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A new species of *Urocleidoides* Mizelle & Price, 1964 from the gills of *Schizodon nasutus* Kner, 1858 (Characiformes, Anostomidae) in southeastern Brazil

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Research Paper

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Abstract

Based on an integrative approach, this study describes a new species of *Urocleidoides* infesting *Schizodon nasutus* in the Paranapanema River basin, Brazil. The new species can be distinguished from its congeners by specific morphological features, including the shape of the male copulatory organ and accessory piece, the ventral bar shape, and the shape and size of the hooks. Molecular analyses, particularly of the 28S rDNA gene, suggest a close relationship between the new species and *Urocleidoides paradoxus*. The phylogenetic and taxonomic arrangement of *Urocleidoides* is discussed, as the analyses of the 28S rDNA and COI mtDNA resolved the genus as non-monophyletic, with *Diaphorocleidus*, *Rhinoxenus*, and *Cacatuocotyle* nested within it. Additional morphological and molecular data of other congeneric species are required to investigate the phylogenetic position and classification of *Urocleidoides*. This study underscores the significance of using integrative approaches in understanding host–parasite associations and phylogenetic relationships, contributing to the description of the freshwater fish parasite biodiversity in South America, particularly in the Paranapanema river basin.

Introduction

Monogeneans stand as the most diverse helminth parasites of freshwater fishes in South America and have been extensively studied in the Neotropical region (Eiras *et al.* 2011; Cohen *et al.* 2013; Luque *et al.* 2017). The family Dactylogyridae Bychowsky, 1933 takes prominence and is known for its wide geographical distribution and by having a significant proportion of their species parasitising characiform fishes (Moreira *et al.* 2015; Rossin and Timi 2016; Franceschini *et al.* 2017; Acosta *et al.* 2017, 2018, 2019; Zago *et al.* 2017, 2018, 2020, 2021; Oliveira *et al.* 2021; Santos Neto and Domingues 2023; Yamada *et al.* 2023). This family currently comprises over 150 genera (WoRMS 2023) – mostly fish parasites on the gills, with others found in nasal cavities, in the mouth, on the body surface, and even in internal organs (Pavanelli *et al.* 2013).

Urocleidoides Mizelle & Price, 1964 is one of the most species-rich among the Neotropical dactylogyrid genera, comprising 52 valid species (Kritsky *et al.* 1986; Zago *et al.* 2020; Santos-Neto and Domingues 2023), and currently includes species parasitising the gills and nasal cavities of fishes of the orders Characiformes, Gymnotiformes, Cyprinodontiformes, and Siluriformes (although this last one still requires revision) (Ferreira *et al.* 2018; Oliveira *et al.* 2020; Zago *et al.* 2020; Freitas *et al.* 2021; Oliveira *et al.* 2021; Santos Neto and Domingues 2023). The genus was proposed by Mizelle and Price (1964) to accommodate *Urocleidoides reticulatus* Mizelle & Price, 1964 (type-species) from *Poecilia reticulata (=Lebistes reticulatus)* Peters, 1859 (Cyprinodontiformes, Poeciliidae) from an aquarium in Sacramento California, USA (probably collected from the Amazon river basin), which was characterised by the presence of a sinistral vagina and copulatory complex comprising an accessory piece and a non-articulated male copulatory organ.

Mizelle *et al.* (1968) proposed an amended morphological diagnosis for the genus, adding traits such as a male copulatory organ with one or more rings and a vagina usually associated with a sclerotized canal, and pointing out that the distribution was restricted to the Neotropical region. Kritsky *et al.* (1986) considered these characteristics as broadly defined, suggested that some species described as *Urocleidoides* represented, in fact, separate genera, and proposed a new amended morphological diagnosis, indicating that the main morphological characteristic was the presence of a hook-shaped sinistral vaginal sclerite, coiled male copulatory organ with counter-clockwise rings, and hook pairs 1 and 5 usually reduced in size. Kritsky *et al.* (1986) also provided a list of 23 species considered as *incertae sedis,* due to the absence of vaginal sclerite. Lately, Zago *et al.* (2020) provided another amended diagnosis of the genus, adding seven species from

Brazilian freshwater fishes. More recently, based on results obtained from partial sequences of the 28S rDNA and COI mtDNA genes, Santos Neto and Domingues (2023) demonstrated that *U. gymnotus* Mizelle, Kritsky & Crane, 1968 and *U. carapus* Mizelle, Kritsky & Crane, 1968, previously considered *incertae sedis* due to the absence of the vaginal sclerite, were valid species. However, with the lack of representative sequences, other species under the *incertae sedis* status are yet to be validated – namely, *U. strictus* Mizelle, Kritsky & Crane, 1968, *U. strombicirrus* (Price & Bussing, 1967) Kritsky & Thatcher, 1974, *U. trinidadensis* Molnar, Hanek & Fernando, 1974, *U. virescens* Mizelle, Kritsky & Crane, 1968, and *U. advenai* Mendoza-Franco & Reina, 2008 from Gymnotiformes; and *U. amazonensis* Mizelle & Kritsky, 1969 and *U. catus* Mizelle & Kritsky, 1969 from Siluriformes.

Within the order Characiformes, the family Anostomidae presents an extensive geographical distribution, encompassing the watersheds of both South and Central America (Nelson *et al.* 2016). This family currently comprises 15 genera and 160 species, many of which are from the Amazon basin, with species of *Schizodon* Agassiz, 1829, *Laemolyta* Cope, 1872, and *Leporinus* Spix, 1829 (Froese and Pauly 2023) acting as significantly common hosts in the life cycle of a diverse array of parasites, including monogeneans (Guidelli *et al.* 2011; Cohen *et al.* 2013; Oliveira *et al.* 2017; Yamada *et al.* 2017).

