Journal of Helminthology

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A new species of *Urocleidoides* Mizelle & Price 1964 (Monogenea: Dactylogyridae) parasite of *Hemiodus orthonops* Eigenmann & Kennedy, 1903 (Hemiodontidae) from the upper Paraná River floodplain

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Research Paper

Cite this article: Hasuike WT, Scorsim B, Arjona IS, Bellay S, de Oliveira AV and Takemoto RM (2024). A new species of *Urocleidoides* Mizelle & Price 1964 (Monogenea: Dactylogyridae) parasite of *Hemiodus orthonops* Eigenmann & Kennedy, 1903 (Hemiodontidae) from the upper Paraná River floodplain. *Journal of Helminthology*, **98**, e5, 1–10
https://doi.org/10.1017/S0022149X23000925

Received: 13 October 2023
Revised: 06 December 2023
Accepted: 06 December 2023

Keywords:
Dactylogyridae; Hemiodontidae; integrative taxonomy; *COI* mtDNA; 28S rDNA fragments

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Abstract

During the study of ectoparasites (Platyhelminthes) of fish in the floodplain of the Upper Paraná River - Brazil, a new species of *Urocleidoides*, present in the gill filaments of *Hemiodus orthonops* (Hemiodontidae), is described using morphological description and molecular data from the mitochondrial region of cytochrome *c* oxidase, subunit 1 (*COI*) and the partial region of the 28S rDNA gene. *Urocleidoides luquei* n. sp. differs from all its congeners by the presence of a filament that joins the accessory piece to the base of the male copulatory organ and resembles *U. paradoxus* and *U. surianoae.* Phylogenetic analyses using molecular data revealed that *U. luquei* n. sp. forms a paraphyletic group concerning the other *Urocleidoides* species. In this way, as well as contributing to the description of a new species, we seek to encourage and contribute to the increase in research using integrative taxonomy, thus making it possible to elucidate some unresolved questions about the genus *Urocleidoides.*

Introduction

It is estimated that there are around 9,000 species of fish distributed throughout the vast bodies of water on the South American continent, however, only 6,200 species of fish have been described (Birindelli and Sidilauskas 2018). Considering the high specificity of fish host–parasite relationships, especially involving monogenean species (Class Monogenea Van Beneden, 1858), it is expected that with the significant increase observed in the last two decades in the description of new fish species (Seidlová *et al.* 2022), there is a growing contribution to the process of knowledge and description of new species of parasites.

Among the monogeneans, the Dactylogyridae family is the most studied, and most of its representatives are ectoparasites of characiform fishes (Luque *et al.* 2017). *Urocleidoides* Mizelle & Price (1964), after the emendation of Kritsky *et al.* (1986) is characterized by species that have overlapping or tandem gonads, a male copulatory organ (MCO) coiled with counterclockwise rings, a sinistral vaginal sclerite, unmodified anchors, similar hooks with dilated shanks, and pairs of hooks 1 and 5 with reduced sizes. Fifty-two species of *Urocleidoides* are considered valid to date and occur especially in Neotropical Characiform fishes (Ferreira *et al.* 2018; Oliveira *et al.* 2020; Zago *et al.* 2020; De Oliveira *et al.* 2021; Santos Neto and Domingues 2023), especially in hosts from the Anostomidae and Erythrinidae (Zago *et al.* 2020). Almeida *et al.* (2021) confirmed the first record of *Urocleidoides* for the gills of *Hemiodus unimaculatus* (Bloch 1794) in the Jari River, a tributary of the Amazon River; however, there has been no description of a species of *Urocleidoides* for this family of fish.

Molecular tools jointly with morphological analyses have been used to improve the understanding of the taxonomic status of *Urocleidoides*, as well as to delimit the diagnosis of the genus, based especially on 28S rDNA and cytochrome *c* oxidase, subunit 1 (*COI*) sequences (Gasques *et al.* 2016; Zago *et al.* 2020; De Oliveira *et al.* 2021, Santos Neto and Domingues 2023). Although the number of descriptions by integrative taxonomy of new species of monogeneans has increased,

more information is still needed to understand the relationships between species in this group (Zago *et al.* 2020), especially concerning species that have already been described without molecular data.

Therefore, this study aimed to describe a new species of *Urocleidoides* parasitic on the gills of *H. orthonops* Eigenmann, Kennedy, 1903 through the analysis of sclerotized structures and internal organs as well to verify the phylogenetic position of the monogenean specimens of the new species through partial sequences of the *COI* mitochondrial region and 28S rDNA.

Material and methods

Study area and host collection

Specimens of *H. orthonops* (n=20) were collected by the PELD site-6 project in the years 2020 to 2021, in the floodplain of the Upper Paraná River, Brazil (-22°,761',100" S -53°,252',067" W), SISBIO collection authorization under n° 52596-5. This region is the last relatively well-preserved natural area of this river, where there is a great diversity of habitats (Thomaz *et al.* 2007), which are of great importance for the conservation of the diversity of organisms.

The fish were caught using gill nets of different mesh sizes. The captured fish were anesthetized with benzocaine and killed under the Practical Guidelines for Euthanasia of the National Council for the Control of Animal Experimentation (CONCEA), with permission from the Ethics Committee for the Use of Animals of the Universidade Estadual de Maringá (CEUA- nº 1420221018). The fish host was identified according to Ota *et al.* (2018).

Parasitological processing

In the Ichthyoparasitology laboratory of the 'Núcleo de Pesquisa em Limnologia Ictiologia and Aquicultura' (Nupélia), the host fish were separated and triaged using two different methodologies. First (morphological characterization), the hosts had their gills removed and fixed in a 5% formalin solution, to be subsequently separated and transferred to 70% ethanol. Each specimen was mounted between a slide and a coverslip containing Hoyer's medium to observe the sclerotized structures, and other specimens were stained with Gomori's Trichrome to observe the internal organs according to Eiras *et al.* (2006). Secondly (molecular characterization), the hosts were triaged according to the methodology of Da Graça *et al.* (2018) in which the monogeneans are kept intact for molecular analysis. To confirm the morphotype, each specimen was photographed showing the main taxonomic characteristics such as the presence of the vaginal sclerite and the morphology of the male copulatory complex. After confirming the morphotypes, all the materials were transferred to 1,5 ml microtubes each containing 20µl of ultrapure water for subsequent DNA extraction.

