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Two new species of Monopisthocotyla (Dactylogyridea: Dactylogyridae) parasitizing the nasal cavities of *Bryconops melanurus* (Characiformes: Iguanodectidae) from coastal drainages of the Eastern Amazon, Brazil

JOÃO F. SANTOS-NETO^{1,2}, SAYMON M. PAIXÃO¹ & MARCUS V. DOMINGUES^{1,2,*}

¹Laboratório de Sistemática e Coevolução, Universidade Federal do Pará, Campus Universitário de Bragança, Instituto de Estudos Containos Tv. Logndro Bilgino de bairos de bairos (2600,000, Bragança, Bará, Brazil

Costeiros; Tv. Leandro Ribeiro, s/n, bairro Aldeia, 68600–000, Bragança, Pará, Brazil

□ joao_netto182@hotmail.com; ◎ https://orcid.org/0000-0002-1814-4884

saymonufpa@gmail.com; https://orcid.org/0009-0002-3755-9070

²Programa de Pós-Graduação em Biologia Ambiental, Universidade Federal do Pará, Brazil

*Corresponding author: 🖃 mvdomingues@ufpa.br; 💿 https://orcid.org/0000-0002-1230-5464

Abstract

The present study integrates molecular and morphological data to support the proposal of new species of *Telethecium* Kritsky, Van Every & Boeger, 1996 and Diaphorocleidus Jogunoori, Kritsky & Venkatanarasaiah, 2004 from the nasal cavities of Bryconops melanurus (Bloch) of the coastal drainages of the Eastern Amazon. Telethecium tiquira sp. n. is characterized by possessing a male copulatory organ (MCO) with two circular sclerotized brims on the base, a coiled tubular shaft having 1 1/2 counterclockwise rings, an accessory piece with enlarged base, pincer-shaped at the distal portion; a sclerotized calyx-shaped vaginal vestibule, and hooks with proximal shank dilatation comprising 3/4 of the shank length. Also, *Telethecium tiquira* sp. n. can be easily distinguished from other species of the genus by the absence of a protruding bag located at the level of the copulatory complex. *Diaphorocleidus forficata* **sp. n.** is characterized by having a MCO with two counterclockwise rings, circular sclerotized tandem brim associated with the base of the MCO; accessory piece non-articulated with the MCO, bifurcate, pincer-shaped; vaginal pore sinistral-ventral with opening marginal, vaginal canal sclerotized, elongated, comprising one loop in the proximal portion before entering to the seminal receptacle; ventral anchor with shaft elongated and evenly curved on the axis; point short and slightly curved, and hooks similar in shape and size, hooks with proximal dilatation comprising approximately ½ of the shank length. Furthermore, D. forficata sp. n. is supported by phylogenetic analysis based on sequences of the partial 28S rDNA gene, which placed D. forficata sp. n. in a well-supported clade of Diaphorocleidus spp. of characiform fishes. Thus, the two new species described here expand our knowledge about the diversity of monopisthocotylan parasites from the nasal cavities of Neotropical fishes. The findings of this study provide valuable insights into the biodiversity of the region and highlight the importance of further research in this area.

Key words: Parasites, Platyhelminthes, Integrative Taxonomy, Phylogeny, 28S rDNA

Introduction

Since Cohen *et al.* (2013) cataloged the diversity of monopisthocotyls in South America, the number of species has notably increased from 651 to over 800 species (Gibson 2023). However, even though \sim 88% of these species have been reported parasitizing the fish gills, the diversity of monopisthocotyls from other infection sites (e.g., body surface, bladder, cloaca, and nasal cavities) is still poorly known.