To date, eight species of *Urocleidoides* have been described from anostomid hosts: *U. digitabulum* Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020 in *Leporinus friderici* (Bloch, 1794*), Leporinus octofasciatus* Steindachner, 1915, and *Megaleporinus elongatus* (Valenciennes, 1850); *U. falxus* Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020 and *U. sapucaiensis* Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020 in *M. elongatus*; *U. jariensis* Oliveira, Santos-Neto, Tavares-Dias & Domingues, 2020 in *Schizodon fasciatus* Spix & Agassiz, 1829; *U. paradoxus* (Kritsky, Thatcher & Boeger, 1986) in *Rhytiodus microlepis* Kner, 1858, *Schizodon nasutus* Kner, 1858, *Megaleporinus macrocephalus* (Garavello & Britski, 1988), *M. elongatus*, *L. friderici*, *Leporinus lacustris* Amaral Campos, 1945 and *Leporinus obtusidens* (Valenciennes, 1837); *U. ramentacuminatus* Oliveira, Santos-Neto, Tavares-Dias & Domingues, 2020 in *Laemolyta proxima* (Garman, 1890); *U. sinus* Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020 in *Leporinus striatus* Kner, 1858, *S. nasutus*, and *Schizodon intermedius* Garavello & Britski, 1990; and *U. solarivaginatus* Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020 in *L. frederici*, *L. octofasciatus* and *L. striatus*.

As part of the studies undertaken in the Paranapanema River basin in Brazil to describe the freshwater fish helminth parasite fauna, a new species of *Urocleidoides* was found infesting the gills of *S. nasutus*. Herein, we described the new species using morphological and molecular features. New insights regarding the current phylogenetic and taxonomic arrangement of *Urocleidoides* spp. were also provided.

Materials and methods

Host and parasite sampling

Nine specimens of *S. nasutus* were collected in the Pardo River (22° 59'22.07" S; 48°26'26.20" W), Paranapanema River basin, municipality of Botucatu, São Paulo state, Brazil, in June 2021. The fish were captured using casting nets and euthanized with sodium thiopental (Thiopentax®). Some specimens were individually stored in plastic bags and frozen to later conduct a necropsy at the laboratory, while others were examined in situ to collect fresh monogeneans, which were placed directly in 96% molecular-grade ethanol for molecular analyses. The gills of the fish were removed, placed in Petri dishes, and analysed for parasites under a stereomicroscope. The specimens were detached from the gills, rinsed in 0.6 saline, and mounted on slides with Hoyer's or Gray and Wess's medium (Kritsky *et al.* 1986).

The morphology and measurements of the monogeneans were analysed using a V3 Leica Application Suite (LAS) computerized system for image analysis adapted to a microscope with differential interference contrast. Drawings were made using a drawing tube. The measurements are made following the scheme of Zago *et al.* (2020).

Holotype and paratypes of the new species were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (Holotype CHIOC 40288a; Paratypes CHIOC 40288b-c), Rio de Janeiro, Brazil. Other vouchers were deposited in the Helminthological Collection of the Institute of Biosciences (CHIBB 725L and 726L), Botucatu, São Paulo, Brazil. Fishes were collected under the authorization of the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO #60640–1). All procedures followed the recommendations and approval of the Ethical Commission for Animal Experimentation from the São Paulo State University (Unesp), Institute of Biosciences, Botucatu, São Paulo state, Brazil (CEUA #9415260520). According to Brazilian laws, species registration for scientific research purposes was carried out at SisGen (A30E9D2).

Molecular and phylogenetic analyses

Genomic DNA was isolated from a freshly collected ethanol-fixed individuals using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. The 28S region of the ribosomal DNA was amplified by polymerase chain reaction (PCR) using the primers 382F (5′-AGCTGGTGG AGTCAAGCTTC-3′) and 1289R (5′-TGCTCACGTTTGAC GATCGA-3′) (Yamada *et al.* 2023), following the specific cycling conditions: initial denaturation of 5 min at 95°C followed by 40 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 2 min, and a final extension of 10 min at 72°C (Yamada *et al.* 2023). The COI region of the mitochondrial DNA was amplified using the primers ASmit1 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) (Bowles *et al.* 1992) and Schisto3 (5′-TAATGCATMGGAAAAAAA CA-3′) (Lockyer *et al.* 2003), following the specific cycling conditions: initial denaturation of 5 min at 95°C followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, and a final extension of 7 min at 72°C (Lockyer *et al.* 2003). PCR amplifications were performed on a final volume of 25 µl containing 12.5 µl of 2× MyFi™ Mix (Bioline, Taunton, MA, USA), 3.0 µl of extracted DNA, 7.5 µl of pure water, and 1.0 µl of each PCR primer. PCR products (2.0 µl) were run on an agarose gel (1%) using GelRed™ fluorescent nucleic acid dye added to BlueJuice™ Gel Loading Buffer (6X) to confirm amplicon size and yield. PCR amplicons were purified using the QIAquick PCR Purification Kit (Qiagen), following the manufacturer's instructions. Automated sequencing was performed directly on purified PCR products using a BigDye v.3.1 Terminator Cycle Sequencing Ready Reaction kit on an ABI3730xl Genetic Analyzer (Applied Biosystems). Forward and reverse sequences were assembled and edited in Sequencher v. 5.2.4 (Gene Codes, Ann Arbor, MI, USA).