The illustrations of the description were prepared with a Nikon Eclipse e200 microscope equipped with a design tube and light phase contrast. All measurements were expressed in micrometers (µm) followed by the mean and amplitude. The ecological descriptors were made according to Bush *et al.* (1997), and the specimens were deposited in the 'Coleção Helmintológica do Instituto Oswaldo Cruz' – Fiocruz.

Molecular identification and characterization

The DNeasy® Blood and Tissue Kit (QIAGEN®) was used to extract the parasites' DNA, following the protocol suggested by the manufacturer. A ProFlex™ 3×32-well thermal cycler was used to carry out the PCR. The DNA amplification reaction consisted of 1U of Taq DNA polymerase (5 U/µL, Invitrogen), Tris-KCL (20 mM Tris-HCl pH 8.4; 50 mM KCl), 1.87 mM $MgCl_2$, 0.1 mM of each dNTP, 4µM of each primer, template DNA (10ng), and Milli-Q water for a final volume of 23µL.

Specific primers were used for the mitochondrial region of cytochrome *c* oxidase, subunit 1 (*COI*), and the 28S rDNA region to amplify the parasites' DNA. The primers used for the *COI* region were COI_Mono_5: 5'TAATWGGTGGKTTTGGTAA-3'; COI_Mono_3: 5'AATGCATMGGAAAAAAACA-3'; and COI_Mono_int3: 5'ACATAATGAAARTGAGC-3' using a protocol adapted from Plaisance *et al.* (2008). The partial region of the 28S gene was amplified with primers U178: 5′-GCACCCG CTGAAYTTAAG-3′ and L1642: 5′-CCAGCGCCATCCATT TTCA-3′ (Lockyer *et al.* 2003), and the internal primer 1500R: 5′-GCTATCCTGAGGGAAACTTCG-3′ (Olson *et al.* 2003) was used for sequencing. Amplification conditions consisted of an initial denaturation at 94°C for 5 min; 30 cycles at 94°C for 30 s, 54°C for 1 min, 72°C for 1 min; and a final extension at 72°C for 5 min.

The PCR products were checked on a 1% agarose gel, and the size of the fragments obtained was estimated using a marker of known molecular weight (100 bp DNA Ladder, Invitrogen 0.5 µg/µL). The products obtained from DNA amplification were purified following the protocol described by Rosenthal *et al.* (1993). Sequencing was carried out by a private company on an Applied Biosystems® AB 3500 Genetic Analyzer and using the BigDye® Terminator kit. Access to genetic heritage was authorized by the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (registration nº AF3A0E7).

The sequences obtained were visualized and manually edited using the BioEdit v7 program (Hall 1999), and the alignment of the sequences was carried out in the MEGA7 software (Kumar *et al.* 2016) by the Clustal W (Thompson *et al.* 1994). Sequences obtained from GenBank and BOLD Systems were added to the analyses for comparison with the sequences obtained in the present study (*COI* mtDNA: 3 and 28S rDNA: 1), totaling 30 sequences for *COI* mtDNA and 17 sequences for 28S rDNA (Table 2).

The JModelTest 2.1.1 software (Darriba *et al.* 2012) was used to select the most suitable evolutionary model for Maximum Likelihood (ML). Based on the Bayesian Information Criterion (BIC), the evolutionary model selected was HKY+G+I for 28S rDNA and HKY +G for *COI* mtDNA. Phylogenetic reconstructions were carried out using the maximum likelihood method with 1000 bootstrap resampling in MEGA7 software and then generated in FigTree v.1.4.3 (Rambaut 2012) and edited in Open Source Inkscape. *Acanthocotyle gurgesiella* Ñacari; Sepulveda; Escribano & Oliva, 2018 (Acanthocotylidae) was used as an outgroup for *COI* (KY379331) and *Pseudorhabdosynochus epinepheli* (Yamaguti, 1938) (Diplectanidae) for 28S (AY553622) according to Zago *et al.* (2020).

The genetic distance *p* was calculated between the species obtained in this study and the sequences available in GenBank using the MEGA7 software. Groups were formed according to the species identified in the database. The sequences obtained in this study have been deposited in GenBank: OR106152 - OR106154 (*COI*); and OR351225 (28S) (Table 1).

Results

Taxonomic summary

Class Monogenea Bychoswky, 1937

Order Dactylogyridea Bychoswky, 1937

https://doi.org/10.1017/S0022149X23000925 Published online by Cambridge University Press

Table 1. List of *Urocleidoides* spp. included in the molecular analyses, with details of the host, locality, and GenBank accession numbers of sequences from the partial 28S rDNA and COI mtDNA genes. New sequences obtained for the present study are in bold

