrus acuminatus* Kritsky, Boeger & Thatcher, 1986 from *Acanthicus hystrix* Spix & Agassiz, 1829, *Trinigyrus mourei* Boeger & Belmont-Jégu, 1994 parasitizing *Squaliforma emarginata* (Valenciennes, 1840) [=*Hypostomus emarginatus*] and *Trinigyrus peregrinus* Nitta & Nagasawa, 2016 from *Pterygoplichthys disjunctivus* (Weber, 1991). Most of the species described were found parasitizing fishes from the municipality of Manaus, Amazonas State, Brazil (*T. tentaculoides*, *T. acuminatus* and *T. mourei*). The type species, *T. hypostomatis*, is naturally distributed in the Talparo River, Trinidad, whereas *T. peregrinus* was introduced in Okinawa-Jima Island, Japan, with its respective alien host, the vermiculated sailfin catfish *P. disjunctivus*. The occurrence of *T. hypostomatis* was also reported in China, parasitizing the gills of the alien fish *Hypostomus plecostomus* (Linnaeus, 1758) from the Pearl River water system, in the municipality of Guangzhou, Guangdong Province (Li & Huang, 2012).

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As part of our long-term studies of the biodiversity of fish parasites from the tributaries of the Upper Paraná River basin in Brazil, two new species of *Trinigyrus* are described from loricariids, supported by morphological and molecular data. New morphological features are added to the diagnosis of the genus. *Trinigyrus peregrinus* is redescribed based on morphological discrepancies found among the original description and the specimens deposited as holotype and paratypes, as well as new specimens collected for this study. For the first time, gene sequences of *Trinigyrus* spp. from Brazil were obtained (partial ribosomal 28S and mitochondrial cytochrome *c* oxidase I (mtCOI)). The phylogenetic relationships among *Trinigyrus* and other monogenean parasites of siluriforms are also evaluated, including sequences of *Hamatopeduncularia* spp. parasites of marine siluriforms, which were previously considered as closely related to *Trinigyrus* spp. by Kritsky *et al.* (1986).

Material and methods

Host sampling and parasitological procedures

We collected 276 specimens of loricariids, from which we extracted 1261 monogenean specimens belonging to *Trinigyrus*. The analysed hosts were as follows: 23 specimens of *Hypostomus margaritifer* (Regan, 1908), 50 specimens of *Hypostomus regani* (Ihering, 1905), 50 specimens of *Hypostomus strigaticeps* (Regan, 1908), 50 specimens of *Hypostomus ancistroides* (Ihering, 1911) and 23 specimens of a new species belonging to *Hypostomus* (C.H. Zawadzki, pers. obs., description in progress), all commonly named as 'suckermouth catfishes'. The *Hypostomus* spp. specimens were collected between March 2012 and December 2013 in the reservoirs of three small hydroelectric power plants (ANEEL, 2008): Palmeiras (20° 32′57.33″S, 47°48′47.26″W), Anhanguera (20°29′38.38″S, 47° 51′33.11″W) and Retiro (20°26′12.5″S, 47°53′18.59″W), in the Sapucaí-Mirim River, a tributary of the Grande River (Upper Paraná River basin), municipality of São Joaquim da Barra, São Paulo State, Brazil. Eighty specimens of *Pterygoplichthys ambrosettii* (Holmberg, 1893), commonly known as 'airplane catfish', were collected in the mouth of the Aguapeí River (21°3′36.20″S, 51°45′38.58″W), a tributary of the Paraná River, municipality of Castilho, São Paulo State, from August 2013 to November 2014. Fishes were collected using a nylon monofilament gill net, under the Permanent License for the Collection of Zoological Material (SISBio 13794-1 and IBAMA 577/2015). The specimens were euthanized by a section of spinal cord, stored individually in plastic bags and placed in a Styrofoam box with ice for transportation to the laboratory where they were necropsied.

The gills were removed and analysed fresh when possible or placed in vials containing hot water (~60°C), shaken to detach the monogeneans of the gill filaments and then absolute ethanol was added to produce a final concentration of 70% ethanol (Boeger & Vianna, 2006). The monogeneans were collected using a stereomicroscope and some specimens were mounted in Hoyer's medium, Gray and Wess' medium or glycerine and picric acid (GAP) to observe sclerotized structures, whereas others were stained with Gömöri trichrome and mounted in permanent slides using Canada balsam for analysis of the internal organs (Ergens, 1969; Humason, 1979; Kritsky *et al.*, 1986). In addition, some specimens of monogeneans obtained from fresh preparation were selected for the molecular analyses (see *Molecular analyses* section).

Morphometrical and morphological analyses were performed with a computerized image analysis system with differential interference contrast (Leica Application Suite, version 3; Leica Microsystems, Wetzlar, Germany). All measurements are presented in micrometres (µm) and expressed as mean, followed by the range and number of specimens measured (*n*) in parentheses. Measurements of some sclerotized structures (bar, anchors and male copulatory complex) were performed according to the scheme shown in fig. 1, and others were taken in accordance with Mizelle & Klucka (1953). Illustrations of the sclerotized structures were obtained with the aid of a camera lucida mounted on a Leica DMLS microscope with phase contrast optics. The prevalence and mean intensity of infestation were calculated according to Bush *et al.* (1997).

Voucher specimens of the fish hosts were deposited in the Ichthyological Collection of the Limnology, Ichthyology and Aquaculture Research Center (NUP) of the State University of Maringá, Paraná State, Brazil. Holotypes and paratypes of the proposed new species were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro State, Brazil. Additional vouchers were deposited in the Helminthological Collection of the Department of Parasitology, Institute of Biosciences, São Paulo State University – UNESP (CHIBB), in the municipality of Botucatu, São Paulo State, Brazil. For comparative purposes, the slides of the holotypes and paratypes of the following species of *Trinigyrus* were examined: *T. mourei* (CHIOC 33052), *T. acuminatus* (National Institute of Amazon Researches – INPA 110 b–c; *T. tentaculoides*: INPA 111–112) and *T. peregrinus* (National Museum of Nature and Science from Japan – NSMT-P1 6196–6203). Additionally, photomicrographs of the holotype of *T. peregrinus* (NSMT-Pl 6195) and paratypes of species of *Trinigyrus* deposited in the Smithsonian US National Museum Helminthological Collection (USNM; *T. acuminatus*: catalogue number 1374541; *T. hypostomatis*: catalogue number 1368749–50; *T. mourei*: catalogue number 1374541; *T. tentaculoides*: catalogue number 1374540) were examined. Scientific names of the hosts follow Froese & Pauly (2019).

Molecular analyses

To confirm parasite identity, each specimen was mounted on a slide with glycerine or a drop of water, covered with a coverslip and photographed. Following morphological identification, specimens were removed from the slide and placed into 96% molecular-grade ethanol for molecular analysis. Conspecific specimens (paragenophores, according to Pleijel *et al.*, 2008) were mounted in Gray and Wess' or Hoyer's medium and deposited in CHIBB (556L, 566L and 569–575L). Total genomic DNA was extracted using the Qiagen Dneasy® Blood and Tissue Kit (Qiagen, California, USA) according to the manufacturer's protocol, and adjusted to a final volume of 30 µl. Partial ribosomal (28S, with divergent domains (D1–D3)) and mtCOI genes were amplified according to the procedures of Mendoza-Palmero *et al.* (2015) and Plaisance *et al.* (2008), respectively. Polymerase chain reaction (PCR) amplifications were performed containing 5 µl of DNA extract, 0.5 µl of each PCR primer and 19 µl of ultrapure water (Sigma, Aldrich, UK), using Ready-to-Go PCR beads (Pure Taq™ Ready-to-Go™ beads, GE Healthcare, Chicago, USA), with a final volume of 25 µl. The thermocycling profile for 28S was an initial denaturation of DNA at 94°C for 3 min, followed by 34 cycles of amplification at 94°C for 30 s, 56°C for 30 s and 72°C for 1.5 min, and a final extension at 72°C for 7 min (Mendoza-Palmero *et al.*, 2015); for mtCOI, it was an initial denaturation of DNA at 94°C for 3 min, followed by

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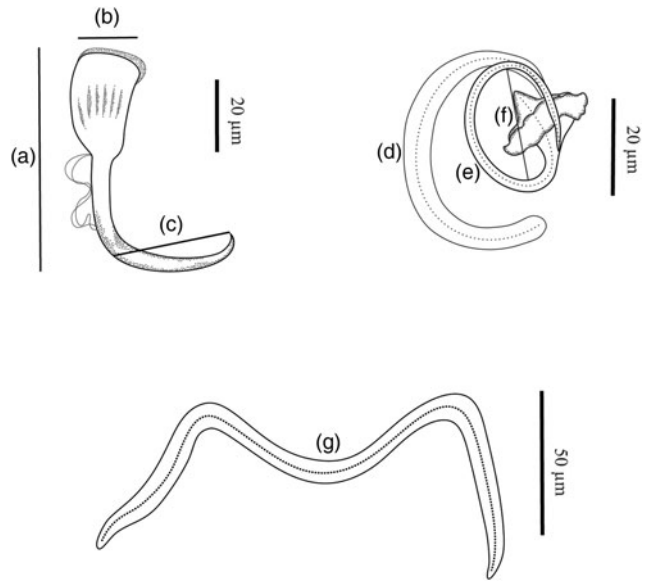

Fig. 1. Scheme of measurements for the species of *Trinigyrus* from this study: (a) anchor length; (b) anchor base width; (c) point length; (d) accessory piece length; (e) male copulatory organ (MCO) length; (f) diameter of the MCO; (g) bar length.