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Two independent datasets of the 28S rDNA and COI mtDNA, including the new sequences and published sequences of closely related genera retrieved from GenBank (Supplementary Table 1), were built using MUSCLE algorithm implemented on Geneious 7.1.3 (Kearse *et al.* 2012) with default settings. Prior to the analyses, the best-fitting model was estimated with JModelTest software (Posada 2008), which was GTR + G + I for both datasets. Phylogenetic relationships were reconstructed for each alignment under Bayesian inference (BI) and maximum likelihood (ML). The BI analyses were performed using MrBayes 3.2 (Ronquist *et al.* 2012) at the online platform CIPRES (Miller *et al.* 2010). The Markov chain Monte Carlo (MCMC) was run with 10^6 generations, saving one tree every 100 generations, with a burn-in set to the first 25% of the trees. The ML analyses were run in RAxML (Guindon and Gascuel 2003) at the online platform CIPRES (Miller *et al.* 2010) with 1000 bootstrap replicates. The BI and ML trees were visualised in FigTree v. 1.3.1 software (Rambaut 2009) and edited in CorelDRAW X6.

Results

Morphological analyses

Thirty-one specimens of a new species of *Urocleidoides* were collected in three of the nine specimens of *S. nasutus*, with 7, 11, and 13 monogeneans in each infested host.

Dactylogyridae Bychowsky, 1933
***Urocleidoides* Mizelle & Price, 1964**
***Urocleidoides curvocuspidis* n. sp.**
Description (Figures 1 and 2)
(urn:lsid:zoobank.org:pub:94A9C4F8-DA08-4FCD-888F-9A3D3827C95B)

[Based on 10 specimens] Body foliform, elongate, robust, total length including haptor 392 (386–399; n = 7); greatest width 155 (133–168; n = 7) near mid-length. Cephalic margin broad; cephalic lobes developed; four bilateral pairs of head organs; indistinct cephalic glands. Eyespots absent; some accessory granules sparse in cephalic region and anterior trunk. Pharynx spherical, 25 (24 and 26; n = 2) in diameter;

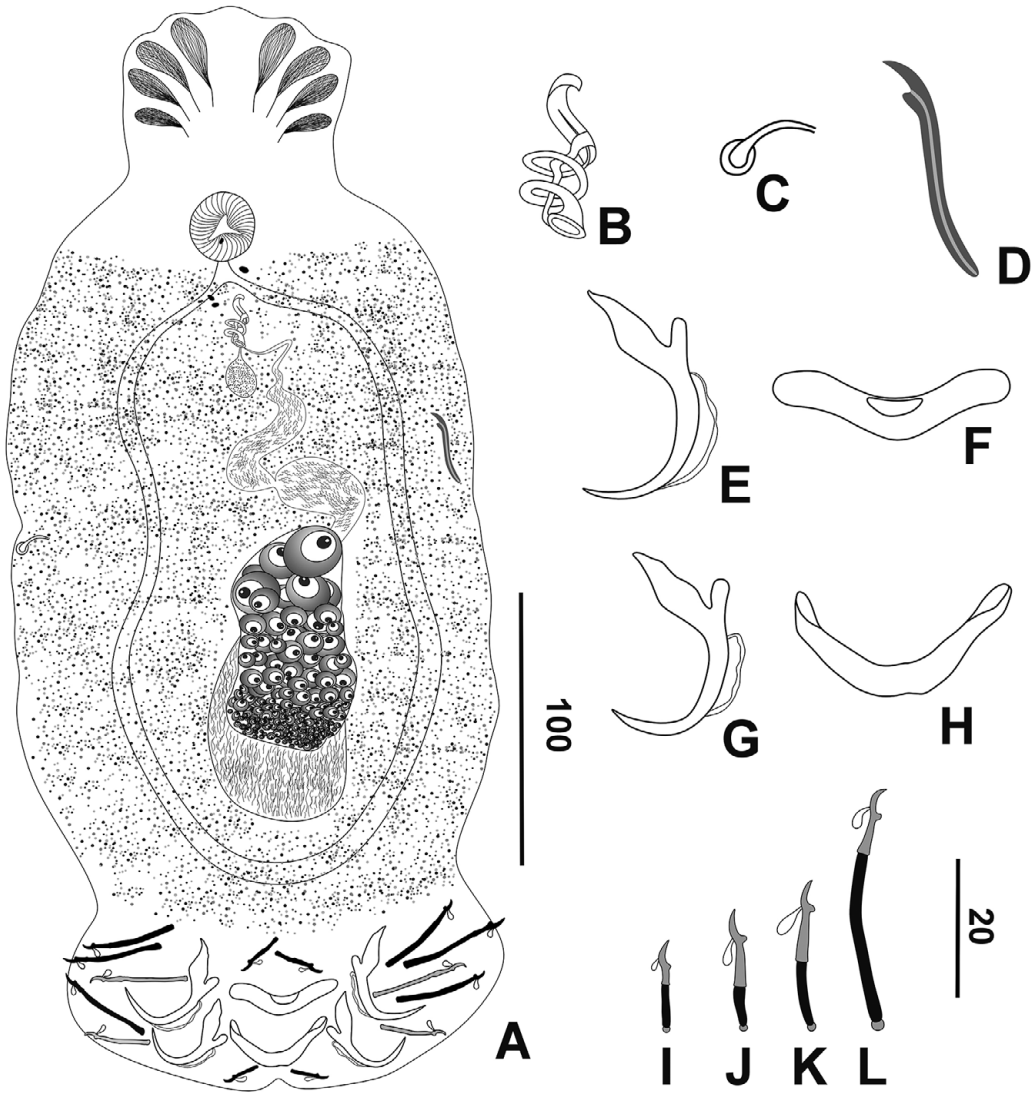

Figure 1. *Urocleidoides curvocuspidis* n. sp. from gills of the *Schizodon nasutus* Kner, 1858 from Pardo River, municipality of Botucatu, São Paulo state, Brazil. (A) composite drawing of whole mount (ventral); (B) copulatory complex; (C) vagina; (D) vaginal sclerite; (E) ventral anchor; (F) ventral bar; (G) dorsal anchor; (H) dorsal bar; (I) hook (pair 5); (J) hook (pair 1); (K) hook (pair 6); (L) hook (pairs 2, 3, 4, 7). Scale bars in micrometres.