Currently, the known diversity of monopisthocotylan parasites of the nasal cavities of neotropical freshwater fish is 24 species belonging to seven genera, as follows: *Rhinoxenus* Kritsky, Boeger & Thatcher, 1988; *Rhinonastes* Kritsky, Thatcher & Boeger, 1988; *Pavanelliella* Kritsky & Boeger, 1998; *Telethecium* Kritsky, Van Every & Boeger, 1996, *Urocleidoides* Mizelle & Price, 1964, *Diaphorocleidus* Jogunoori, Kritsky & Venkatanarasaiah, 2004 and *Characithecium* Mendoza-Franco, Reina & Torchin, 2009 (Table 1). Of this total, 20 species have been reported in Brazilian waters, of which the following ten species have been found parasitizing fishes from the Amazon region: *Rhinoxenus piranhus* Kritsky, Boeger & Thatcher, 1988; *R. arietinus* Kritsky, Boeger & Thatcher, 1988; *R. nyttus* Kritsky, Boeger & Thatcher, 1988; *R. euryxenus* Domingues & Boeger, 2005; *R. cachorra* Soares & Domingues, 2019; *Rhinonastes pseudocapsaloideum* Kritsky, Thatcher & Boeger, 1988; *Telethecium nasalis* Kritsky, Van Every & Boeger, 1996; *T. paniculum* Kritsky, Van Every & Boeger, 1996; *Pavanelliella pavanellii* Kritsky & Boeger, 1998, and *P. jarii* Aguiar, Maia, Silva, Ceccarelli, Domingues & Adriano, 2017 (Cohen *et al.* 2013; Aguiar *et al.* 2017; Soares *et al.* 2019).

	V 1				
Parasite	Type host	Host order	Type locality	References	
Characithecium paranapanemense	Psalidodon paranae	Characiformes	Upper Paraná River basin, Brazil	Zago <i>et al.</i> (2021)	
Characithecium costaricensis	Psalidodon fasciatus	Characiformes	Taquari River, São Paulo, Brazil	Acosta et al. (2015)	
Diaphorocleidus kabatai	Psalidodon fasciatus	Characiformes	Taquari River, São Paulo, Brazil	Acosta <i>et al.</i> (2015)	
Urocleidoides naris	Hoplias malabaricus	Characiformes	Cuiabá, State Brazil	Rosim et al. (2011)	
Rhinoxenus anaclaudiae	Triportheus cf. nematurus	Characiformes	Miranda River, Mato Grosso do Sul, Brazil	Domingues & Boeger (2005)	
Rhinoxenus arietinus	Schizodon fasciatum	Characiformes	Solimões River, Amazonas, Brazil	Kritsky et al. (1988)	
Rhinoxenus bulbovaginatus	Salminus maxillosus	Characiformes	Paraná River, Paraná, Brazil	Boeger et al. (1995)	
Rhinoxenus curimbatae	<i>Prochilodus</i> cf. <i>lineatus</i>	Characiformes	Represa Capivari- Cachoeira, Paraná, Brazil	Domingues & Boeger (2005)	
Rhinoxenus euryxenus	Serrasalmus marginatus	Characiformes	Medalha Basin, Matogrosso do Sul, Brazil	Domingues & Boeger (2005)	
Rhinoxenus guianensis	Curimata cyprinoides	Characiformes	Iracoubo, Degrad Forian, French Guiana	Domingues & Boeger (2005)	
Rhinoxenus nyttus	Schizodon fasciatum	Characiformes	Solimões River, Amazonas, Brazil	Kritsky et al. (1988)	
Rhinoxenus piranhus	Serrasalmus nattereri	Characiformes	Solimões River, Amazonas, Brazil	Kritsky et al. (1988)	
Rhinoxenus cachorra	Hydrolycus armatus	Characiformes	Xingu River, Altamira, Pará, Brazil	Soares et al. (2019)	
Rhinoxenus paranaensis	Serrasalmus maculattus	Characiformes	Paraná River, Entre Ríos, Argentina	Rossin et al. (2019)	
Rhinoxenus argentinensis	Pygocentrus nattereri	Characiformes	Colastiné River, Santa Fé, Argentina	Rossin et al. (2019)	
Rhinonastes pseudocapsaloideum	Prochilodus nigricans	Characiformes	Janauacá Lake, Amazonas, Brazil	Kritsky et al. (1988)	
Rhinonastes curimatae	Prochilodus argenteus	Characiformes	São Francisco River, Minas Gerais, Brazil	Monteiro & Brasil-Sato (2014)	
Pavanelliella pavanellii	Pseudoplatystoma corruscans	Siluriformes	Baia River, Mato Grosso do Sul, Brazil	Kritsky & Boeger (1998)	
Pavanelliella scaphiocotylus	Rhamdia guatemalensis	Siluriformes	Ixin-há Cenote, Yucatán, Mexico	Kritsky & Mendoza- Franco (2003)	
Pavanelliella takemotoi	Pimelodus maculatus	Siluriformes	Mogi Guaçu River, São Paulo, Brazil	Aguiar et al. (2011)	
Pavanelliella laertei	Pimelodus heraldoi	Siluriformes	Mogi Guaçu River, São Paulo, Brazil	Aguiar et al. (2011)	
Pavanelliella jarii	Brachyplatystoma rousseauxii	Siluriformes	Tapajós river, Santarém, Pará, Brazil	Aguiar et al. (2017)	

TABLE 1. Monopisthocotylan parasites of the nasal cavity of Neotropical freshwater fishes.