35 cycles of amplification at 94°C for 30 s, 44°C for 30 s and 72°C for 2 min, and a final extension at 72°C for 7 min (Plaisance *et al.*, 2008). Primers used for amplification and sequencing of partial 28S ribosomal DNA (rDNA) fragments were U178 (5′-GCACCCGCTGAAYTTAAG-3′) and L1642 (5′-CCAGCGCCATCCATTTTCA-3′) (Lockyer *et al.*, 2003), and L1200R (5′-GCATAG TTCACCATCTTTCGG-3′) for sequencing (Littlewood *et al.*, 2000). For amplification and sequencing of mtCOI, the primers used were COI_Mono_5: 5′-TAATWGGTGGKTTTGGTAA-3′ and COI_Mono_3: 5′-TAATGCATMGGAAAAAAACA-3′ (Plaisance *et al.*, 2008). PCR products were run on an agarose gel using GelRed™ (Biotium, Hayward, USA), and loading buffer and purified using the QIAquick PCR Purification Kit (Qiagen, California, USA). Automated sequencing in both directions was performed directly on the purified PCR products using the BigDye version 3.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA). Sequences were read on an Applied Biosystems ABI 3500 DNA genetic analyser. Contiguous sequences were assembled with Sequencher™ version 5.2.4 (Gene Codes, Ann Arbor, Michigan, USA) and submitted to GenBank (accession numbers presented in table 1).

Phylogenetic analyses

Six newly generated sequences of partial genes (three sequences of 28S rDNA and three sequences of mtCOI) were aligned with sequences obtained previously from monogeneans of catfishes (table 1). *Murraytrema pricei* Bychowsky & Nagibina, 1977 (DQ157672), *Pseudorhabdosynochus lantauensis* (Beverley-Burton & Suriano, 1981) Kritsky & Beverley-Burton, 1986 (AY553624) and *Pseudorhabdosynochus epinepheli* (Yamaguti, 1938) Kritsky & Beverley-Burton, 1986 (AY553622) (Diplectanidae) were used as outgroup for the 28S rDNA; and *Tetrancistrum nebulosi* Young, 1967 (KJ001360) was used as outgroup for the mtCOI. Accession numbers, species and hosts of the sequences used in this study are shown in table 1. Newly obtained sequences from both data sets (28S rDNA and mtCOI) were aligned using MUSCLE implemented in Geneious version 11.1.4 (Kearse *et al.*, 2012) with the extremes of the alignment trimmed. The index of substitution saturation (I_{ss}) was estimated in DAMBE 5 to evaluate the occurrence of substitution saturation (Xia, 2013).

Genetic divergence was calculated for partial 28S rDNA and mtCOI genes using the uncorrected *p*-distances model in MEGA7 software (Kimura, 1980; Tamura *et al.*, 2013). The alignment of mtCOI was 534 bp, with no stop codons and translation on frame 2, flatworm mitochondrial code.

The alignment of 28S rDNA gene was 617 bp long and the model of nucleotide substitution selected was GTR + I + G. The most appropriate evolutionary model for maximum likelihood (ML) and Bayesian inference (BI) was selected by JModelTest 2.1.1 programme (Posada, 2008) using the Akaike information criterion. ML analysis was performed using the program RAxML version 8 (Guindon & Gascuel, 2003), and BI using MrBayes 3.2 (Ronquist & Huelsenbeck, 2003). Bootstrap support values for ML were determined by performing 1000 repetitions. Markov Chain Monte Carlo chains were run for 50 million generations and the log-likelihood scores plotted. The burn-in was set to the first 25% of generations discarded and the consensus tree (majority rules) was estimated using the remaining topologies. MrBayes and RaxML analyses were carried out on the computational resource CIPRES (Miller *et al.*, 2010). Phylogenetic trees were visualized and edited in FigTree version 1.3.1 (Rambaut, 2009).

Results

Dactylogyridae Bychowsky, 1933
Trinigyrus *Hanek, Molnar & Fernando (1974)*

Taxonomic summary

Type species, host and locality. Trinigyrus hypostomatis Hanek, Molnar & Fernando (1974), from *H. robinii* Valenciennes, 1840, Talparo River, Trinidad.

Other species. Trinigyrus tentaculoides, *T. acuminatus*, *T. mourei*, *T. peregrinus*, *Trinigyrus anthus* n. sp. and *Trinigyrus carvalhoi* n. sp.

Diagnosis. Body pyriform, divisible into cephalic region, trunk and haptor (peduncle absent). Tegument thin, smooth. Cephalic lobes, head organs, cephalic glands present. Eyespots absent. Mouth subterminal, midventral; pharynx muscular, glandular; oesophagus short. Two intestinal caeca confluent posterior to gonads; diverticula absent. Gonads intercaecal, overlapping; testis dorsal or dorsoposterior to germarium. Vas deferens looping left intestinal caecum; seminal vesicle as an enlargement of the vas deferens; two prostatic reservoirs. Copulatory complex comprising accessory piece and tubular male copulatory organ (MCO). Weakly sclerotized fringe on wide base of the MCO present or absent. Oviduct short; uterus delicate or well developed; vagina dextral, vaginal tube sclerotized or not; exterior vaginal appendage present or absent; seminal receptacle lying diagonally to the right of midline. Genital pore midventral. Vitelline follicles scattered throughout trunk, absent in region of reproductive organs, coextensive with intestinal caeca. Haptor with ventral anchor/bar complex composed of one pair of anchors, anchors lacking roots distinction (flattened base), one bar, haptoral appendages. Sclerotized basal border on anchor base present or absent. Haptoral glandular reservoirs present (variable number) or absent. Hooks similar in shape and size, proximal dilation of

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Table 1. Monogeneans included in the phylogenetic analyses. New sequences obtained for the present study are in bold.