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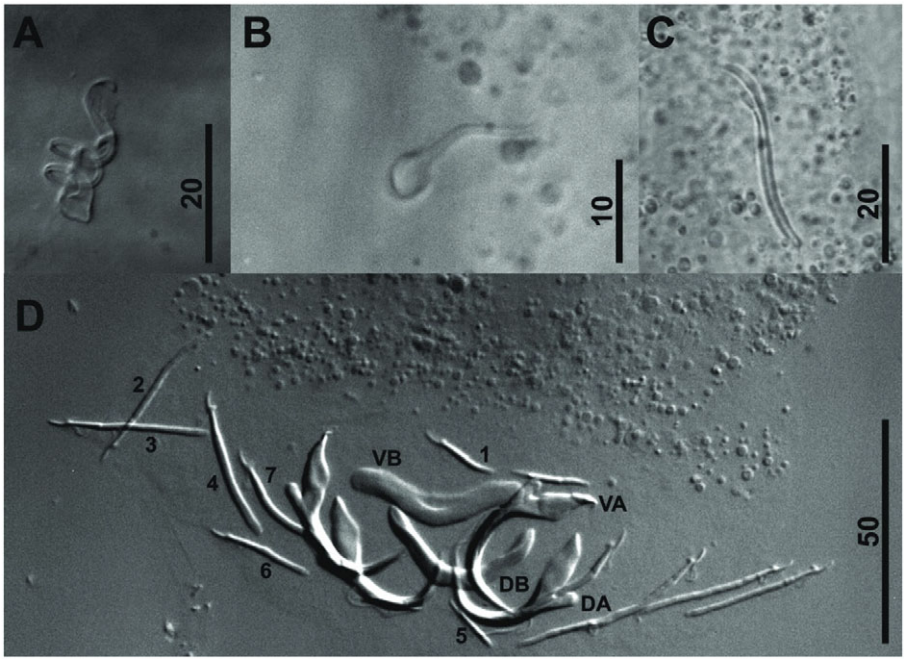

Figure 2. *Urocleidoides curvocuspidis* n. sp. from gills of the *Schizodon nasutus* Kner, 1858 from Pardo River, municipality of Botucatu, São Paulo state, Brazil. Photomicrographs of the sclerotized pieces: (A) copulatory complex; (B) vagina; (C) vaginal sclerite; (D) haptor, highlighting hook pairs (1 to 7), ventral anchor (VA), ventral bar (VB), dorsal anchor (DA), and dorsal bar (DB). Scale bars in micrometres.

short esophagus. Peduncle short; haptor robust, slightly subhexagonal 57 (52 and 63; n = 2) long, 78 (71 and 151; n = 2) wide. Ventral anchor (Figure 2D) 35 (32–37; n = 10) long, 18 (15–21; n = 10) wide, with a robust base, well-distinguishable deep and superficial roots, elongated shaft and point. Dorsal anchor (Figure 2D) 26 (25–28; n = 10) long, 12 (9–14; n = 10) wide, with well-developed deep and superficial roots, elongated shaft and point. Ventral bar (Figure 2D) 41 (37–49; n = 10) long, 8 (5–10; n = 10) total wide, 4 (3–6; n = 10) median wide; slightly V-shaped. Dorsal bar (Figure 2D) 32 (29–37; n = 6) long, 8 (7–9; n = 6) wide, 4 (4–5; n = 6) median wide; curved, U-shaped with rounded terminations. Hooks (Figure 2D) different in size and morphology (proportion between the shank subunits and extension of filamentous hook loop), with a slightly recurved shaft and delicate point, protruding thumb, shank comprising two subunits, and with dilation proximally. Hook pairs 1 and 5 reduced in size. Hook pair 5, 13 (13–14; n = 10) long, first subunit 1/4 shank length, and with filamentous hook (FH) loop reaching thumb level. Hook pair 1, 18 (17–18; n= 10) long, first subunit 1/2 shank length, and FH loop extending to 1/2 first subunit of shank. Hook pair 6, 21 (21–21; n = 10) long, first subunit 1/5 shank length, and FH loop extending to 1/2 first subunit of shank. Hook pairs 2, 3, 4, and 7 similar in size, 29 (25–37; n = 10) long, first subunit 1/6 shank length, and FH loop extending to 1/2 first subunit of shank. Vitellaria scattered throughout trunk, absent in reproductive organ regions. Eggs not observed. Male copulatory organ (Figure 2A) as a coiled tube with approximately 2½ counterclockwise rings, with its diameter reducing along its extension, ending with acute point, 8 (7–9; n = 6) first ring diameter and enlarged base; proximal ring diameter 8 (7–10; n = 10). Accessory piece 9 (7–12; n = 10) long, connected with the base of the MCO by a sinuous ligament, that ends a little distance from base of enlarged and elongated proximal part of accessory piece, that ends pointed, curving backwards. Gonads intercaecal, overlapping. Testis partially dorsal to germarium, not completely visualised. Seminal vesicle as a distal enlargement of the vas deferens; one elongated prostatic reservoir. Germarium 47 (29 and 65; n = 2) long, 15 (11 and 19; n = 2) wide. Vaginal aperture dextral (Figure 2B); vagina slightly sclerotized, with rounded opening followed by a short and slightly curved tube, opening close to the body margin; seminal receptacle, oviduct, ootype and uterus not observed. Vaginal sclerite sinistral (Figure 2C), robust, 24 (21–28; n = 10) long, composed of a grooved rod with a distal hook.