Parasite species	Host/host family	Locality	GenBank ID		Reference
			COI	28S	
***Urocleidoides luquei* n.sp. (U1.3)**	***Hemiodus orthonops*/ Hemiodontidae**	**Brazil: Upper Paraná River Floodplain**	**OR106152**	**---**	**Present study**
***Urocleidoides luquei* n.sp. (U1.2)**	***Hemiodus orthonops*/ Hemiodontidae**	**Brazil: Upper Paraná River Floodplain**	**OR106154**	**OR351225**	**Present study**
***Urocleidoides luquei* n.sp. (U2.3)**	***Hemiodus orthonops*/ Hemiodontidae**	**Brazil: Upper Paraná River Floodplain**	**OR106153**	**---**	**Present study**
Urocleidoides cuiabai	*Hoplias* aff. *malabaricus*/ Erythrinidae	Brazil: Upper Paraná River	KT625591 - KT625595	---	Gasques *et al.* (2016)
Urocleidoides malabaricusi	*Hoplias* aff. *malabaricus*/ Erythrinidae	Brazil: Upper Paraná River	KT625587 - KT625590	---	Gasques *et al.* (2016)
Urocleidoides uncinus	*Gymnotus inaequilabiatus*/ Gymnotidae	Brazil: Upper Paraná River basin	MT594473	MT556798	Zago *et al.* (2020)
Urocleidoides tenuis	*Apareiodon piracicabae*/ Parodontidae	Brazil: Upper Paraná River basin	MT594475	MT556797	Zago *et al.* (2020)
Urocleidoides tenuis	*Parodon nasus*/ Parodontidae	Brazil: Upper Paraná River basin	---	OK465455	Oliveira et al. (2021)
Urocleidoides digitabulum	*Megaleporinus elongatus*/ Anostomidae	Brazil: Upper Paraná River basin	MT594400	MT556796	Zago *et al.* (2020)
Urocleidoides strombicirrus	Characidae	Panama	MF939830, MF939838, MF939748, MF939854, MF939876	---	Unpublished
Urocleidoides cultellus	Hipopomidae	Panama	MF939723, MF939848, MF939762	---	Unpublished
Urocleidoides paradoxus	*Leporinus friderici*/ Anostomidae	Brazil: Upper Paraná River basin	---	MT556795	Zago *et al.* (2020)
Urocleidoides sinus	*Schizodon nasutus*/ Anostomidae	Brazil: Upper Paraná River basin	MT594474	MT556799	Zago *et al.* (2020)
Urocleidoides naris	*Hoplias malabaricus*/ *Erythrinidae*	Brazil: Itabocal River, Irituia, Pará	OR285308	OR270163	Neto e Domingues (2023)
Urocleidoides carapus	*Gymnotus carapo*/ *Gymnotidae*	Brazil: Guamá River, Ourém, Pará	OR270816	OR270166	Neto e Domingues (2023)
Urocleidoides gymnotus	*Gymnotus carapo*/ *Gymnotidae*	Brazil: Guamá River, Ourém, Pará	OR270814	OR270734	Neto e Domingues (2023)
Urocleidoides nataliapasternakae	*Brachyhypopomus brevirostris*/ *Hypopomidae*	Brazil: Guamá River, Ourém, Pará	OR270823	OR270733	Neto e Domingues (2023)
Urocleidoides vanini	*Erythrinus erythrinus*/ *Erythrinidae*	Brazil: São Domingos do Capim, Pará	0R285309	OR270736	Neto e Domingues (2023)
Urocleidoides macrosoma	*Hoplias malabaricus*/ *Erythrinidae*	Brazil: Quatipurú River, Taurí, Pará	OR270815	OR270735	Neto e Domingues (2023)
Urocleidoides atilaiamarinoi	*Hoplerytrinus unitaeniatus*/ *Erythrinidae*	Brazil: Guamá River, Ourém, Pará	---	OR270164	Neto e Domingues (2023)
Urocleidoides brasiliensis	*Hoplias malabaricus*/ *Erythrinidae*	Brazil: Itabocal River, Irituia, Pará	---	OR270165	Neto e Domingues (2023)
Urocleidoides parodoni	*Parodon nasus*/ *Parodontidae*	Brazil: Upper Paraná River basin	---	OK482867	Oliveira et al. (2021)
Urocleidoides indianensis	*Parodon nasus*/ *Parodontidae*	Brazil: Upper Paraná River basin	---	OK482868	Oliveira et al. (2021)

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Table 2. Genetic distance *p* obtained from sequences of the 28S rDNA region for species of the genus *Urocleidoides.* The sequence obtained in this study is in bold

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. U. atilaiamarinoi (OR270164)																
2. U. brasiliensis (OR270165)	0.221															
3. U. carapus (OR270166)	0.183	0.178														
4. U. digitabulum (MT556796)	0.242	0.242	0.230													
5. U. gymnotus (OR270734)	0.192	0.196	0.025	0.233												
6. U. indianensis (OK482868)	0.226	0.212	0.208	0.257	0.212											
7. U. parodoni (OK482867)	0.251	0.201	0.210	0.282	0.214	0.131										
8. U. macrosoma (OR270735)	0.219	0.090	0.169	0.248	0.176	0.221	0.233									
9. U. naris (OR270163)	0.219	0.047	0.187	0.251	0.194	0.226	0.214	0.097								
10. U. nataliapasternakae (OR270733)	0.181	0.172	0.097	0.248	0.117	0.205	0.217	0.176	0.185							
11. U. paradoxus (MT556795)	0.273	0.248	0.244	0.117	0.237	0.262	0.255	0.255	0.257	0.257						
12. U. sinus (MT556799)	0.260	0.246	0.233	0.097	0.233	0.237	0.269	0.251	0.257	0.246	0.135					
13. U. tenuis (OK465455)	0.244	0.203	0.212	0.271	0.214	0.133	0.038	0.230	0.205	0.210	0.248	0.255				
14. U. tenuis (MT556797)	0.242	0.205	0.212	0.275	0.214	0.131	0.032	0.233	0.208	0.212	0.251	0.262	0.007			
15. U. uncinus (MT556798)	0.223	0.194	0.108	0.233	0.102	0.205	0.208	0.190	0.203	0.151	0.237	0.223	0.201	0.201		
16. U. vanini (OR270736)	0.102	0.219	0.203	0.244	0.210	0.255	0.264	0.221	0.217	0.203	0.266	0.264	0.255	0.255	0.233	
***17. U. luquei* n. sp. (OR351225)**	**0.287**	**0.230**	**0.239**	**0.266**	**0.242**	**0.298**	**0.260**	**0.237**	**0.239**	**0.262**	**0.282**	**0.266**	**0.262**	**0.266**	**0.244**	**0.289**