...Continued on the next page

TABLE 1. (Continued)

Parasite	arasite Type host Host order Type locality		Type locality	References
Telethecium nasalis	Osteoglossum bicirrhosum	Osteoglossiformes	Furo do Catalão, Manaus, Amazonas, Brazil	Kritsky et al. (1996)
Telethecium nasalis	Pterygoplychtys ambrosettii	Siluriformes	Upper Paraná River, São Paulo/Mato Grosso, Brazil	Acosta et al. (2020)
Telethecium nasalis	Rhinelelepis aspera	Siluriformes	Upper Paraná River, São Paulo/Mato Grosso, Brazil	Acosta et al. (2020)
Telethecium paniculum	Pellona flavipinnis	Clupeiformes	Rio Solimões, Manaus, Amazonas, Brazil	Kritsky et al. (1996)
Telethecium tiquira	Bryconops melanurus	Characiformes	Guamá River, Ourém, Pará, Brazil	Present Study
Diaphorocleidus forficata	Bryconops melanurus	Characiformes	Guamá River, Bonito, Pará, Brazil	Present Study

In the present study, two new species of Monopisthocotyla are described (*Telethecium tiquira* **sp. n.** and *Diaphorocleidus forficata* **sp. n.**) from the nasal cavities of *Bryconops melanurus* (Bloch). In addition, partial 28S rDNA gene sequence were used to analyze the phylogenetic position of the *D. forficata* **sp. n.** with others congeneric species.

Material and Methods

Host collection

Specimens of *B. melanurus* were collected with the use of trawls and dip nets in three locations in the Northeastern Pará mesoregion: Igarapé Cururutuia (Caeté River), municipality of Bragança, Pará, Brazil (1°4'44.55"S, 46°44'18.54"W); Balneário Aracu (Guamá River), municipality of Ourém, Pará, Brazil (1°34'1.02"S, 47°9'52.35"W) and Balneário Risca Faca (Guamá River), municipality of Bonito, Pará, Brazil (1°29'12.96"S 47°21'7.34"W). All applicable institutional, national, and international guidelines for the care and use of animals were followed (CEUA-UFPA 3764211223). Specimens were collected under the license for collection of biological material (43381) granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

Parasitological procedures

The nasal cavities of each specimen of *B. melanurus* were opened with dissection scissors and washed with heated water (~65°C) in a Petri dish, and the contents were examined under a stereoscopic microscope (LEICA S6D, Leica Microsystems, Wetzlar, Germany) to detect monopisthocotyls parasites. The parasites found were removed with small probes, and were fixed in 5% formalin for morphological study, or in 96% ethanol for molecular characterization. Specimens intended for studies of internal structures were stained with Gomori's trichrome (Humason 1979; Boeger & Vianna 2006) and mounted in Dammar gum. For the study of sclerotized structures, the specimens were mounted in Hoyer's or Gray & Wess medium (Humason 1979; Boeger & Vianna 2006).

All measurements were made in micrometers following Mizelle & Klucka (1953) and using Leica LAS Interactive Measurement software. Dimensions of organs and other structures represent the greatest measurement in ventral view; lengths of curved or bent structures (anchors, bars, accessory piece) represent the straight-line distances between extreme ends; and the total length of the male copulatory organ (MCO) was measured using the freehand tool in ImageJ software (Rasband 2022). Hooks were classified according to Mizelle & Price (1963). The average measurement is followed by the ranges and the number (n) of specimens measured in parentheses. Illustrations were prepared with the aid of a drawing tube on a microscope with differential interference contrast and phase-contrast optics (LEICA DM 2500, Leica Microsystems, Wetzlar, Germany). Definitions of prevalence

and mean intensity were calculated according to Bush *et al.* (1997). Type specimens, vouchers, and hologenophores presented in this study were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC, Portuguese acronym), Rio de Janeiro, Rio de Janeiro State, Brazil. Acting in accordance with the regulations in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature, details of the new taxa have been submitted to ZooBank.