Parasite species	Host	Host family	Locality	GenBank ID	Reference
Ameloblastella chavarrai	*Rhamdia quelen*	Heptapteridae	Catemaco Lake, Mexico	KP056252	Mendoza-Palmero *et al.* (2015)
Ameloblastella unapinoides	*Sorubim lima*	Pimelodidae	Iquitos, Peru	KP056254	Mendoza-Franco *et al.* (2016)
Ameloblastella edentensis	*Hypophthalmus edendatus*	Pimelodidae	Nanay River, Peru	KP056255	Mendoza-Franco *et al.* (2016)
Ancyrocephalinae sp.	–	–	Panama	MF939844*	Unpublished
Ancyrocephalinae sp.	–	–	Panama	MF939692*	Unpublished
Ancyrocephalinae sp.	–	–	Panama	MF939804*	Unpublished
Ancyrocephalinae sp.	–	–	Panama	MF939733*	Unpublished
Ancyrocephalinae sp.	–	–	Panama	MF939685*	Unpublished
Ancyrocephalinae sp.	–	–	Panama	MF939865*	Unpublished
Aphanoblastella aurorae	*Goeldiella eques*	Heptapteridae	Santa Clara, Peru	KP056239	Mendoza-Palmero *et al.* (2015)
Aphanoblastella magna	*Pimelodella avanhandavae*	Heptapteridae	Upper Paraná River, basin, Brazil	MH688484	Yamada *et al.* (2018)
Aphanoblastella travassosi	*Rhamdia guatemalensis*	Heptapteridae	Lake Catemaco, Mexico	MK358458	Acosta *et al.* (2019)
Aphanoblastella travassosi	–	–	Panama	MF939731*	Unpublished
Aphanoblastella sp.	–	–	Panama	MF939823*	Unpublished
Characitecium sp.	–	–	Panama	MF939687*	Unpublished
Cosmetocleithrum bifurcum	*Hassar orestis*	Doradidae	Aquarium Momón, Iquitos, Peru	KP056216	Mendoza-Palmero *et al.* (2015)
Cosmetocleithrum bulbocirrus	*Pterodoras granulosus*	Doradidae	Upper Paraná River, basin, Brazil	MG001324	Acosta *et al.* (2018)
Demidospermus anus	*Loricariichthys platymetopon*	Loricariidae	Upper Paraná River basin, Brazil	KY766957	Franceschini *et al.* (2018)
Demidospermus mortenthaleri	*Brachyplatystoma juruense*	Pimelodidae	Santa Clara, Peru	KP056245	Mendoza-Palmero *et al.* (2015)
Demidospermus prolixus	*Loricaria prolixa*	Loricariidae	Upper Paraná River basin, Brazil	KY766955	Franceschini *et al.* (2018)
Demidospermus rhinelepisi	*Rhinelepis aspera*	Loricariidae	Upper Paraná River, basin, Brazil	MG001324	Acosta *et al.* (2018)
Demidospermus spirophallus	*Loricaria prolixa*	Loricariidae	Upper Paraná River, basin, Brazil	KY766954	Franceschini *et al.* (2018)
Demidospermus sp. 11	*Brachyplatystoma vaillantii*	Pimelodidae	Nanay River, Peru	KP056235	Mendoza-Palmero *et al.* (2015)
Demidospermus sp. 23	*Brachyplatystoma vaillantii*	Pimelodidae	Nanay River, Peru	KP056236	Mendoza-Palmero *et al.* (2015)
Diaphorocleidus sp.	–	–	Panama	MF939827*	Unpublished
Diaphorocleidus sp.	–	–	Panama	MF939714*	Unpublished
Heteropriapulus anchoradiatus	*Pterygoplychthys ambrosettii*	Loricariidae	Upper Paraná River basin, Brazil	MF116371	Acosta *et al.* (2017)
Heteropriapulus heterotylus	*Pterygoplychthys ambrosettii*	Loricariidae	Upper Paraná River basin, Brazil	MF116370	Acosta *et al.* (2017)
Heteropriapulus simplex	*Pterygoplychthys ambrosettii*	Loricariidae	Upper Paraná River basin, Brazil	MF116372	Acosta *et al.* (2017)
Heteropriapulus sp.	*Pterygoplychthys ambrosettii*	Loricariidae	Upper Paraná River basin, Brazil	MF116373	Acosta *et al.* (2017)
Nanayella aculeatrium	*Sorubim lima*	Pimelodidae	Iquitos, Peru (fish market in Belén)	KP056228	Acosta *et al.* (2019)
Nanayella amplofalcis	*Hemisorubim platyrhynchos*	Pimelodidae	Upper Paraná River basin, Brazil	MG001325	Acosta *et al.* (2019)
Nanayella megorchis	*Sorubim lima*	Pimelodidae	Iquitos, Peru	MK367407	Acosta *et al.* (2019)

Nanayella processusclavis	*Hemisorubim platyrhynchos*	Pimelodidae	Upper Paraná River basin, Brazil	MG001328	Acosta *et al.* (2019)
Trinigyrus peregrinus	*Pterygoplychthys disjunctivus*	Loricariidae	Okinawa-jima, Japan	LC104308	Nitta & Nagasawa (2016)
Trinigyrus peregrinus	***Pterygoplychthys ambrosettii***	**Loricariidae**	**Upper Paraná River basin, Brazil**	**MN944890; MN913212***	**Present study**
***Trinigyrus anthus* n. sp.**	***Hypostomus regani***	**Loricariidae**	**Upper Paraná River basin, Brazil**	**MN947622; MN916719***	**Present study**
***Trinigyrus carvalhoi* n. sp.**	***Hypostomus ancistroides***	**Loricariidae**	**Upper Paraná River basin, Brazil**	**MN947608; MN922321***	**Present study**
Unibarra paranoplatensis	*Aguarunichthys torosus*	Pimelodidae	Santa Clara, Peru	KP056219	Mendoza-Palmero *et al.* (2015)
Unilatus unilatus	*Pterygoplychthys disjunctivus*	Loricariidae	Okinawa-jima, Japan	LC104307	Nitta & Nagasawa (2016)
Unilatus unilatus	*Pterygoplychthys ambrosettii*	Loricariidae	Upper Paraná River basin, Brazil	MF102106	Acosta *et al.* (2017)
Vancleaveus janauacaensis	*Pterodoras granulosus*	Doradidae	Itaya River, Peru	KP056247	Mendoza-Palmero *et al.* (2015)
Walteriella conica	*Platynematichthys notatus*	Pimelodidae	Nanay River, Peru	MK834513	Mendoza-Palmero *et al.* (2019)
Walteriella ophiocirrus	*Platystomatichthys sturio*	Pimelodidae	Iquitos, Peru	MK834515	Mendoza-Palmero *et al.* (2019)
Chauhanellus boegeri	*Genidens genidens*	Ariidae	Antonina, Paraná, Brazil	KP056241	Mendoza-Palmero *et al.* (2015)
Hamatopeduncularia arii	*Arius jella*	Ariidae	Andhra Pradesh, India	KF676629	Unpublished
Hamatopeduncularia bagre	*Bagre marinus*	Ariidae	San Francisco, Mexico	KF676637	Mendoza-Franco *et al.* (2018)
Hamatopeduncularia elongata	*Arius jella*	Ariidae	Andhra Pradesh, India	KF676630	Unpublished
Hamatopeduncularia thalassini	*Arius jella*	Ariidae	Andhra Pradesh, India	KF676631	Unpublished
Hamatopeduncularia sp. 2	*Arius dussumieri*	Ariidae	Andhra Pradesh, India	KF676638	Unpublished
Schilbetrema sp.	*Pareutropius debauwi*	Schilbeidae	Aquarium from Czech Republic, origin West Africa	KP056243	Mendoza-Palmero *et al.* (2015)
Tetrancistrum nebulosi	*Siganus fuscescens*	Siganidae	China	KJ001360[a]*	Wang *et al.* (2014)
Thaparocleidus asoti	*Silurus asotus*	Siluridae	Chongqing City, China	DQ157669	Wu *et al.* (2006)
Thaparocleidus siluri	*Silurus glanis*	Siluridae	River Morava, Czech Republic	AJ969940	Šimková *et al.* (2006)
Urocleidoides flegomai	–	–	Panama	MF939741*	Unpublished
Urocleidoides sp.	–	–	Panama	MF939814*	Unpublished
Murraytrema pricei[a]	*Nibea albiflora*	Scianidae	Panyu, China	DQ157672	Wu *et al.* (2006)
Pseudorhabdosynochus epinepheli[a]	*Epinephelus bruneus*	Serranidae	Huidong, China	AY553622	Wu *et al.* (2006)
Pseudorhabdosynochus lantauensis[a]	*Epinephelus bruneus*	Serranidae	Huidong, China	AY553624	Wu *et al.* (2006)

[a]Species used as outgroups.
*Sequences used for the nucleotide divergence (*p*-distance) analyses using mtCOI (supplementary table S2).

shank; four pairs of hook-bearing appendages: two bilateral pairs bearing hooks pairs 2, 7; single posteroventral pair branched, bearing hook pairs 3, 4; pair of posterodorsal appendages bearing hook pair 6; hook pairs 1, 5 sessile. Sclerotized basal border on the base of anchor present or absent; double filament of anchor. Egg operculate, ovate, with a long, delicate and convoluted filament at proximal pole.

Remarks

The generic diagnosis of *Trinigyrus* is presented, adding new features for placement of the new species in the genus. The new features include the presence of a sclerotized basal border on the base of anchor, and a weakly sclerotized fringe on the wide base of the MCO of *T. peregrinus*, *T. anthus* n. sp. and *T. carvalhoi* n. sp., which was not described earlier in other congeners. *Trinigyrus peregrinus* is redescribed based on morphological discrepancies found among the original description presented by Nitta & Nagasawa (2016) and the specimens deposited as holotype and paratypes, as well as specimens newly collected for this study.

Trinigyrus anthus n. sp.

Taxonomic summary

Type host. *Hypostomus regani* (Ihering, 1905) (Siluriformes: Loricariidae) (NUP 15217).

Other hosts. *Hypostomus strigaticeps* (Regan, 1908) (NUP 14990), *H. margaritifer* (Regan, 1908) (NUP 15216) and *Hypostomus* sp. (NUP14997).

Site in host. Gills.

Type locality. Sapucaí-Mirim River (20°29′38.38″S, 47° 51′33.11″W), municipality of São Joaquim da Barra, São Paulo State, Brazil.

Prevalence (P) and mean intensity of infestation (MII). *Hypostomus regani*: P = 60%, MII = 16.2 ± 3.3 (1.0–73.0); *H. strigaticeps*: P = 64%, MII = 11.6 ± 2.7 (1.0–79.0); *H. margaritifer*: P = 17.4%, MII = 3.2 ± 1.3 (1.0–7.0); *Hypostomus* sp.: P = 13%, MII = 3.7 ± 2.2 (1.0–8.0).

Type material. Holotype CHIOC (39249), paratypes CHIOC (39250–39253), vouchers CHIBB (554–560L).