Taxonomic summary

Type-host: *Schizodon nasutus* Kner, 1958 (Characiformes: Anostomidae).

Type-locality: Pardo River (22°59'22.07" S; 48°26'26.20" W), Paranapanema River basin, municipality of Botucatu, São Paulo State, Brazil.

Material deposited: Holotype CHIOC 40288a, paratypes CHIOC 40288b-c.

Site of infestation: Gill filaments.

Infestation level: three of the nine specimens of *S. nasutus* were infested, with 7, 11, and 13 monogeneans per host, respectively.

Etymology: The specific epithet refers to the curved shape of the distal end of the accessory piece of the male copulatory complex (from the Latin *curvo* = arched, curved or bent plus *cuspidis* = tip or point).

Remarks

Urocleidoides curvocuspidis n. sp. can be distinguished from most of its congeners mainly by the combination of the following features: the morphology of its midventral curved and pointed accessory piece, the morphology of the dextral vagina, and the morphology and size of hooks.

The new species morphologically resembles other monogenean species exhibiting a dextral vagina and sinistral vaginal sclerite.

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Among them, *U. paradoxus* is morphologically similar and genetically close-related. However, *Urocleidoides curvocuspidis* n. sp. is easily distinguished from *U. paradoxus* by the shape of the accessory piece and the number of MCO rings (grooved, with two proximal arms, and MCO with 2 rings in *U. paradoxus*, whereas *Urocleidoides curvocuspidis* n. sp. exhibit an accessory piece curved and pointed, and MCO with 2½ counterclockwise rings).The other species with sinistral vaginal sclerite located on the opposite side of the vaginal opening are *U. digitabulum*, *U. ramentacuminatus*, *U. sapucaiensis*, *U. solarivaginatus*, and *U. sinus*. The new species differs from *U. digitabulum* mainly by the shape of the accessory piece (glove finger in *U. digitabulum* vs. curved and pointed in *Urocleidoides curvocuspidis* n. sp.) and vaginal aperture (distal ovate bulb guarding the vaginal aperture in *U. digitabulum* vs. rounded in the new species). In relation to *U. ramentacuminatus*, the new species differs in the shape of MCO (one counterclockwise ring in *U. ramentacuminatus* vs. 2½ counterclockwise rings in *Urocleidoides curvocuspidis* n. sp.) besides the vaginal sclerite (robust in *U. ramentacuminatus* vs. thin in *Urocleidoides curvocuspidis* n. sp.). The new species can be distinguished from *U. sapucaiensis* mainly by the morphology of the accessory piece (V-shaped in *U. sapucaiensis* and curved vs. pointed in *Urocleidoides curvocuspidis* n. sp.) and vagina (a delicate tube with dilatation in the distal portion in *U. sapucaiensis* vs. rounded in *Urocleidoides curvocuspidis* n. sp.). *Urocleidoides sinus* differs from *Urocleidoides curvocuspidis* n. sp. in the morphology of the accessory piece (sigmoid shaped in *U. sinus* and curved vs. pointed in the new species) and vagina (sac-shaped vagina in *U. sinus* vs. rounded in the new species). Finally, the new species can be easily distinguished from *U. solarivaginatus* by the morphology of the vagina (a distal bulb with projections, resembling a spherical shape with the flames of the sun in *U. solarivaginatus* and rounded in *Urocleidoides curvocuspidis* n. sp.) and accessory piece (sinuous shape in *U. solarivaginatus* and curved and pointed in *Urocleidoides curvocuspidis* n. sp.).

Molecular analyses

A partial sequence of the 28S rDNA gene was obtained for one specimen of *Urocleidoides curvocuspidis* n. sp. (1,439 bp long; Genbank accession number OR583687). The final alignment included 74 sequences of members of Dactylogyridae and Diplectanidae; after trimming to the shortest sequence, the alignment was 765 bp long. The ML and BI analyses recovered identical tree topologies with most nodes highly supported (Figure 3). The 28S