DNA extraction, amplification, and sequencing

For identification, the parasite specimens subjected to molecular analysis was divided using fine needles under a dissecting microscope. The anterior half of the body was placed in a 1.5 ml microtube with 96% ethanol for genomic DNA extraction. The posterior region of the parasite body was mounted in Hoyer's medium between the slide and cover slip to identify the species. Genomic DNA was extracted using Qiagen Dneasy ® Blood and Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol, with a final volume of 30 µl. Concentration of the DNA was verified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA).

The partial gene 28S rDNA (D1–D3 region) was amplified using a two-round of polymerase chain reaction (PCR). In the first round, DNA was amplified with the primer 1200F (Littlewood & Olson 2001) and D2 (Wu *et al.* 2006). In the second round, for the nested PCRs, the primer combinations were C1 (Wu *et al.* 2006) and D2. The PCR was performed for an initial denaturation step of 94°C for 5 min, followed by 35 cycles at 94°C for 45s, 50°C for 30s, 72°C for 90s, and a final elongation at 72°C for 7 min. Nested PCR was conducted with 1µl of the product of the PCRs, diluted 1:1 in ultrapure water, with the same PCR profile. PCRs were performed in a Matercycler® nexus (Eppendorff, Hamburg, Germany) with a final volume of 25µl and with the DreamTaq Green PCR Master Mix (2×) (Thermo Scientific Wilmington, USA), following the manufacturer's recommendations. A quantity of 0.5 mM of each primer and 3µl of the extracted DNA was used in the reactions.

Amplicons were electrophoresed in 2% agarose gel stained with GelRed (Biotium Inc., Hayward, California, USA), and DNA quality was evaluated on an ultraviolet transilluminator. PCR products were purified using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and sequencing was performed with BigDye® Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, California, USA) in ABI 3500 XL automatic sequencer (Applied Biosystems, California, USA) at the Instituto de Estudos Costeiros (IECOS), Universidade Federal do Pará (UFPA), Pará, Brazil. Sequencing was performed using primers C1 and D2.

Phylogenetic inferences

The sequenced partial 28S rDNA gene obtained in the present study was aligned with 32 sequences of Dactylogyridae, and 3 sequences of Diplectanidae were used as outgroups (*Murraytrema pricei* Bychowsky & Nagibina, 1977, *Pseudorhabdosynochus lantauensis* (Beverley-Burton & Suriano, 1981) Kritsky & Beverley Burton, 1986 and *Pseudorhabdosynochus epinepheli* (Yamaguti, 1938) Kritsky & Beverley-Burton, 1986) retrieved from GenBank (see Table 2). The alignment was generated using default parameters of MUSCLE implemented in Geneious version 7.1.3 (Kearse *et al.* 2012); the extremes of the alignment were trimmed resulting in 622 bp long.

Genetic divergence was calculated using MEGAX software (Kumar *et al.* 2018). JModelTest 2.1.1 software (Posada 2008) was used to select the most appropriate evolutionary model for Maximum Likelihood (ML) and Bayesian Inference (BI) based on the Akaike information criterion (AIC). The chosen evolutionary model was GTR+I+G. The PhyML 3.1 software (Guindon *et al.* 2010) was used to carry out the ML analysis with the bootstrap confidence determined by performing 1000 replications implemented via the web service at ATGC - Montpellier Bioinformatics Platform (http://www.atgc-montpellier.fr/) (Guindon *et al.* 2010). Bayesian Inference (BI) analysis was done using MrBayes v.3.2 (Ronquist & Huelsenbeck 2003) implemented via the computational resource CIPRES (Miller *et al.* 2010). The model indicated by JModelTest was implemented in Mr. Bayes. The analysis posterior probabilities estimated from 1 million generations with two independent runs of four simultaneous Markov Chain Monte Carlo (MCMC) algorithms, sufficient to keep the average standard deviation below 0.001. The MCMC algorithms with the 1000th tree saved and diagnostics for every 1000th generation with burn-in periods were set to

the first 25,000 generations. The Tracer v. 1.6 (Rambaut *et al.* 2014) was used to verify convergence and confirm the effective sample size (ESS) to provide reasonable estimates of variance in model parameters (i.e., ESS values > 200). Trees were visualized using Figtree 1.3.1 (Rambaut 2012) and figures prepared using CorelDraw 2019.