Representative DNA sequences. 1519-bp-long sequence of the 28S rDNA gene – GenBank accession number MN947622; 780-bp-long sequence of the mtCOI gene – GenBank accession number MN916719.

ZooBank registration. urn:lsid:zoobank.org:act:44F08315-A52C-4C43-911D-3C014F42CCBA, according to the regulations of the International Code of Zoological Nomenclature (ICZN, 2012).

Etymology. The specific epithet is from the Latin and is derived from the flower-petal-like fringe on the wide base of the MCO (*anthus* = flower).

Description

Based on eight specimens mounted in Gray and Wess' medium, two specimens in Hoyer's medium and three specimens stained with Gömöri's trichrome (fig.2a–f). Body pyriform, 604 (426–781; $n = 13$) long, 191 (126–327; $n = 13$) wide. Two terminal cephalic lobes, with three bilateral pairs of well-developed head organs; cephalic glands inconspicuous. Pharynx muscular 47 (36–55; $n = 9$) in diameter. MCO 65 (54–74; $n = 13$) long, and delicate tube, slightly curved, base with a flower-petal-like fringe, non-articulated with accessory piece. Accessory piece 68 (54–89; $n = 13$) long, rod-shaped, tapering discretely in proximal portion, slightly recurved distally, serving as guide of distal portion of MCO. Gonads intercaecal. Testis dorsal to germarium, elongated 97 (95–99; $n = 2$) long, 39 (35–43; $n = 2$) wide. Vas deferens looping left intestinal caecum. Germarium 193 (143–282; $n = 4$) long, 87 (42–140; $n = 4$) wide. Oviduct, ootype and uterus not observed. Conspicuous glands in middle part of body (possibly surrounding ootype region). Egg ovate, 60 (49–72; $n = 2$) long and 34 (30–39; $n = 2$) wide, with a proximal filament, long, delicate and convoluted filament. Vagina dextral, non-sclerotized; sac-like seminal receptacle. Haptor 98 (56–174; $n = 13$) long, 292 (208–443; $n = 13$) wide, an expanded portion of the body, variable according to disposition of haptoral appendages. Ten haptoral appendages relatively long, bearing hook pairs 2, 3, 4, 6 and 7; hook pairs 1 and 5 sessile. Anchors 54 (41–60, $n = 13$) long, lacking roots, base 16 (14–19; $n = 13$) wide; conspicuous sclerotized basal border on base of the anchor; short shaft, elongate point 31 (27–35; $n = 13$) long, recurved tip; anchor filament double. Bar M-shaped, 207 (172–267; $n = 13$) long, longitudinal groove along its length and pointed ends. Hooks similar, 13 (11–15; $n = 43$) long, with recurved shaft, shank proximally dilated, weakly sclerotized; erect thumb. Filamentous hooklet loop approximately two-thirds of shank length. Four pairs of hook-bearing appendages: two bilateral pairs, bearing hook pairs 2, 7; single posteroventral pair branched, bearing hook pairs 3, 4; pair of posterodorsal appendages bearing hook pair 6; hook pairs 1, 5 sessile.

Remarks

Trinigyrus anthus n. sp. shares the morphological features of the genus, like one pair of anchors, one bar M-shaped, haptoral appendages, vagina aperture dextral and overlapping gonads. The proposed new species differs from other congeners mainly by the shape of MCO, which is a delicate tube with base containing a flower-petal-like fringe. The accessory piece of the new species is similar in shape of that observed in *T. tentaculoides* but differs in its length (larger in new species – see table 2; see Kritsky *et al.* (1986) for details on *T. tentaculoides*). The haptoral appendages of the new species are conspicuous, when compared with the type species *T. hypostomatis* and *T. acuminatus*; however, they are smaller than *T. tentaculoides*, according to the description of Kritsky *et al.* (1986). The bars of *T. anthus* n. sp. and *T. tentaculoides* are morphologically different, including the absence of a flat posteromedial projection (present in *T. tentaculoides*), and the accentuated M-shaped bar observed in the new species. Moreover, the anchors of the new species present a base with a subrectangular shape versus a 'tear-drop' shape in *T. tentaculoides*. The seminal receptacle of *T. anthus* n. sp. was often filled with spermatozoa, as described for *T. acuminatus* and *T. tentaculoides* by Kritsky *et al.* (1986).

Trinigyrus carvalhoi n. sp.

Taxonomic summary

Type host. *Hypostomus ancistroides* (Ihering, 1911) (Siluriformes: Loricariidae) (NUP 15003).

Site in host. Gills.

Type locality. Sapucaí-Mirim River (20°29′38.38″S, 47° 51′33.11″W), municipality of São Joaquim da Barra, São Paulo State, Brazil.

Prevalence and mean intensity of infestation. P = 34%, MII = 5.0 ± 0.9 (1.0–14.0).

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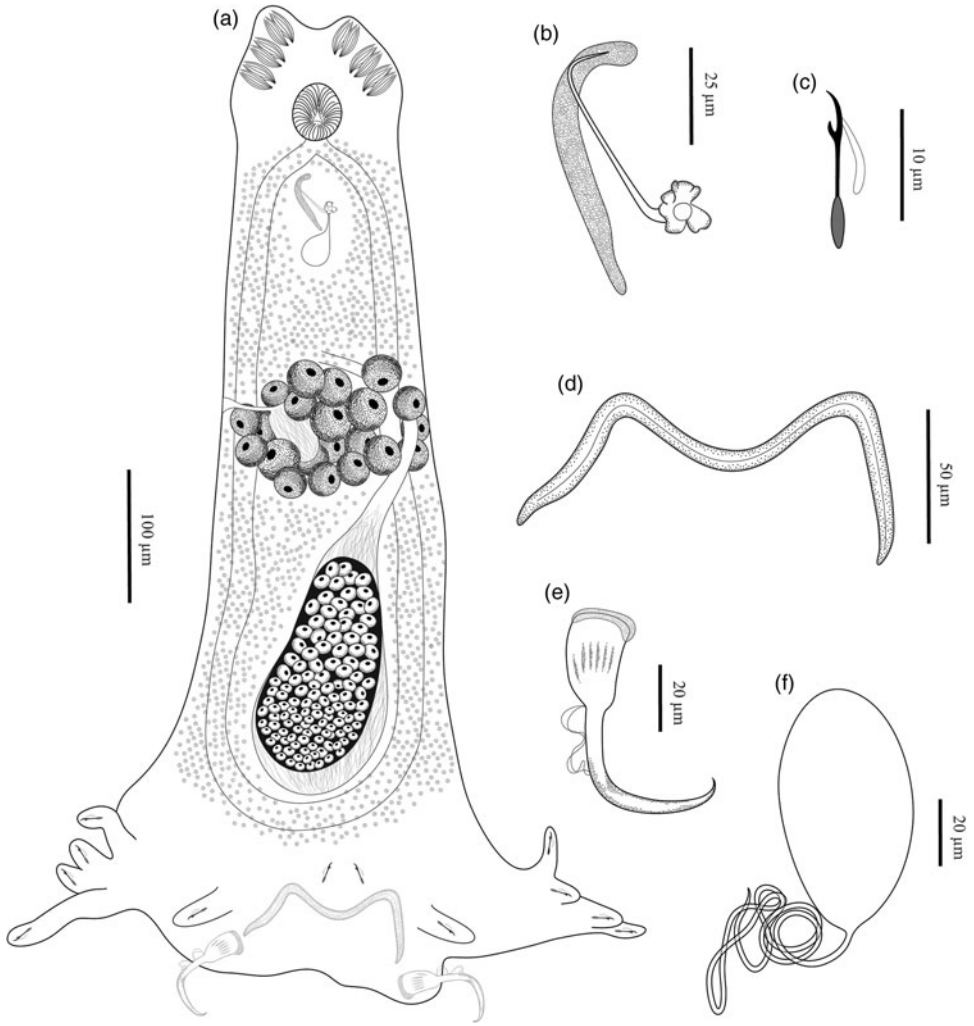

Fig. 2. *Trinigyrus anthus* n. sp. of *Hypostomus regani* (Ihering, 1905) from the Sapucaí-Mirim River, São Paulo State, Brazil: (a) entire body, ventral view (composite); (b) male copulatory complex, ventral view; (c) hook; (d) bar; (e) anchor; (f) egg.

Type material. Holotype CHIOC (39254), paratypes CHIOC (39255–39259), vouchers CHIBB (561–568L).

Representative DNA sequences. 1380-bp-long sequence of the 28S rDNA gene – GenBank accession number MN947608; 757-bp-long sequence of the mtCOI gene – GenBank accession number MN922321.

ZooBank registration. urn:lsid:zoobank.org:act:9D20E486-2B1C-4023-A976-C5D8B7C73BFB, according to the regulations of the International Code of Zoological Nomenclature (ICZN, 2012).