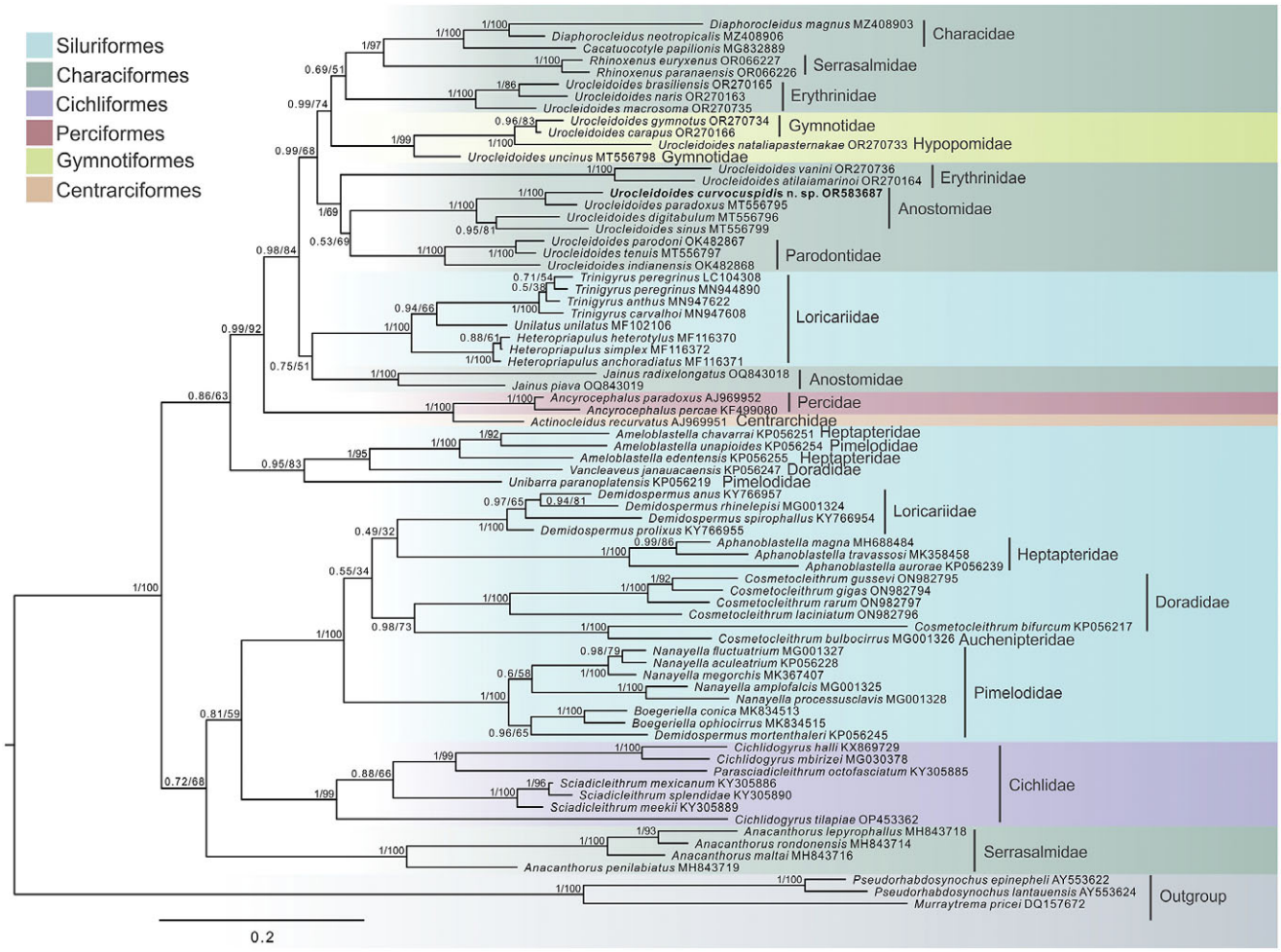

Figure 3. Bayesian topology based on partial 28S rDNA sequences of Dactylogyridae Bychowsky, 1933 and Diplectanidae Monticelli, 1903 species. GenBank accession numbers are after species names. The newly sequenced species are in bold. The support values are included above the nodes as follows: posterior probabilities for BI analysis, followed by bootstrap for the ML analysis. Only nodes with posterior probabilities > 0.95 and bootstrap scores > 70 are considered well supported. Dashes before nodes represent clades that were not recovered by both analyses. Branch length scale bar indicates the number of substitutions per site.

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rDNA sequences of *Urocleidoides* included in this study were not yielded as a monophyletic assemblage since they were placed in three separate clades. The sequences of *U. brasiliensis*, *U. naris*, and *U. macrosoma*, all parasites of Erythrinidae, were grouped together (pp = 1; bootstrap = 100) and positioned as sister taxa to the clade formed by the sequences of *Rhinoxenus* spp., *Diaphorocleidus* spp., and *Cacatuocotyle papilionis* Zago, Franceschini, Müller & da Silva, 2018, albeit with no nodal support (pp = 0.69; bootstrap = 51). The *Urocleidoides* sequences obtained from Gymnotiformes (i.e., *U. gymnotus*, *U. carapus*, *U. nataliapasternakae*, and *U. uncinus*) were grouped together in a strongly supported clade (pp = 1; bootstrap = 99), which was placed as sister taxa to the clade formed by sequences of *U. brasiliensis*, *U. naris* and *U. macrosoma* + *Rhinoxenus* spp., *Diaphorocleidus* spp., and *C. papilionis.* Although highly supported only by the BI analysis (pp = 1; bootstrap = 69), the remaining sequences of *Urocleidoides* parasitising Characiformes (families Erythrinidae, Anostomidae and Paradontidae), were resolved in a monophyletic clade (i.e., *Urocleidoides curvocuspidis* n. sp., *U. paradoxus*, *U. digitabulum*, *U. sinus*, *U. paradoni*, *U. tenuis*, *U. indianensis*, *U. vanini*, and *U. atilaiamarinoi*). The new species was recovered as the sister species of *U. paradoxus.*

A partial sequence of the COI mtDNA gene was obtained for *Urocleidoides curvocuspidis* n. sp. (562 bp long; Genbank accession number OR582424). The final alignment was 338 bp long and comprised 32 sequences of members of Dactylogyridae and Acanthocotylidae. The ML and BI analyses recovered slightly different tree topologies, and most nodes were poorly supported (Figure 4). As with the results obtained for the 28S rDNA gene, the COI mtDNA sequences of *Urocleidoides* included in this study were resolved as non-monophyletic; the analyses grouped the sequences of the new species, *U. malabaricusi*, *U. vanini*, *U. sinus*, *U. tenuis, U. digitabulum, U. naris*, and *U. macrosoma*, all parasites of Characiformes (Erythrinidae, Anostomidae and Parodontidae), contrasting with the 28S rDNA phylogenetic analyses (see Figure 3) where these last two species, *U. naris* and *U. macrosoma,* were placed in a separate clade from the other *Urocleidoides* species. A clade formed by all *Urocleidoides* spp. parasites of Gymnotiformes was, however, resolved as monophyletic (i.e., *U. gymnotus*, *U. carapus*, *U. nataliapasternakae*, *U. uncinus* and *U. cultellus* Mendoza-Franco & Reina, 2008) with high to moderate support values (pp = 1; bootstrap = 79). *Urocleidoides strombicirrus* appeared nested with the sequences of *Diaphorocleidus magnus* and *D. neotropicalis*, and sister to the sequences of *Jainus* spp.