Parasite species	Host species	Host Family	Locality	Genbank ID	Reference	
Dactylogyridae						
Ameloblastella chavarrai	Rhamdia quelen	Heptapteridae	Catemaco Lake, Mexico	KP056251	Mendoza-Palmero et al. (2015)	
Ameloblastella edentensis	Hypophthalmus edentatus	Pimelodidae	Nanay River, Iquitos, Peru	KP056255	Mendoza-Palmero et al. (2015)	
Aphanoblastella eurorae	Goeldiella eques	Heptapteridae	Santa Clara, Iquitos, Peru	KP056239	Mendoza-Palmero et al. (2015)	
Aphanoblastella magna	Pimelodella avanhandavae	Heptapteridae	11		Mendoza-Palmero et al. (2015)	
Aphanoblastella travassosi	Rhamdia guatemalensis	Heptapteridae	Lake Catemaco, Mexico	MK358458	Acosta <i>et al.</i> (2019)	
Cosmetocleithrum bulbocirrus	Pterodoras granulosus	Doradidae	Upper Paraná River basin, Brazil	MG001326	Acosta <i>et al.</i> (2018)	
Cosmetocleithrum bifurcum	Hassar orestis	Doradidae	Aquarium Río Momón, Iquitos, Peru	KP056217	Mendoza-Palmero et al. (2015)	
Demidospermus mortenthaleri	Brachyplatystoma juruense	Pimelodidae	Santa Clara, Peru	KP056245	Mendoza-Palmero et al. (2015)	
Demidospermis prolixus	Loricaria prolixa	Loricariidae	Upper Paraná River basin, Brazil	Upper Paraná River KY766955		
Demidospermus rhinelepisi	Rhinelepis áspera	Loricariidae	Upper Paraná River, MG001324 basin, Brazil		Acosta <i>et al.</i> (2018)	
Dactylogyridae gen. sp. 13	Hypophthalmus edentatus	Pimelodidae	Nanay River, Iquitos, Peru	KP056229	Mendoza-Palmero <i>et al.</i> (2015)	
Heteropriapulus anchoradiatus	Pterygoplychthys ambrosettii	Loricariidae	Upper Paraná River basin, Brazil			
Heteropriapulus heterotylus	Pterygoplychthys ambrosettii	Loricariidae	Upper Paraná River basin, Brazil	MF116370	Acosta <i>et al.</i> (2017)	
Nanayella aculeatrium	Sorubim lima	Pimelodidae	Fish Market, Iquitos, Peru	KP056228	Acosta <i>et al.</i> (2019)	
Nanayella fluctuatrium	Sorubim lima	Pimelodidae	Upper Paraná River basin, Brazil	MG001327	Acosta <i>et al.</i> (2019)	
Trinigyrus anthus	Hypostomus regain	Loricariidae	Upper Paraná River basin, Brazil	MN947622	Francischini et al.(2020)	
Unibarra paranoplatensis	Aguarunichthys torosus	Pimelodidae	Santa Clara, Iquitos, KP056219 Peru		Mendoza-Palmero et al. (2015)	
Unilatus unilatus	Pterygoplychthys ambrosettii	Loricariidae	Upper Paraná River basin, Brazil	MF102106	Acosta <i>et al.</i> (2017)	
Characithecium paranapanemense	Psalidodon paranae	Characidae	Upper Paraná River basin, Brazil	MZ408907 MZ408902 MZ408908	Zago et al. (2021)	
Diaphorocleidus magnus	Astyanax lacustris	Characidae	Upper Paraná River MZ408903 basin, Brazil		Zago et al. (2021)	
Diaphorocleidus neotropicalis	Astyanax lacustris	Characidae	Upper Paraná River basin, Brazil	MZ408906 MZ408909	Zago et al. (2021)	
<i>Diaphorocleidus forficata</i> sp. n.	Bryconops melanurus	Iguanodectidae	Guamá River basin, Brazil	PP081811	Present study	

TABLE 2. List of monopisthocotyls included in the phylogenetic analyses.