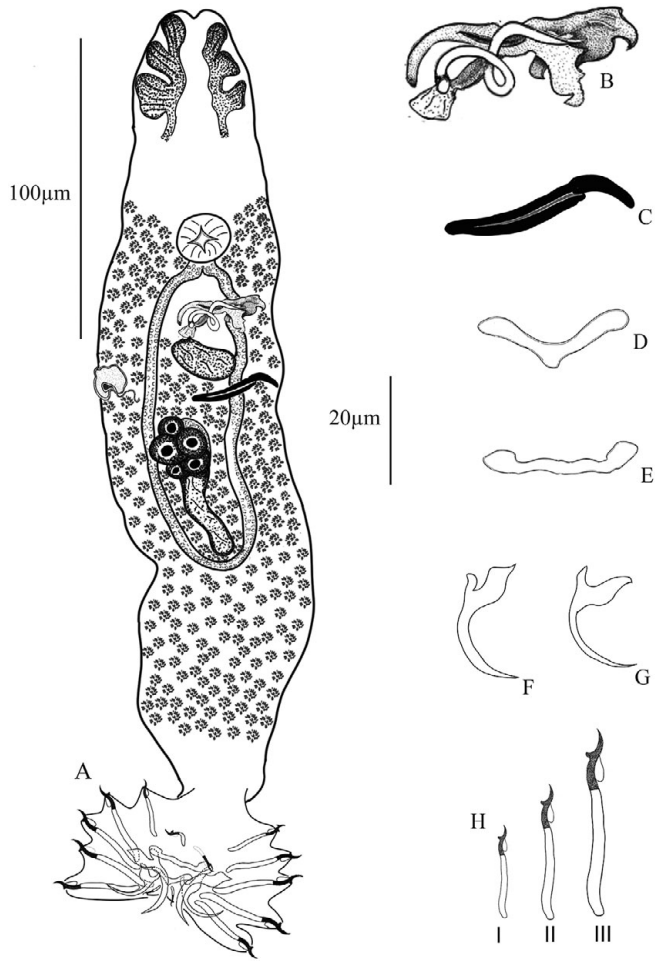

Figure 1. A) *Urocleidoides luquei* n. sp. (ventral view); B) Copulatory complex; C) Vaginal sclerite; D) Dorsal bar; E) Ventral bar; F) Dorsal anchors; G) Ventral anchors; H) Hooks I: pairs 1 and 5, II: pairs 2, 3, 4, III: pairs 6 and 7.

Family Dactylogyridae Bychowsky, 1933

Genus *Urocleidoides* Mizelle & Price, 1964

Species: *Urocleidoides luquei* n. sp. (Figure 1)

Type of host: *Hemiodus orthonops* Eigenmann, Kennedy, 1903

Location type: Upper Paraná River floodplain (-22°,761',100" S -53°, 252',067" W)

Infestation site: gill filaments.

Infestation rate: 50% prevalence, 20 hosts analyzed, 10 parasitized; total parasites found: 29; mean abundance: 1.45 ± 2.38 (1-9); mean intensity of infection: 2.9 ± 2.64.

Specimens deposited (Holotype CHIOC 40270 a), (Paratypes CHIOC 40270 b-j).

Etymology: The specific name refers to a tribute to Dr. José Luis Fernando Luque Alejos for his great contributions to fish parasitology studies in Brazil.

Morphological descriptions of new species

Description: (composite drawing). Based on 7 specimens mounted unstained in Hoyer's medium and 2 stained with Gomori´s trichrome: body elongated, smooth and thin tegument, divided into cephalic region, trunk, peduncle, and haptor. Fusiform body 296.2 (240–84) long and 66.8 (48–96) wide from a central region of the trunk. The cephalic lobes comprises three bilateral pairs of head organs, eyespots, and accessory chromatic granules absent. Mid-ventral subspherical muscular pharynx. Vitellaria dense are distributed throughout the trunk (except in the cephalic region, copulatory complex, peduncle, and haptor). The male copulatory complex is composed of male copulatory organ (MCO) connected to the accessory piece by laminar ligament attached to the base of the MCO; MCO 28 (24.5–34.3) long 1½ rings counterclockwise, base of MCO with sclerotized cap; accessory piece 30.3 (21.5–32.2) long, robust, comprising 2 subunits; anterior subunit serving as guide for de MCO, posterior subunit clamp-shaped; ornamented distal part. Vaginal sclerite 33 (29.4–35.2) long, sigmoidal in shape with single groove striated longitudinally, thumb short, point long. Semi-ovoidal prostatic gland, long and narrow. Testicle partially in tandem with the ovary, Mehlis' glands and seminal vesicle not observed. Vaginal pore dextral ventral; vaginal vestibule slightly sclerotized, vaginal canal comprising slim delicate tube. Sub-hexagonal haptor 76.4 (60–88.8) wide and 57.9 (45.6–74.5) long equipped with seven pairs of hooks according to the distribution of dactylogyrid (Mizelle 1936), similar in shape; Pairs 1 and 5 reduced size; Pair 1 10.2 (7.8–12.7) long, Pair 5 14.6 (12.7–15.6) long; Pairs 2-3-4-6 and 7 similar sizes 34 (29.4–39.2) long; Dorsal anchor 20.5 (17.6–22.5) long, base 8.1 (6.8–8.8) wide, well-developed superficial root and deep root, straight shaft and recurved point. Dorsal bar 'V' shaped 28.4 (24.5–29.4) length. Ventral anchors 22.8 (19.6–24.5) high and 8.6 (4.9–9.8) wide the base, well-developed superficial root and deep root, straight shaft, blade and recurved tip longer than the superficial root. Ventral bar 32 (29.4–35.2) long, with expanded ends wider than long. Ventral bar 32 (29.4–35.2) long, with expanded ends wider than long.

Remarks: *Urocleidoides luquei* n. sp. resembles *U. paradoxus* Kritsky, Thatcher & Boeger, 1986 and *U. surianoae* Rossin & Timi 2016. Both species have a laminar ligament connecting the base of the MCO to the accessory piece, with the main significant difference from the other *Urocleidoides* spp. described as valid. However, it can be easily distinguished from *U. paradoxus* by the shape of the very ornate accessory piece distally. When compared to *U. surianoae*, both species are similar in terms of the morphology of the accessory piece; however, it can be easily distinguished by the morphology of the bars. *U. surianoae* has a large medial antero-posterior development on the ventral bar, a feature not found in the new species.

Molecular data

The partial sequence of the 28S rDNA, with 951 base pairs (bp) after editing and alignment, was obtained for the new species, showing *p*-distance values ranging 23% to 29.8% compared to other sequences in the database, being closer to *U. brasiliensis* Rosim, Mendoza-Franco & Luque, 2011 and more distant from *U. indianensis* De Oliveira *et al.* 2021. The genetic distance values involving the new species were slightly higher when compared to genetic distance values between other species of the genus (Table 2). Based on the 28S rDNA gene tree (Figure 2), *U. luquei* n. sp. was positioned in a distinct clade, even separated from *U. paradoxus*, a morphologically closer species due to the linkage structure between the base of the MCO and the accessory piece.