Etymology. The species is named after Edmir Daniel Carvalho (*in memoriam*), professor and researcher at the São Paulo State University, Institute of Biosciences, Campus of Botucatu, São Paulo State, Brazil, who dedicated his life to the study of ecology and environmental impacts on rivers and reservoirs caused by anthropic actions, and actively participated in the development of our project.

Description

Based on nine specimens mounted in Gray and Wess' medium, two in Hoyer's medium, three mounted in GAP and one stained with Gömöri's trichrome (fig. 3a–e). Body pyriform, stout, 525 (432–661; $n = 8$) long, 151 (88–203; $n = 8$) wide. Two terminal cephalic lobes, head organs, cephalic glands poorly developed. Pharynx subspherical, muscular, 43 (35–46; $n = 3$) in diameter. MCO 61 (54–66; $n = 9$) long, counterclockwise C-shaped, measuring 19 (19–20; $n = 5$) in diameter; weakly sclerotized fringe surrounding wide base of MCO with presence of lateral flap, non-articulated with accessory piece. Accessory piece 52 (46–58; $n = 9$) long, C-shaped, relatively robust. Gonads intercaecal. Testis dorsoposterior to germarium, elongated 47 (33–57; $n = 3$) long, 16 ($n = 1$) wide. Vas deferens looping left intestinal caecum. Germarium 96 (68–150; $n = 4$) long, 43 (30–60; $n = 3$) wide. Egg elliptical 63 (58–69; $n = 4$) long, 40 (36–46; $n = 4$) wide, with proximal filament, delicate and convoluted. Vagina dextral, non-sclerotized; sac-like seminal receptacle. Haptor 97 (81–123; $n = 6$) long, 246 (179–288; $n = 6$) wide, an expanded portion of the body, with haptoral appendages relatively long. Anchors 49 (44–51; $n = 9$) long, lacking roots, base 13 (11–14; $n = 9$) wide, elongate; conspicuous sclerotized basal border on base of the anchor; short shaft, elongate point 29 (27–31; $n = 9$) long, recurved tip; anchor filament double. Bar M-shaped, 195 (182–218; $n = 9$) long, longitudinal groove along its length, pointed ends. Hooks similar, 14 (13–15; $n = 16$) long, shank proximally dilated, weakly sclerotized,

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Table 2. Morphometric comparison of species of *Trinigyrus* from loricariid fish.

Parasite	*T. hypostomatis*[a]	*T. acuminatus*[b]	*T. tentaculoides*[b]	*T. mourei*[c]	*T. peregrinus*[d]	*T. peregrinus* (present study)	*T. anthus* n. sp.	*T. carvalhoi* n. sp.
Type host	*Hypostomus robinii*	*Acanthicus hystrix*	*Hypoptopoma thoracatum*	*Squaliforma emarginata*	*Pterygoplichthys disjunctivus*	*Pterygoplichthys ambrosettii*	*Hypostomus regani*	*Hypostomus ancistroides*
Number of specimens	10	32	19	9	23	15	13	14
Body length	300 (280–325)	320 (190–407)	222 (165–307)	448 (390–500)	647 (460–819)	757 (540–963)	604 (426–781)	525 (432–661)
Body width	167 (110–189)	–	–	116 (67–152)	217 (148–321)	197 (139–294)	191 (126–327)	151 (88–203)
Haptor length	–	93 (63–129)	59 (42–73)	96 (74–124)	158 (130–195)	189 (109–374)	102 (47–206)	97 (81–123)
Haptor width	60 (51–82)	164 (110–196)	110 (81–145)	213 (182–236)	342 (230–435)	389 (282–519)	300 (162–540)	246 (179–288)
Anchor length	51 (55–61)	41 (33–45)	47 (43–50)	59 (55–65)	64 (59–71)	60 (54–65)	54 (41–60)	49 (44–51)
Anchor root base width	–	9 (7–11)	10 (8–11)	12 (11–15)	17 (15–20)	20 (16–24)	16 (14–19)	13 (11–14)
Anchor tip length	–	–	–	–	–	32 (31–35)	31 (27–35)	29 (27–31)
Bar length	120 (97–130)	66 (60–79)	75 (60–93)	154 (153–155)	154 (126–178)	321 (229–358)	207 (172–267)	195 (182–218)
Hook length	11 (10–11)	13 (11–16)	10 (7–12)	8–9	13 (11–14)	14 (13–15)	13 (10–16)	14 (13–15)
MCO length	28 (27–32)	98 (94–101)	29–30	36 (35–39)	60 (55–64)	72 (67–78)	65 (54–74)	61 (54–66)
MCO shape	Tube curved with lumen uniform	Elongate slender tube with sinistral loop and base reduced	Curved shaft arising from a simple base	J-shaped tube, very robust, with truncate distal end	Coiled tube forming a circle	Coiled tube forming a counterclockwise circle; weakly sclerotized fringe surrounding wide base	Delicate tube, slightly curved, with a flower-petal-like shape fringe on the wide base	C-shaped tube (incomplete circle), counterclockwise; weakly sclerotized fringe surrounding wide base
Accessory piece length	26 (24–27)	24 (19–36)	28 (22–33)	42 (40–45)	31 (25–38)	71 (61–77)	68 (54–89)	52 (46–58)
Vagina	Non-sclerotized (muscular)	Sclerotized tube, with exterior flower-like appendage	Small funnel; irregularly sclerotized tube	Non-sclerotized	Non-sclerotized	Weakly sclerotized tube	Non-sclerotized	Non-sclerotized
Egg length	50 (46–53)	–	–	–	63 (50–72)	57	60 (49–72)	63 (58–69)
Egg width	35 (28–37)	–	–	–	32 (28–36)	28 (27–29)	34 (30–39)	40 (36–46)

[a]Hanek *et al.* (1974).
[b]Kritsky *et al.* (1986).
[c]Boeger & Belmont-Jégu (1994), except the bar length and accessory piece length, which were obtained from the analysis of the slides deposited in museums.
[d]Nitta & Nagasawa (2016). Measurements of the anchors, copulatory complex and bars correspond to fig. 1.

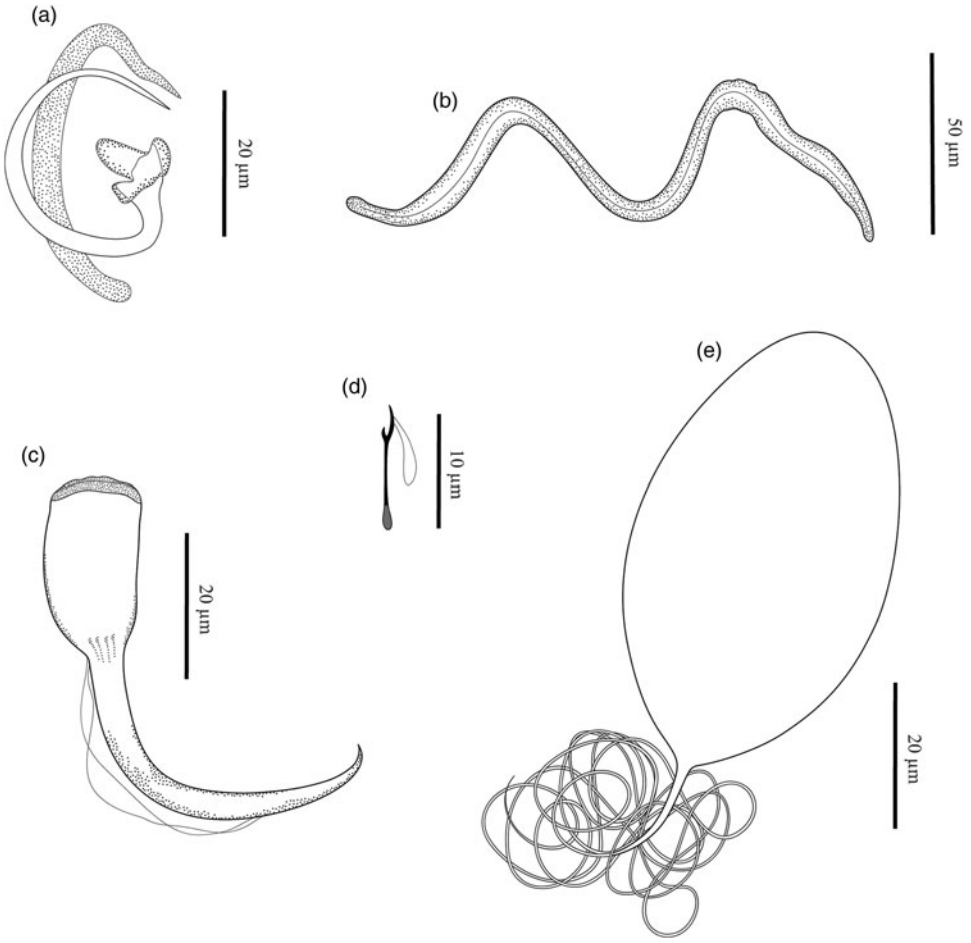

Fig. 3. *Trinigyrus carvalhoi* n. sp. (composite) of *Hypostomus ancistroides* (Ihering, 1911) from the Sapucaí-Mirim River, São Paulo State, Brazil, showing: (a) male copulatory complex, dorsal view; (b) bar; (c) anchor; (d) hook; (e) egg.

erect thumb. Filamentous hooklet loop approximately half the shank length. Four pairs of hook-bearing appendages: two bilateral pairs bearing hooks pairs 2, 7; single posteroventral pair branched, bearing hook pairs 3, 4; pair of posterodorsal appendages bearing hook pair 6; hook pairs 1, 5 sessile.