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TABLE 2. (Continued)

Parasite species	Host species	Host Family	Locality	Genbank ID	Reference	
Urocleidoides digitabulum	Megaleporinus elongates	Anostomidae	Upper Paraná River basin, Brazil	MT556796	Zago et al. (2020)	
Urocleidoides paradoxus	Leporinus friderici	Anostomidae	Upper Paraná River basin, Brazil	MT556795	Zago et al. (2020)	
Urocleidoides sinus	Schizodon nasutus	Anostomidae	Upper Paraná River basin, Brazil	MT556799	Zago et al. (2020)	
Urocleidoides tenuis	Apareiodon piracicabae	Parodontidae	Upper Paraná River basin, Brazil	MT556797	Zago et al. (2020)	
Urocleidoides indianensis	Parodon nasus	Parodontidae	Capivara River, Upper Paraná River basin	OK482868	Oliveira <i>et al.</i> (2021)	
Urocleidoides parodoni	Parodon nasus	Parodontidae	Capivara River, Upper Paraná River basin	OK482867	Oliveira <i>et al.</i> (2021)	
Vancleavus Janauacaensis	Pterodoras granulosus	Doradidae	Itaya River, Iquitos, Peru	KP056247	Mendoza-Palmero <i>et al.</i> (2015)	
Boegeriella ophiocirrus	Platystomatichths sturio	Pimelodidae	Iquitos, Peru	MK834515	Mendoza-Palmero <i>et al.</i> (2019)	
Diplectanidae						
Murraytrema pricei*	Nibea albiflora	Scianidae	Panyu, China	DQ157672	Wu et al. (2006)	
Pseudorhabdosynochus epinepheli*	Epinephelus bruneus	Serranidae	Huidong, China	AY553622	Wu et al. (2006)	
Pseudorhabdosynochus lantauensis*	Epinephelus bruneus	Serranidae	Huidong, China	AY553624	Wu <i>et al.</i> (2006)	

*Outgroup

Results

Two new species of dactylogyrids have been identified based on the morphological and morphometric features of parasites collected from the nasal cavities of *B. melanurus* from three different areas in the Northeast region of Para state, Brazil. These new species belong to the genera *Diaphorocleidus* and *Telethecium*. The identification of the new species *Diaphorocleidus* is supported by molecular data based on sequences of the partial 28S rDNA gene.

Class Monopisthocotyla Brabec, Salomaki, Kolisko, Scholz & Kuchta, 2023

Order Dactylogyridea Bychowsky, 1937

Dactylogyridae Bychowsky, 1933

Telethecium Kritsky, Van Every & Boeger, 1996

Amended diagnosis

Body comprising cephalic region, trunk, peduncle, haptor. Tegument thin and smooth, with no scales. Cephalic lobes undifferentiated or poorly developed; head organs present; cephalic glands multicellular, lateral or posterolateral to pharynx. Eyespots four; granules ovate to elongate ovate; accessory granules, absent or numerous in cephalic region. Mouth subterminal, midventral, pre-pharyngeal; pharynx comprising muscular, glandular bulb; esophagus present; intestinal ceca two, confluent posterior to gonads, lacking diverticula. Common genital pore midventral near level of intestinal bifurcation. Gonads intercecal, overlapping or slightly tandem; testis, dorsal to germarium. Vas deferens looping left intestinal cecum; seminal vesicle a dilation of vas deferens; one prostatic reservoir. Copulatory

complex comprising male copulatory organ (MCO) and accessory piece. Male copulatory organ consisting of sclerotized coiled tube with counterclockwise rings; accessory piece sclerotized, articulated to MCO base, bifurcated, with distal portion serving as guide for MCO. Protruding bag containing distal portion of copulatory complex, midventral, present or absent. Vagina single, vaginal pore sinistral, marginal or submarginal; vaginal vestibule sclerotized; vaginal canal sclerotized, elongated, straight or sinuous. Seminal receptacle pre-germarial. Vitellaria well developed, coextensive with intestinal ceca, absent from regions of other reproductive organs. Haptor globose or posteroventrally concave; 14 similar hooks (12 submarginal and two subcentral), with slightly protruding or depressed thumb, shank comprised of 2 subunits. Anchor/bar complexes absent. Parasites of the nasal cavities of freshwater Neotropical fishes.