Furthermore, it is possible to verify a phylogenetic relationship between the species *U. sinus* Zago *et al.* 2020, *U. digitabulum* Zago *et al.* 2020, and *U. paradoxus*, all of which parasitize Anostomidae's fishes. This relationship between parasites that share the same host family can also be seen for *U. tenuis* De Oliveira *et al.* 2021, *U. parodoni* De Oliveira *et al.* 2021, and *U. indianensis* De Oliveira

https://doi.org/10.1017/S0022149X23000925 Published online by Cambridge University Press

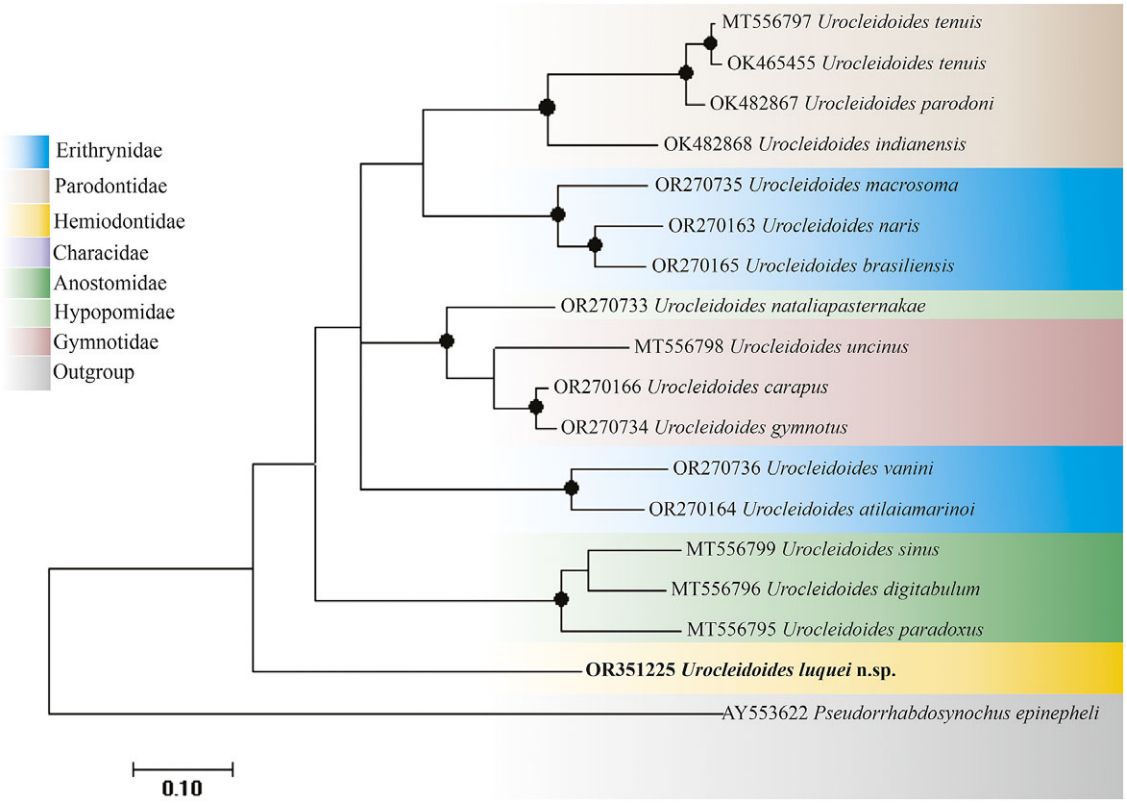

Figure 2. Gene tree constructed using the maximum likelihood method with 1000 bootstrap resamplings for the 28S molecular marker, where *Pseudorhabdosynochus epinepheli* (Yamaguti, 1938) was used as the outgroup and the nucleotide substitution model used was HKY+G+I. The sequence highlighted in bold is the one obtained in this study. Support values above 85 are highlighted with circles, and the colors refer to the host fish's family.

et al. 2021, fish parasites from the Parodontidae. Parasites of the Gymnotidae (*U. uncinus* Zago *et al.* 2020, *U. carapus* Mizelle, Kritsky & Crane, 1968, and *U. gymnotus* Mizelle, Kritsky & Crane, 1968) also formed a distinct clade, closer to *U. nataliapasternakae* Santos Neto & Domingues, 2023, the only representative of Hypopomidae for this marker.

Parasites of the Erythrinidae were the only ones that did not group, forming two distinct clades, one including the species *U. macrossoma* Santos Neto & Domingues, 2023, *U. naris* Rosim, Mendoza-Franco & Luque, 2011, and *U. brasiliensis* and the other with *U. vanini* Santos Neto & Domingues, 2023 and *U. atilaiamarinoi* Santos Neto & Domingues, 2023.

Partial sequences of the *COI* region, with 671 bp after editing and alignment, were obtained for the new species, which were identical to each other and had distances ranging from 58.5% to 65.9% to the other *Urocleidoides* specimens obtained from the database, being closer to *U. vanini* and more distant from *U. cuiabai* Rosim, Mendoza-Franco & Luque, 2011 (Table 3). Considering the high genetic distance values obtained for *U. luquei* n. sp. When compared to the other species of the genus, the mitochondrial marker shows that the new species is differentiated genetically from the others, which is also reflected in the gene tree (Figure 3).

The gene tree for *COI* shows that the new species of *Urocleidoides* (highlighted in bold) constituted a clade distinct from the others. All the sequences analyzed from *U. strombicirrus* (Price & Bussing 1967), the only parasite representing the Characidae, were grouped. Parasites of the Gymnotidae (*U. uncinus*, *U. carapus*, and *U. gymnotus*) also formed a distinct clade, closer to Hypopomidae representatives.

Just like the ribosomal marker, parasites of the Erythrinidae did not group together and formed two distinct clades, one including the species *U. macrossoma*, *U. naris*, *U. vanini*, and *U. malabaricusi* Rosim, Mendoza-Franco & Luque, 2011 and the other with *U. cuibai* Rosim, Mendoza-Franco & Luque, 2011. Finally, representatives of Anostomidae (*U. digitabulum* and *U. sinus*) were also not placed in the same clade.

Discussion

The current study presents a description of a new species of *Urocleidoides* Mizelle & Price (1964), rising to 53 the number of valid species parasitizing various types of hosts recorded in other localities on the Neotropical continent (Zago *et al.* 2020; Santos Neto and Domingues, 2023). In molecular aspects, *U. luquei* n. sp. is genetically differentiated from the species that have sequences available in databases, mainly concerning the mitochondrial marker.