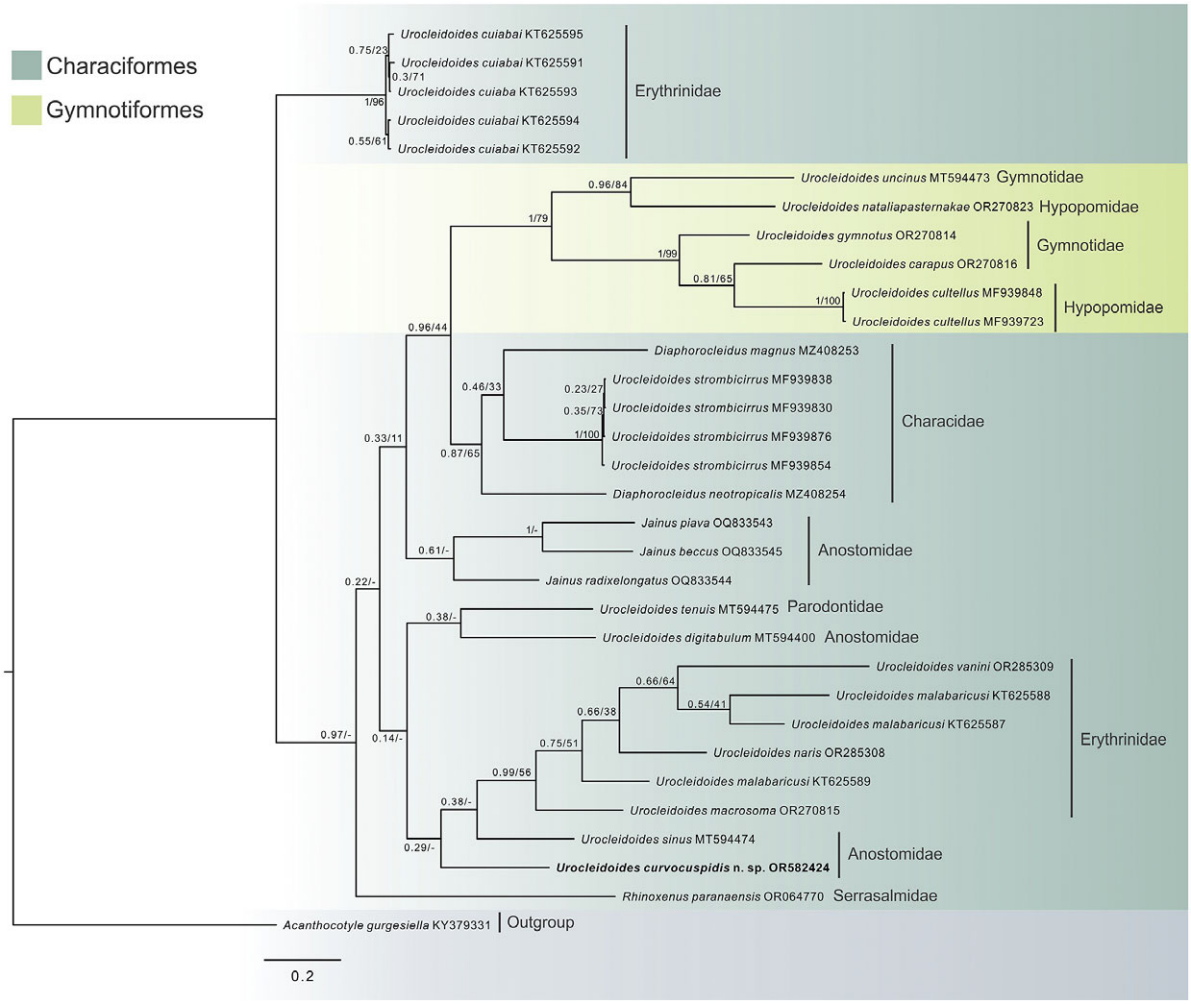

Figure 4. Bayesian topology based on partial COI mtDNA sequences of Dactylogyridae Bychowsky, 1933 and Acanthocotylidae Monticelli, 1903 species. GenBank accession numbers are after species names. The newly sequenced species are in bold. The support values are included above the nodes as follows: posterior probabilities for BI analysis, followed by bootstraps for the ML analysis. Only nodes with posterior probabilities > 0.95 and bootstrap scores > 70 are considered well supported. Dashes before nodes represent clades that were not recovered by both analyses. Branch length scale bar indicates the number of substitutions per site.

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Finally, the sequences of *U. cuiabai* were placed as the early divergent clade of all the other sequences included in the analysis.