Remarks: *Telethecium* was proposed by Kritsky *et al.* (1996) to accommodate their new species from Central Amazon, *T. nasalis* (type-species) and *T. paniculum* from the nasal cavities of *Osteoglossum bicirrhosum* (Cuvier) (Osteoglossiformes, Osteoglossidae) and *Pellona flavipinnis* (Valenciennes) (Clupeiformes, Pristigasteridae), respectively. The genus was characterized by having an antero-midventral protruding bag containing distal portion of copulatory complex, accessory piece bifurcated and articulated to the male copulatory organ base, sinistral vaginal aperture, overlapping gonads, haptor posteroventrally concave without anchor/bar complexes, and hooks distribution (12 marginal, 2 subcentral). However, monopisthocotyls collected from nasal cavities of Characiformes during the present survey possessed a mix of these morphological features, suggesting that re-evaluation of the genus was necessary. In the species recovered from *B. melanurus*, the protruding bag containing the terminal portion of the copulatory complex is absent, as well as the form of the haptor is globose, and not posteroventrally concave as reported for the two known species. Although these two features seem to be important requirements for *Telethecium*, we have adopted a conservative approach by considering the new species as member of *Telethecium* mainly because the new species shares with other species of *Telethecium* the morphology of the copulatory complex, haptor without anchor/bar complex, hooks distribution, and site of infection.

Telethecium tiquira sp. n.

(Figs. 1A-D)

Type-host: Bryconops melanurus (Bloch).

Type locality: Balneário Aracu (Basin of the Guamá River), municipality of Ourém, Pará, Brazil (1°34'1.02"S, 47°9'52.35"W).

Site of infection: Nasal cavity.

Prevalence: 27% of 18 hosts examined.

Mean intensity: 1.2 parasites per infected host.

ZooBank registration: 07E0F90B-F2CC-42C5-B95F-A80B505A18CC.

Specimens deposited: Holotype, CHIOC 40408; paratypes, CHIOC 40409–40411.

Etymology: The specific name is derived from the local name of the host, "tiquira", used by the people of the Northeast Amazon, Brazil.

Description: (Based on six adult specimens; three mounted in Gomori's trichrome, and three mounted in Hoyer's medium). Body fusiform, 164 (125–230; n=5) long, 85 (52–120; n=5) wide, length excluding haptor (Fig. 1A). Cephalic margin possessing 2 terminal cephalic lobes poorly developed (Fig. 1A). Three bilateral pairs of head organs moderately developed. Eyespots, equidistant, posterior pair larger than the anterior pair; accessory granules absent or sparse in cephalic area; granule elongate ovate. Pharynx subovate, 14 (13–17; n=3) long, 14 (13–17; n=3) wide; (Fig. 1A). Genital pore opening midventral next to level of cecal bifurcation. Gonads overlapping. Male copulatory organ (MCO) with two circular sclerotized brims on base, coiled tubular shaft having 1½ counterclockwise rings, 100 (n=2) long (Fig. 1B). Accessory piece articulated with MCO, comprising complex sheath with proximal portion expanded, distal portion bifid, pincer-shaped (Fig. 1B). Protruding bag containing the terminal portion of the copulatory complex absent. Testis ovate, 20 (17–22; n=3) long, 15 (12–19; n= 3) wide. Vas deferens looping left intestinal cecum anterior to germarium (observed only in paratypes). Seminal vesicle with loop before entering the MCO base (Fig. 1A). Prostatic reservoir sigmoid, lying posterior to MCO. Vaginal pore ventral, sub-marginal; vaginal vestibule sclerotized, calyx-shaped; vaginal canal sclerotized (Fig. 1C). Germarium bacilliform, 22 (20–25; n=3) long, 13 (10–15; n=3) wide. Seminal receptacle pre-germarial, sacculiform (Fig. 1A). Mehlis' glands, ootype, eggs, and uterus not observed. Peduncle short; haptor globose, sub-hexagonal, 36 (27–42; n=5) long, 60 (45–89;