Morphology has undergone changes about the genus. Kritsky *et al.* (1986) based on the genus review proposed a new diagnostic criterion (the presence of a vaginal sclerite); although this characteristic is adopted as a definitive criterion, it has generated doubts because some species are described as representatives of *Urocleidoides* and do not have vaginal sclerite as a diagnostic feature (Kritsky *et al.* 1986; Mendoza-Franco and Reina 2008; Cohen

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Table 3. Genetic distance *p* obtained from *COI* sequences for species of the genus *Urocleidoides.* The sequences obtained in this study are in bold

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	number of individuals
1. U. uncinus																	1
2. U. tenuis	0.282																1
3. U. sinus	0.259	0.266															1
4. U. malabaricusi	0.268	0.244	0.232														4
5. U. digitabulum	0.329	0.223	0.229	0.238													1
6. U. cuiabai	0.284	0.248	0.254	0.242	0.254												5
7. U. cultellus	0.283	0.268	0.275	0.291	0.300	0.295											3
8. U. strombicirrus	0.264	0.244	0.256	0.259	0.268	0.266	0.259										5
9. U. naris	0.243	0.259	0.196	0.176	0.256	0.238	0.300	0.246									1
10. U. carapus	0.233	0.279	0.266	0.291	0.306	0.270	0.228	0.239	0.286								1
11. U. gymnotus	0.233	0.269	0.286	0.288	0.312	0.262	0.234	0.249	0.272	0.173							1
12. U. nataliapasternakae	0.206	0.236	0.236	0.263	0.266	0.252	0.270	0.270	0.233	0.256	0.243						1
13. U. vanini	0.262	0.239	0.259	0.189	0.282	0.254	0.279	0.244	0.179	0.266	0.266	0.219					1
14. U. macrosoma	0.264	0.243	0.213	0.187	0.249	0.221	0.262	0.215	0.179	0.243	0.256	0.236	0.196				1
***15. U. luquei* n.sp. (OR106152)**	**0.615**	**0.611**	**0.625**	**0.602**	**0.598**	**0.659**	**0.628**	**0.631**	**0.618**	**0.628**	**0.645**	**0.625**	**0.585**	**0.648**			**1**
***16. U. luquei* n.sp. (OR106153)**	**0.615**	**0.611**	**0.625**	**0.602**	**0.598**	**0.659**	**0.628**	**0.631**	**0.618**	**0.628**	**0.645**	**0.625**	**0.585**	**0.648**	**0.000**		**1**
***17. U. luquei* n.sp. (OR106154)**	**0.615**	**0.611**	**0.625**	**0.602**	**0.598**	**0.659**	**0.628**	**0.631**	**0.618**	**0.628**	**0.645**	**0.625**	**0.585**	**0.648**	**0.000**	**0.000**	**1**

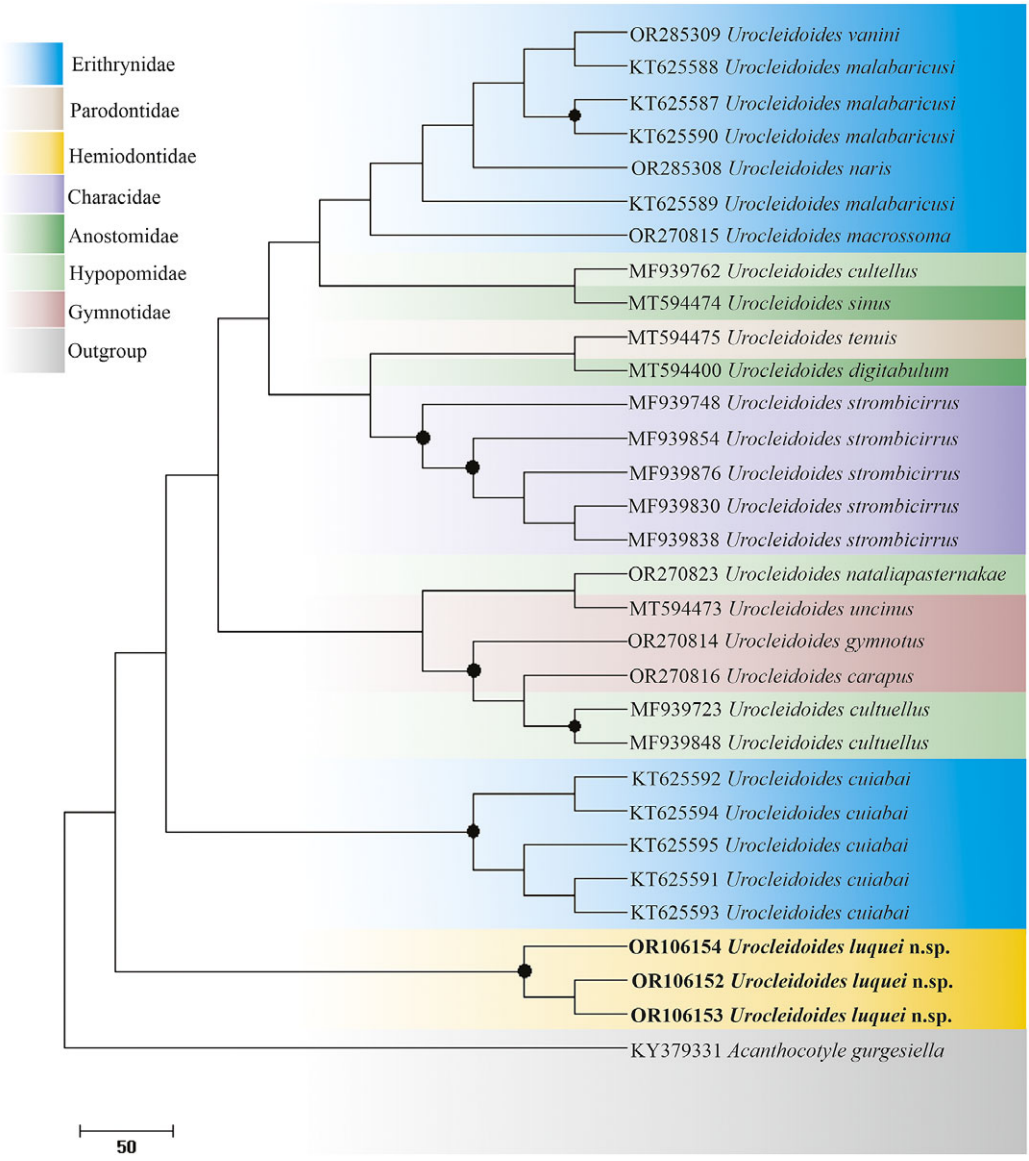

Figure 3. Gene tree constructed using the maximum likelihood method with 1000 bootstrap resamplings for the *COI* molecular marker. *Acanthocotyle gurgesiella* Ñacari *et al.*, 2017 was used as an outgroup, and the nucleotide substitution model used was HKY+G. The sequences highlighted in bold are those obtained in this study. Support values above 85 are highlighted with circles, and the colors refer to the host fish's family.

et al. 2013; Santos Neto and Domingues, 2023). However, it was suggested by Santos Neto and Domingues (2023) that the isolated presence or absence of the vaginal sclerite is insufficient for the diagnosis of the species of the genus. Concerning that, in our analysis, there is no molecular separation, regarding 28S rDNA, between *U. vanini* Santos Neto & Domingues, 2023 (absence of vaginal sclerite) and *U. atilaiamarinoi* Santos Neto & Domingues, 2023 (presence of vaginal sclerite), both grouping in the same clade.