Remarks

Although the copulatory complex of this new species resembles in shape that of *T. peregrinus* and *T. mourei*, the MCO of *T. mourei* is the shortest and more robust among them (median values: 36 to *T. mourei*, 61 to *T. carvalhoi* n. sp., 60 and 72 to *T. peregrinus* from Japan and Brazil, respectively). Differences in shape of the MCO are also present: J-shaped in *T. mourei*, one counterclockwise circle in *T. peregrinus* (see *Redescription* section) and C-shaped (curved, but not forming a circle) in *T. carvalhoi* n. sp. The sclerotized fringe on the wide base of the MCO present in *T. carvalhoi* n. sp. and *T. peregrinus* (more evident in *T. carvalhoi* n. sp.) is not present in *T. mourei*. Comparatively, the accessory piece of *T. carvalhoi* n. sp. is shorter than that of *T. peregrinus* (52 (46–58) versus 71 (61–77), respectively).

The base of the anchor of *T. carvalhoi* n. sp. is slightly more elongate than that observed in *T. peregrinus* from Brazil, and more similar to that described in *T. mourei*, although differences in measurements can be verified (see table 2). In contrast, the sclerotized basal border on the base of the anchor is more evident in *T. peregrinus* when compared to *T. carvalhoi* n. sp., and apparently absent in *T. mourei*. *Trinigyrus carvalhoi* n. sp. and *T. peregrinus* can be distinguished from each other based on the sclerotization of the vagina (absent in the new species), differences in size and shape of the eggs (63 (58–69) × 40 (36–46), elliptical in *T. carvalhoi* n. sp. versus 57 × 28 (27–29), ovate in *T. peregrinus*), differences in size of the haptoral bar (195 (182–218) in *T. carvalhoi* n. sp. versus 321 (229–358) in *T. peregrinus*) and differences in total body size (525 (432–661) in *T. carvalhoi* n. sp. versus 757 (540–963) in *T. peregrinus*).

Trinigyrus peregrinus *Nitta & Nagasawa, 2016*

Taxonomic summary

Type host. *Pterygoplichthys disjunctivus* (Weber, 1991) (Siluriformes: Loricariidae).

Other host. *Pterygoplichthys ambrosettii* (Holmberg, 1893) (Siluriformes: Loricariidae).

Site in host. Gills.

Type locality. Hija River, Misato, Okinawa city, Japan.

Other localities. Sembaru Reservoir, Sembaru, Nishihara town, Okinawa-jima Island, Okinawa Prefecture, Japan (Nitta & Nagasawa, 2016), and Aguapeí River, municipality of Castilho

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(Paraná River basin), São Paulo State, Brazil (21°3′36.20″S, 51°45′38.58″W) (present study).

Specimens studied. Holotype NSMT-Pl 6195, paratypes NSMT-P1 6196–6203 and newly collected specimens from *P. ambrosettii* from the Aguapeí River.

Prevalence and mean intensity of infestation. P = 12% and MII = 29.4 ± 24.0 (1.0–246.0) from hosts from the Aguapeí River.

Material deposited (present study). Vouchers CHIOC (39260–39263) and CHIBB (569–577L).

Representative DNA sequences. 954-bp-long sequence of the 28S rDNA gene – GenBank accession number LC104308 (Nitta & Nagasawa, 2016); and 1514-bp-long sequence of the 28S rDNA gene – GenBank accession number MN944890 (present study); 746-bp-long sequence of the mtCOI gene – GenBank accession number MN913212 (present study).

Redescription

Based on 12 specimens mounted in Hoyer's medium, one in GAP medium and two specimens stained with Gömöri's trichrome (fig. 4a–f). Body robust, pyriform, 757 (540–963; $n = 12$) long, 197 (139–294; $n = 12$) wide. Two terminal cephalic lobes poorly developed. Pharynx subspherical, muscular, 59 (50–72; $n = 6$) in diameter. Oesophagus short. MCO 72 (67–78; $n = 12$) long, forming one counterclockwise circle measuring 23 (22–23; $n = 4$) in diameter; weakly sclerotized fringe surrounding wide base of MCO with presence of lateral flap, non-articulated with accessory piece. Accessory piece 71 (61–77; $n = 12$) long, robust, C-shaped. Vitelline follicles scattered throughout trunk, absent in region of reproductive organs, coextensive with intestinal caeca. Testis dorsal to germarium, elongated 75 ($n = 1$) long, 22 ($n = 1$) wide. Vas deferens looping left intestinal caecum. Seminal vesicle elongated, as an enlargement of the vas deferens; two prostatic reservoirs subovate. Germarium 145 (97–193; $n = 2$) long, 63 (40–85; $n = 2$) wide. Conspicuous glands in middle part of body (possibly surrounding ootype region). Egg ovate, 57 ($n = 2$) long, 28 (27–29; $n = 2$) wide, with proximal filament, delicate and convoluted. Vagina dextral, sclerotized tube; sac-like seminal receptacle. Haptor 189 (109–374; $n = 12$) long, 389 (282–519; $n = 10$) wide, an expanded portion of the body, with measurements varying according to arrangement of haptoral appendages; variable number of glandular reservoirs, conspicuous in stained specimens. Ventral anchor/bar complex composed of one pair of anchors, one bar, haptoral appendages relatively long and robust. Anchors 60 (54–65, $n = 12$) long, base 20 (16–24; $n = 12$) wide, lacking roots; conspicuous sclerotized basal border on anchor base; short shaft, elongate point with 32 (31–35; $n = 12$) long and sharply recurved tip; anchor filament double. Bar M-shaped 321 (229–358; $n = 12$) long, longitudinal conspicuous groove along its length, pointed ends. Hooks similar, 14 (13–15; $n = 25$) long, with shank proximally dilated, weakly sclerotized; delicate, erected thumb. Filamentous hooklet loop approximately two-thirds of the shank length. Four pairs of hook-bearing appendages: two bilateral pairs, bearing hook pairs 2, 7; single posteroventral pair branched, bearing hook pairs 3, 4; pair of posterodorsal appendages bearing hook pair 6; hook pairs 1, 5 sessile.

Remarks

Trinigyrus peregrinus was described by Nitta & Nagasawa (2016) for dactylogyrids from the gills of *P. disjunctivus* native to South America that were introduced to Japan. The morphology of the holotype (NSMT-Pl 6195) and all paratypes of *T. peregrinus* deposited in the National Museum of Nature and Science in Japan (NSMT-P1 6196–6203) corresponded to that of the species of *Trinigyrus* found in this study from *P. ambrosettii* in Brazil. However, several discrepancies were found among the original description (text and drawings) and the examined specimens.

Some morphological characteristics of *T. peregrinus* that are not represented in Nitta & Nagasawa (2016) were detected in the holotype and paratypes, and also in the specimens collected in Brazil. These characteristics include the presence of a weakly sclerotized fringe on the wide base of the MCO; sclerotized vaginal tube; conspicuous glands in the middle part of the body (possibly surrounding ootype region); overlapping gonads (testis dorsal to germarium as opposed to posterior to ovary in Nitta & Nagasawa, 2016); glandular reservoirs in the haptor (visible in the stained specimens); conspicuous sclerotized basal border on the base of the anchor; bar with longitudinal groove along its length; and hooks with shank proximally dilated. The discrepancies among the specimens described by Nitta & Nagasawa (2016) and the specimens analysed in the present study can also be made when comparing the line drawings (fig. 4a–f) of the present study, and consulting Nitta & Nagasawa (2016). Differences in size of some structures between *T. peregrinus*, *P. disjunctivus* and *P. ambrosettii* are presented in table 2.

The two sequences of the partial ribosomal 28S of *T. peregrinus*, one from *P. disjunctivus* introduced to Japan and one from *P. ambrosettii* from Brazil, showed genetic divergence of 6 bp (1% for 28S rDNA analysis). Although discrepancies were observed among the line drawings of *T. peregrinus* specimens represented by Nitta & Nagasawa (2016) and those of the present study, when analysing the museum's paratypes and considering the new molecular data, it was concluded that these discrepancies are a consequence of incongruities in the graphic representativeness of the specimens. This fact reinforces the importance of consulting specimens deposited in collections during the course of taxonomic studies, minimizing the possibility of making incorrect and incomplete descriptions.