Discussion

Our findings based on morphological and molecular analyses provide validation for the recognition of *Urocleidoides curvocuspidis* n. sp. as a new species parasitising *S. nasutus* in the Paranapanema River basin. *Urocleidoides curvocuspidis* n. sp. is morphologically distinguished from its congeners mainly by the combination of features such as the midventral pointed and curved accessory piece, the dextral vaginal aperture, the sinistral vaginal sclerite located on the opposite side of the vaginal opening, a wide U-shaped ventral bar with enlarged terminations, and hooks of four different sizes and shape. The integrative approach employed in this study revealed that the new species bears a closer resemblance to *U. paradoxus*, which is distinguished by the shape of the accessory piece and the sclerotized structures of the haptor (see 'Remarks' section for details); in addition, molecular data resolved these as closely related species, particularly through the 28S rDNA analyses since no COI sequences are available for *U. paradoxus.*

Based on our phylogenetic analyses of the 28S rRNA and the COI mtDNA genes, *Urocleidoides*, as it is presently taxonomically arranged, should not be considered as a natural group, since the sequences used herein were resolved as non-monophyletic, in agreement with previous studies (Oliveira *et al.* 2021; Santos Neto and Domingues 2023). Moreover, in the two studies referred above, the phylogenetic reconstructions lacked sequences of species of *Diaphorocleidus* and *Rhinoxenus*, which were probably not available at the time. The inclusion of these taxa in our phylogenetic reconstructions revealed that some species of *Urocleidoides* spp. infecting Gymnotidae and Erythrinidae were nested with *Diaphorocleidus* spp., *Rhinoxenus* spp., and *C. papilionis.*

In our 28S rRNA and COI mtDNA phylogenetic analyses, the sequences of *Urocleidoides* spp. infecting Gymnotiformes were recovered as monophyletic assemblages with high nodal support and were nested with *Diaphorocleidus* spp., *Rhinoxenus* spp., and *C. papilionis.* Santos-Neto and Domingues (2023) also found the same results in their 28S rDNA and COI mtDNA analyses. In the study of Mendoza-Franco and Reina (2008), authors detected that *Urocleidoides* spp. described from Gymnotiformes shared some common morphological characteristics, such as the absence of eyes, dorsal level ornamentations, and midventral vaginal aperture (see Mendoza-Franco and Reina (2008) for a complete review), implying that such shared traits may be suggested as a potential evolutionary relationship among *Urocleidoides* spp. parasitising Gymnotiformes. Therefore, based on our phylogenetic analyses and the morphological observations of Mendoza-Franco and Reina (2008), we hypothesise that the *Urocleidoides* spp. From Gymnotiformes should be probably reallocated to a new or different genus. Nevertheless, an extensive morphological revision along with more sequence data of *Urocleidoides* spp. From Gymnotiformes is required to thoroughly test this hypothesis, which is beyond the scope of the present study.

Our 28S rDNA phylogenetic analysis grouped the sequences of *U. brasiliensis*, *U. naris*, and *U. macrosoma*, which parasitise erythrinids, in a monophyletic clade with strong nodal support; this result agrees with the findings of Santos Neto and Domingues (2023). This clade appeared more closely related to *Rhinoxenus*, *Diaphorocleidus*, and *Cacatuocotyle* than to other *Urocleidoides* spp., albeit with low nodal support in both BI and ML analyses. Moreover, these species were not grouped with the other *Urocleidoides* spp. parasitising erythrinids (i.e., *U. vanini* and *U. atilaiamarinoi*). A different result was obtained in the COI mtDNA analyses, in which the sequences of *U. naris* and *U. macrosoma* were allocated with all other sequences of *Urocleidoides* parasites of Characiformes (except for *U. strombicirrus* and *U. cuiabai*) and closely related to the other species from erythrinids (i.e., *U. vanini* and *U. malabaricusi*). Nevertheless, most clades in the COI mtDNA analyses were unsupported. These conflicting results and the separation of clades from species that occur in erythrinids, even unsupported, give rise to uncertainties regarding their coevolutionary processes and phylogenetic position and assignation. Santos-Neto and Domingues (2023) obtained similar findings regarding the phylogenetic position of *U. brasiliensis*, *U. naris*, and *U. macrosoma* and suggested that the separation of *Urocleidoides* spp. occurring in erythrinids into separate clades may be associated with ecological host-shifting events. However, it would be ideal to reassess the phylogenetic reconstructions of *Urocleidoides*, adding more sequences of congeneric species sampled from erythrinid fishes and close-related genera, especially for the COI mtDNA gene, along with morphological data to recover more accurate results regarding these species and their position within *Urocleidoides.*

Most of the species of *Urocleidoides* spp. parasitising Characiformes used in this study, including the new species, were recovered in monophyletic clades in both the 28S rDNA and COI mtDNA analyses. These clades were subdivided into groups apparently related to host families (i.e., Anostomidae, Erythrinidae, and Parodontidae) (except for *U. digitabulum* in the COI mtDNA analyses, however unsupported). This result may probably be associated with host specificity. Molecular data for *U. reticulatus*, the type-species of the genus, is currently unavailable. Certainly, the addition of this particular sequence will shed light on the phylogenetic arrangement and species boundaries within *Urocleidoides.* It would also be interesting to add molecular data for the 28S rDNA gene for *U. strombicirrus,* which was recovered nested with *Diaphorocleidus* spp., and for *U. cuiabai*, which was resolved as the early divergent clade of all the other sequences used (all *Urocleidoides* spp., *Diaphorocleidus* sp., *Jainus* spp., and *R. paranaensis*). New morphological and molecular data along with host ecological information should be gathered to confirm the aforementioned hypotheses.

Our study contributes to our understanding of the biodiversity of freshwater fish monogeneans in South America, particularly in the Paranapanema River basin, and sheds light on the importance of the description of the parasite biodiversity of these hosts using morphology and molecular data to enhance comprehension of host–parasite association and phylogenetic relationships.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X23000962.

Data availability statement. All data will be available for consultation. Whenever necessary, they should be requested from the corresponding author. The specimens used for this work will be available in specialized scientific collections.

Authors' contribution. All authors contributed to the study's conception and design. Material preparation, data collection, and analyses were performed by MBE, MMOP, and RJS. The first version of the manuscript was written by MBE, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical standard. 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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