Considering molecular and morphological aspects, there are differences between species. Morphologically, *U. luquei* n. sp. is closer to *U. paradoxus* and *U. surianoae* due to a laminar ligament that connects the base of the MCO to the accessory piece. However, concerning genetic similarity, *U. luquei* was closer to *U. brasiliensis* and *U. vanini*, considering 28S and *COI* markers, respectively. Currently, for *COI* mtDNA, there are 27 sequences available in the database representing 26.92% of the total species described so far for the genus *Urocleidoides* spp. (14 of 52 species) and 16 sequences of 28s rDNA, representing only 28.84% of the species described (of 52 species). So this study used all the sequences available for the genus in the databases. However, the unavailability of sequences to the same species, both for COI and 28S marker, did not allow us to infer, with information from both regions of the DNA, which species is phylogenetically closest to *U. luquei* n. sp.

Based on 28S sequences, the phylogenetic analyses with the marker show close molecular relationships between the species *U. digitabulum*, *U. sinus,* and *U. paradoxus*, all of which parasitize Anostomidae, as well as the similarity between *U. sinus* and *U. digitabulum* has also been observed in previous studies (Zago *et al.* 2020; Santos Neto and Domingues 2023). Furthermore, it is possible to note the relationship between *U. tenuis*, *U. parodoni*, and *U. indianensis*, the three parasites of Parodontidae, as observed by Santos Neto and Domingues (2023). Finally,

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U. nataliapasternakae is the only representative of Hypopomidae, so it is not possible to verify the relationship of parasites belonging to this family.

The species of *Urocleidoides* reported for Erythrinid fish, as observed by Zago *et al.* (2020) and Santos Neto and Domingues (2023), do not represent a monophyletic group, being divided into two clades in the analysis carried out (Figure 2 and 3, blue color) for both markers. This separation may be associated with host exchange events as well as their geographical distribution, which can contribute to shaping the sharing of these parasites (Braga *et al.* 2015; Santos Neto and Domingues 2023), and this pattern can be observed for both studied markers.

Urocleidoides tenuis, being the only representative of Parodontidae for this marker, does not group with the other species; this pattern was also observed by Zago *et al.* (2020) and Santos Neto and Domingues (2023). Moreover, Gymnotidae and Hypopomidae were mixed according to Santos Neto and Domingues (2023); however, in our analysis, although there was also this mixture, for Hypopomidae there was no clumping pattern since one of the sequences was separated from the rest; the same happened with the representatives of Anostomidae.

Finally, this study contributes to the knowledge of the diversity of the parasitic fauna present in the floodplain of the upper Paraná River, Brazil, especially concerning the hosts of the genus *Urocleidoides*. In addition, because *U. luquei* n. sp. is the first species described parasitizing *H. orthonops*, which belongs to the Hemiodontidae family, more studies are needed to monitor the occurrence of this monogenean in the family and elucidate unresolved phylogenetic issues. Just as Santos Neto and Domingues (2023) pointed out, we believe that the phylogenetic relationships in *Urocleidoides* could be elucidated in future studies, with the possible inclusion of DNA sequences of all the species described in the analyses.

Acknowledgements. The authors are grateful to Capes and CNPq for funding the study, to the Núcleo de Pesquisa em Limnologia Ictiologia e Aquicultura - NUPÉLIA-UEM for providing the infrastructure, and to PELD site 6 for the samples obtained in this work.

Financial support. PELD site 6 and Conselho Nacional do Desenvolvimento Científico e Tecnológico supported this work.

Data availability. The datasets generated and analyzed during the study are available in the GenBank repository under the following accession numbers: OR106152, OR106153, OR106154 (COI) and OR351225 (28S).

Competing interest. The authors declare that they have no conflict of interest.

Ethical standard. All applicable guidelines for the care and use of animals were followed.

References

Almeida OM, Oliveira MSB, and Tavares-Dias M (2021) Community and infracommunities of metazoan parasites in *Hemiodus unimaculatus* (Hemiodontidae) from Jari River basin, a tributary of Amazon River (Brazil). *Revista Brasileira de Parasitologia Veterinária* **30**, 1–9.

Birindelli JLO and Sidlauskas BL (2018) Preface: how far has neotropical ichthyology progressed in twenty years? *Neotropical Ichthyology* **16**, 1–8.

Braga MP, Razzolini E, and Boeger WA (2015) Drivers of parasite sharing among Neotropical freshwater fishes. *Journal of Animal Ecology* **84**, 487–497.

Bush AO, Lafferty KD, Lotz JM, and Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. *revisited.* Journal of Parasitology **83**, 575–583.

Cohen SC, Justo MCN, and Kohn A (2013) *South American Monogenoidea parasites of fishes, amphibians and reptiles.* Rio de Janeiro, Oficina de livros.

Da Graça RJ, Fabrin TM, Gasques LS, Prioli SM, Balbuena JA, Prioli AJ, and Takemoto RM (2018) Topological congruence between phylogenies of *Anacanthorus* spp. (Monogenea: Dactylogyridae) and their Characiformes (Actinopterygii) hosts: a case of host–parasite cospeciation. *PLoS One* **13**, 1–14.

Darriba D, Taboada GL, Doallo R, and Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772–772.

De Oliveira GS, Da Silva RJ, Vieira FEG, and Acosta AA (2021) *Urocleidoides* spp. (Monogenea: Dactylogyridae) from the gills of *Parodon nasus* (Characiformes: Parodontidae) from a Brazilian stream with descriptions of two new species. *Zootaxa* **5081**, 535–550.

Eiras JDC, Takemoto RM, and Pavanelli GC (2006) *Métodos de estudo e técnicas laboratoriais em parasitologia de peixes*. 2nd ed. Maringá, Eduem.