Phylogenetic relationships

The BLAST search performed using each of the generated sequences of the new species did not match any other monogenean sequences available in GenBank. The estimates for evolutionary divergences with the partial 28S rDNA gene were compared using the sequences of species of *Trinigyrus* with 38 other sequences of dactylogyrids and the three sequences of diplectanids used as outgroup retrieved from GenBank, with data varying from 1 to 38% (see supplementary table S1). The genetic divergences among the new species and *T. peregrinus* varied from 2 to 3% (6–18 bp), among *Trinigyrus* spp. and *Heteropriapulus* spp. ranged from 16 to 17% (96–105 bp), and from *Trinigyrus* spp. and *Unilatus unilatus* Mizelle & Kritsky, 1967 varied from 13 to 15% (83–96 bp). See supplementary table S1 for information on the genetic divergence values among *Trinigyrus* spp. using the 28S rDNA gene and each species used in the phylogenetic analyses. The estimates for evolutionary divergences using the mtCOI gene were compared using the species of *Trinigyrus* with 14 species of dactylogyrids retrieved from GenBank, with data varying from 7 to 12% (1–132 bp) (see supplementary table S2). The genetic divergences among the new species of *Trinigyrus* and *T. peregrinus* varied from 6 to 7% (83–92 bp).

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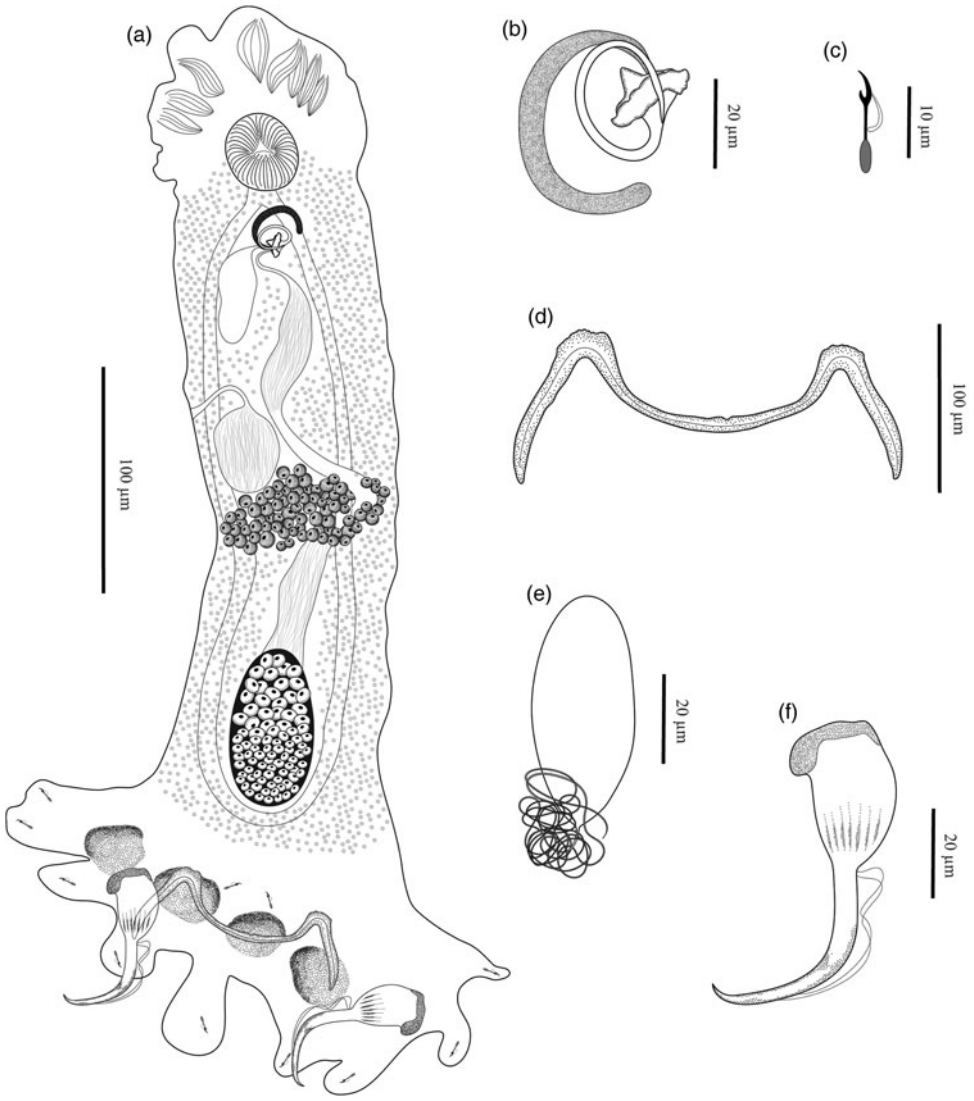

Fig. 4. *Trinigyrus peregrinus* Nitta & Nagasawa, 2016 of *Pterygoplichthys ambrosettii* (Holmberg, 1893) from the Aguapeí River, São Paulo State, Brazil, showing: (a) entire body, ventral view (composite); (b) male copulatory complex, dorsal view; (c) hook; (d) bar; (e) egg; (f) anchor.

Both ML and BI phylogenetic analyses converged in similar topologies with highly supported nodes, with the two main clades labelled as A and B (fig. 5). The I_{ss} indicated no saturation in either transitions or transversions. Critical index of substitution saturation ($I_{ss.c}$) values were greater than the I_{ss} values.

Clade A is strongly supported from both analyses and is divided into two well-supported subclades (A1 and A2). Clade A1 comprises *Ameloblastella* spp. (from heptapterids and pimelodids), *Vancleaveus janauacaensis* Kritsky, Thatcher & Boeger 1986 (from doradids) and *Unibarra paranoplatensis* Suriano & Incorvaia, 1995 (from pimelodids). Clade A2 comprises species that parasitize exclusively loricariids: *U. unilatus*, *Heteropriapulus* spp. and *Trinigyrus* spp. Species of *Trinigyrus* formed a lineage sister to *Heteropriapulus* spp. with high support values (fig. 5).

Clade B is also strongly supported and is divided into two well-supported clades: clade B1 (subdivided into B1′ and B1″) and clade B2 comprising *Thaparocleidus* spp. (from silurids), which forms the basal group of the main clade B1. Clade B1′ (not supported) comprises *Cosmetocleithrum* spp. (from doradids) and a closely related clade that includes different species of monogenean parasites of pimelodids from Brazil and Peru (*Demidospermus* spp., *Walteriella* spp. and *Nanayella* spp.). The clade B1″ (not supported) includes *Demidospermus* spp. (from Brazilian loricariids), *Aphanoblastella* spp. (from heptapterids) and monogenean parasites of marine catfishes, as *Hamatopeduncularia* spp. and *Chauhanellus boegeri* Domingues & Fehlauer, 2006 (from ariids) that are closely related to *Schilbetrema* sp. from freshwater catfishes (Schilbeidae).

Discussion

The erection of the new species proposed is supported by a combination of the differences observed in the morphological and molecular data among *Trinigyrus* spp. To date, 26 valid species belonging to four dactylogyrid genera, *Demidospermus* Suriano, 1983 *sensu stricto* (five species), *Unilatus* (six species), *Trinigyrus* (seven species, including the new species described herein) and *Heteropriapulus* Kritsky, 2007 (eight species) have been commonly reported from loricariid catfishes in the