Eigenmann CH and Kennedy CH (1903) On a collection of fishes from Paraguay, with a synopsis of the American genera of cichlids. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 497–537.

Ferreira KDC, Rodrigues ARO, Cunha JM, and Domingues MV (2018) Dactylogyrids (Platyhelminthes, Monogenoidea) from the gills of *Hoplias malabaricus* (Characiformes: Erythrinidae) from coastal rivers of the Oriental Amazon Basin: species of *Urocleidoides* and *Constrictoanchoratus* n. gen. *Journal of Helminthology* **92**, 353–368.

Gasques LS, Graça RJ, Prioli SMAP, Takemoto RM, and Prioli AJ (2016) Molecular characterization of *Urocleidoides cuiabai* and *U. malabaricusi* (Monogenea: Dactylogyridae) from the trahira fish *Hoplias* aff. *malabaricus* in the Paraná River, Brazil. *Journal of Helminthology* **90**, 693–697.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Kritsky D, Thatcher V, and Boeger W (1986) Neotropical Monogenea. VIII: revision of *Urocleidoides* (Dactylogyridae, Ancyrocephalinae). *Proceedings of the Helminthological Society of Washington* **53**, 1–37

Kumar S, Stecher G, and Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Lockyer AE, Olson PD, and Littlewood DTJ (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society* **78**, 155–171.

Luque JL, Pereira FB, Alves PV, Oliva ME, and Timi J (2017) Helminth parasites of South American fishes: current status and characterization as a model for studies of biodiversity. *Journal of Helminthology* **91**, 150–164.

Mendoza-Franco EF and Reina RG (2008) Five new species of *Urocleidoides* (Monogenoidea) (Mizelle and Price 1964) Kritsky, Thatcher, and Boeger, 1986, parasitizing the gills of Panamanian freshwater fishes. *Journal of Parasitology* **94**, 793–802.

Mizelle JD (1936) New species of trematodes from the gills of Illinois fishes. *American Midland Naturalist* **17**, 785–806.

Mizelle JD, Kritsky DC, and Crane JW (1968) Studies on monogenetic trematodes. XXXVIII. Ancyrocephalinae from South America with the proposal of *jainus* gen. n. *American Midland Naturalist* **80**, 186–198.

Mizelle JD and Price CE (1964) Studies on monogenetic trematodes. XXVII. Dactylogyrid Species with the Proposal of *Urocleidoides* gen. n. *Journal of Parasitology* **50**, 579.

Ñacari LA, Sepulveda FA, Escribano R, and Oliva ME (2018) Acanthocotyle gurgesiella n. sp.(Monogenea: Acanthocotylidae) from the deep-sea skate Gurgesiella furvescens (Rajidae) in the south-eastern Pacific. *Journal of helminthology*, **92**, 223–227.

Oliveira MSB, Santos-Neto JF, Tavares-Dias M, and Domingues MV (2020) New species of *Urocleidoides* (Monogenoidea: Dactylogyridae) from the gills of two species of anostomidae from the Brazilian Amazon. *Revista Brasileira de Parasitololgia Veterinária* **29**, 1–14.

Olson PD, Cribb TH, Tkach VV, Bray RA, and Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* **33**, 733–755.

Ota RR, Deprá GdeC, Graça WJda, and Pavanelli CS (2018) Peixes da planície de inundação do alto rio Paraná e áreas adjacentes: revised, annotated and updated. *Neotropical Ichthyology* **16**, 1–111.

https://doi.org/10.1017/S0022149X23000925 Published online by Cambridge University Press

Plaisance L, **Rousset V**, **Morand S, and Littlewood TDJ** (2008) Colonization of Pacific islands by parasites of low dispersal ability: phylogeography of two monogenean species parasitizing butterflyfishes in the South Pacific Ocean. *Journal of Biogeography* **35**, 65–87.

Price CE and Bussing WA (1967) Monogenean parasites of Costa Rican fishes. Part I. Descriptions of two new species of *Cleidodiscus* Mueller, 1934. *Rivista di Parassitologia* **28**, 81–86.

Rambaut A (2012) *FigTree v1. 4. Molecular evolution, phylogenetics and epidemiology*. Edinburgh: University of Edinburgh, Institute of Evolutionary Biology.

Rosenthal A, **Coutelle O, and Craxton M** (1993) Large-scale production of DNA sequencing templates by microtitre format PCR. *Nucleic Acids Research* **21**, 173.

Rosim DF, **Mendoza-Franco EF, and Luque JL** (2011) New and previously described species of *Urocleidoides* (Monogenoidea: Dactylogyridae) infecting the gills and nasal cavities of *Hoplias malabaricus* (Characiformes: Erythrinidae) from Brazil. *Journal of Parasitology* **97**, 406–417.

Rossin MA and Timi JT (2016) Dactylogyrid monogeneans parasitising *Cyphocharax voga* (Hensel) (Teleostei: Curimatidae) from the Pampas region, Argentina: new and previously described species. *Systematic Parasitology* **93**, 701–714.

Santos Neto JF and Domingues MV (2023) Integrative taxonomy of *Urocleidoides* spp. (Monogenoidea: Dactylogyridae) parasites of characiform and gymnotiform fishes from the coastal drainages of the Eastern Amazon, Brazil. *Journal of Helminthology* **97**, e64.

Seidlová L, **Benovics M, and Šimková A** (2022) Gill monogeneans of neotropical cichlid fish: diversity, phylogenetic relationships, and host–parasite cophylogenetic associations. *International Journal for Parasitology* **52**, 603–615.

Thomaz SM, **Bini LM, and Bozelli RL** (2007) Floods increase similarity among aquatic habitats in river-floodplain systems. *Hydrobiologia* **579**, 1–13.

Thompson JD, **Higgins DG, and Gibson TJ** (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.

Yamaguti S (1938) Studies on the helminth fauna of Japan. Part 24. Trematodes of fishes, V. *Japanese Journal of Zoology* **8**, 15–74.

Zago AC, **Yamada FH**, **de Oliveira Fadel Yamada P**, **Franceschini L**, **Bongiovani MF, and da Silva RJ** (2020) Seven new species of *Urocleidoides* (Monogenea: Dactylogyridae) from Brazilian fishes supported by morphological and molecular data. *Parasitology Research* **119**, 3255–3283.

https://doi.org/10.1017/S0022149X23000925 Published online by Cambridge University Press