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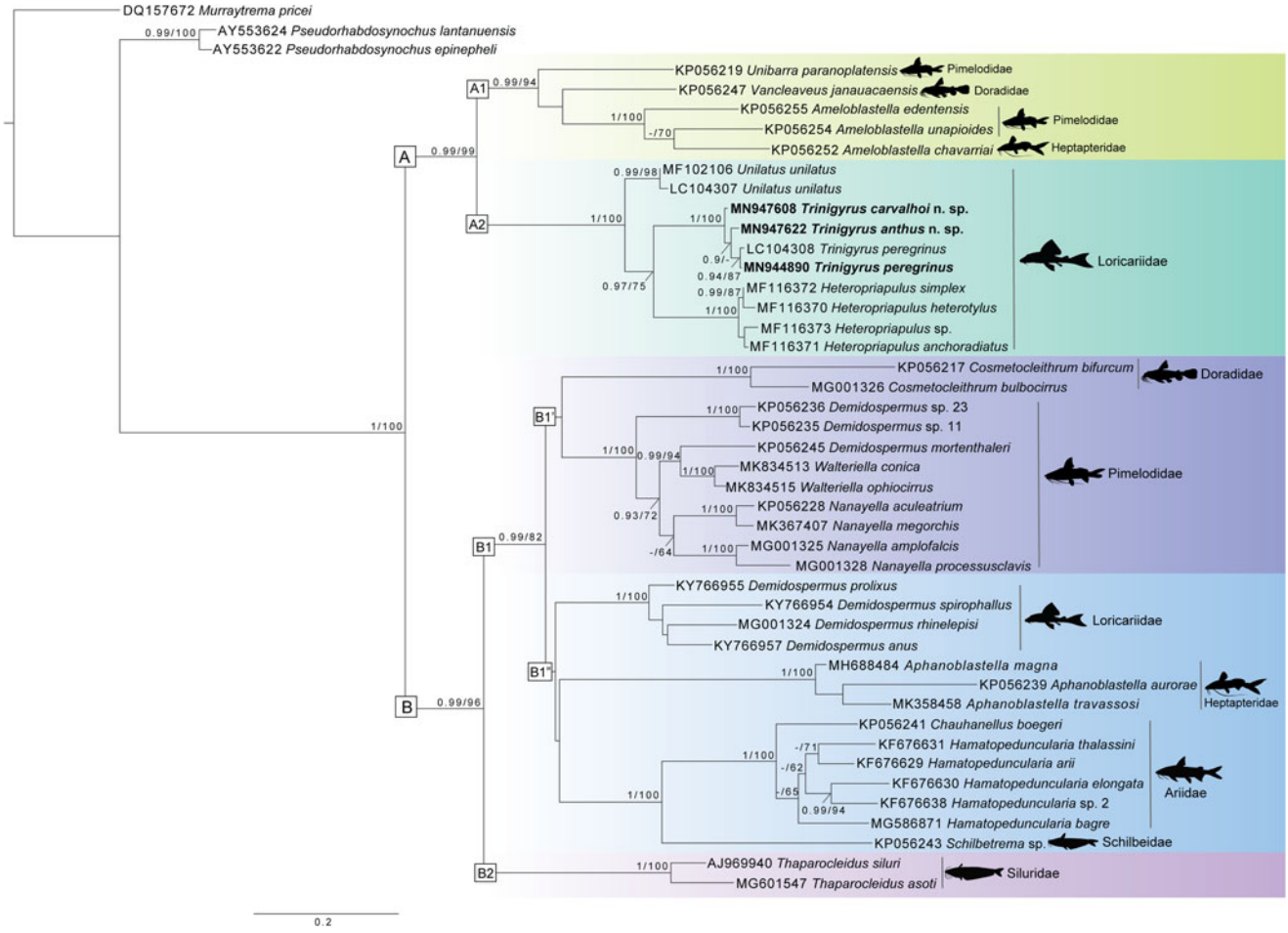

Fig. 5. Maximum likelihood topology based on partial 28S ribosomal DNA sequences of monogenean parasites of siluriforms. GenBank accession numbers precede species names. New sequences obtained for the present study are in bold. *Murraytrema pricei*, *Pseudorhabdosynochus lantauensis* and *Pseudorhabdosynochus epinepheli* (Diplectanidae) were used as outgroup. Support values are above nodes: posterior probabilities <0.90 and bootstrap scores <60 are not shown or are represented by a dash. Branch-length scale bar indicates number of substitutions per site (see supplementary table S1).

Neotropical region (natural distribution) and from areas where they were co-introduced with their hosts (Nitta & Nagasawa, 2016; Acosta *et al.*, 2017, 2018; Franceschini *et al.*, 2018 and references therein).

Negrelli *et al.* (2017) reported the occurrence of *Trinigyrus* sp. parasitizing the gills of *Astyanax lacustris* (Lütken, 1875) (=*Astyanax altiparanae* Garutti & Britski, 2000) from the Batalha River, State of São Paulo, Brazil. However, the occurrence of *Trinigyrus* sp. was not reported by the same research group in a revision about parasites of characiforms from the Batalha River collected at a concomitant period, with the specimens analysed by Negrelli *et al.* (2017) (see Dias *et al.*, 2017 and references therein). In their checklist, the authors reported the occurrence of *Trinibaculum altiparanae* (Abdallah, Azevedo & Silva, 2013) instead of *Trinigyrus* sp. in *Astyanax* specimens. In both studies, the authors did not deposit voucher specimens in any museum collection as they stated.

According to Boeger & Kritsky (1993), one pair of ventral anchors in the haptor is a synapomorphy for the Class Monogenea (=Class Monogenoidea), whereas two ventral pairs of anchors in the haptor developed later as a synapomorphy for the Order Dactylogyridea (Boeger *et al.*, 1997). The occurrence of a single anchor pair in some dactylogyrid species apparently represents multiple examples of independent and secondary loss of either the ventral or dorsal pairs in the evolutionary history of the Dactylogyridae (see Kritsky & Kulo, 1992; Boeger *et al.*, 1997 and references therein), such as that observed in *Trinigyrus*. Besides the loss of the dorsal anchor/bar complex in *Trinigyrus* spp., it is possible to identify other derived characters, including loss of eyespots, the presence of confluent intestinal caeca and the development of haptoral appendages (Kritsky *et al.*, 1986; Boeger & Kritsky, 1993).

Supported by several shared morphological characters, Kritsky *et al.* (1986) proposed the phylogenetic relationship of *Trinigyrus* with *Hamatopeduncularia* Yamaguti, 1953, both genera parasitizing freshwater and marine siluriforms, respectively. Although species of *Hamatopeduncularia* have retained more primitive characteristics when compared to those of *Trinigyrus* (Kritsky *et al.*, 1986), species of both genera possess haptoral appendages, glandular reservoirs in the haptor (e.g. *T. tentaculoides*, *T. peregrinus* and most species belonging to *Hamatopeduncularia*, such as *Hamatopeduncularia arii* Yamaguti, 1953, *Hamatopeduncularia major* Kearn & Whittington, 1994 and *Hamatopeduncularia pearsoni* Kearn & Whittington, 1994) and a flat posteromedial projection on bar, a common character in some species of *Hamatopeduncularia* (e.g. *Hamatopeduncularia thalassini*

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Bychowsky & Nagibina, 1969 and *H. arii*), which was also described in *T. tentaculoides* (Kritsky *et al.*, 1986).

In the present study, phylogenetic analyses based on partial 28S rDNA sequences, considering monogenean parasites of siluriform fishes, showed that *Trinigyrus* (in the main clade A) and *Hamatopeduncularia* (in the main clade B) were not closely related, as proposed by Kritsky *et al.* (1986). *Trinigyrus* species clustered together as a sister group to *Heteropriapulus* spp., and closely related to *Unilatus* spp., forming a well-supported clade of monogenean parasites of loricariids, specifically fishes belonging to the Hypostominae, from Neotropical freshwater environments (fig. 5). Jogunoori *et al.* (2004) proposed a phylogenetic link among *Unilatus*, *Trinigyrus* and *Heteropriapulus* based only on morphologically shared features. Although *Trinigyrus*, *Unilatus* and *Heteropriapulus* share morphological characters (see Jogunoori *et al.*, 2004 and references therein), *Trinigyrus* spp. can be easily recognized because they are the unique representatives of this clade, with a single anchor/bar complex (ventral), bar M-shaped and a redistribution of hooks in haptoral appendages (except the sessile pairs 1 and 5). The phylogenetic relationships among these three genera confirm the phylogenetic link suggested by Jogunoori *et al.* (2004) based on comparative haptoral morphology, but refute the proposal of Kritsky *et al.* (1986), once *Trinigyrus* and *Hamatopeduncularia* are not closely related.

According to the 'Fahrenholz rule', parasites and their hosts speciate in synchrony, with the phylogeny of parasite groups usually corresponding directly to the natural relationships of their hosts, including the closeness of the phylogenetic relationships among them, since the majority of hosts are susceptible to a specific group of these parasites (Eichler, 1948; Kritsky *et al.*, 1986; Kritsky & Kulo, 1992; Thatcher, 2006; Braga *et al.*, 2014). Considering that siluriforms from the Neotropical region, specifically, do not represent a monophyletic group (Sullivan *et al.*, 2006; Braga *et al.*, 2014), monophyly is also not observed in some groups of monogeneans that parasitize fishes belonging to this order, such as *Demidospermus* spp. (Mendoza-Palmero *et al.*, 2015, 2019; Acosta *et al.*, 2018, 2019; Franceschini *et al.*, 2018).

So far, monophyly is proposed for some genera of monogenean parasites of siluriforms, such as *Heteropriapulus*, *Ameloblastella*, *Aphanoblastella* (Acosta *et al.*, 2019; Mendoza-Palmero *et al.*, 2019) and herein the monophyly is also suggested for *Trinigyrus*. Thereby, the strongly supported clade comprising the species of *Trinigyrus*, *Unilatus* and *Heteropriapulus*, which parasitize only loricariids belonging to the Suborder Loricarioidei (the deepest group of catfish from the Neotropical region – see Kappas *et al.*, 2016), enable us to propose that these genera of monogeneans may share an ancient history with their respective hosts.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X20000097.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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