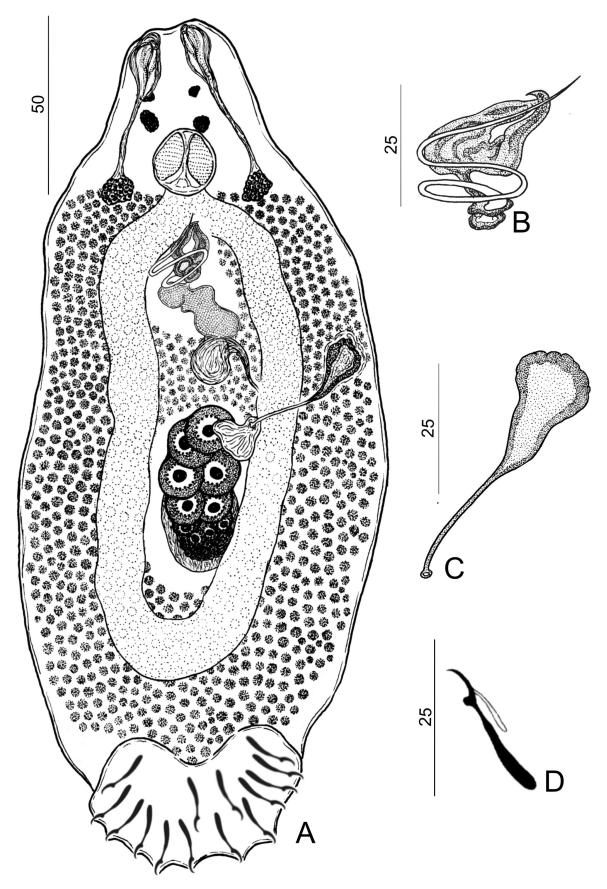


FIGURE 1. *Telethecium tiquira* sp. n. A. Holotype, whole-body (ventral); B. MCO; C. Vagina; D. Hooks. Scales: $A = 50 \mu m$; $B-D = 25 \mu m$.

n=5) wide (Fig. 1A). Hooks similar in shape and size, 20 (17–21; n=3) long, with proximal dilatation comprising 3/4 of the shank length, thumb truncate, elongate slightly curved shaft, delicate point, filament hook loop extended to near beginning of shank dilation (Fig. 1D).

Remarks: The new species proposed here shares with species of *Telethecium* the morphology of the copulatory complex, the position of the vaginal aperture, hooks distribution, and the absence of anchor/bar complex. Based on these features, we decided to include the new species as a member of *Telethecium*, even though the new species does not present the protuberant bag associated with the copulatory complex. *Telethecium tiquira* **sp. n.** is most similar to *T. nasalis* by sharing the morphology of the accessory piece (i.e., pincer-shaped distal portion) and hooks (i.e., slightly protruding broad thumb). However, the new species can be easily distinguished from this species and other congeners by having a globose haptor (concave in other species), a MCO with two circular sclerotized brims (absent in the other species), and a copulatory complex not associated with a protruding bag (copulatory complex located inside a bag that protrudes from the anteroventral surface of the trunk in the other species).

Diaphorocleidus Jogunoori, Kritsky & Venkatanarasaiah, 2004

Diaphorocleidus forficata sp. n.

(Figs. 2A-H)

Type-host: Bryconops melanurus (Bloch).

Type-locality: Balneário Risca Faca (Basin of the Guamá River), municipality of Bonito, Pará, Brazil (1°29'12.96"S, 47°21'7.34"W).

Other-localities: *Bryconops melanurus* (Prevalence: 100% of three hosts examined; mean intensity: one parasite per host), Igarapé Cururutuia (Basin of the Caeté River), municipality of Bragança, Pará, Brazil (1°4'44.55"S, 46°44'18.54"W).

Site of infection: Nasal cavity.

Prevalence: 75% of 10 hosts examined.

Mean intensity: 1.33 parasites per host.

ZooBank registration: 6D0A3290-0C24-489D-87D4-8B26EE688565.

Representative DNA sequence: 722 bp long of partial sequence of the 28S rDNA (Genbank accession number PP081811).

Specimens deposited: Holotype, CHIOC 40297a; paratypes, CHIOC 40297b–c, 40298, 40299a–c, 40300a-b, 40401–40403; vouchers, CHIOC 40404–40406; hologenophore, CHIOC 40407.

Comparative measurements: Table 3.

Etymology: The specific epithet is derived from Latin (*forficat* = bifurcated) and refers to the morphology of the accessory piece, which is bifurcated.

Description: (Based on 12 adult specimens; five mounted in Gomori's trichrome, five mounted in Hoyer's medium, two mounted in Grey & Wess). Body fusiform, 310 (221-460; n=10) long, 174 (92-323; n=10) wide, length excluding haptor (Fig. 2A). Cephalic lobes poorly developed, bilateral; three pairs of head organs moderately developed; cephalic glands not observed (Fig. 2A). Eyespots with posterior pair larger than anterior pair; granules ovate; accessory granules sparse in cephalic area; (Fig. 2A). Pharynx subspherical, 21 (19–24; n=3) long, 19 (18–21; n=3) wide; esophagus elongate (Fig. 2.A). Male copulatory organ 189 (164–216; n=6) long (Fig. 2B) coiled, tubular with two counterclockwise rings, circular sclerotized tandem brim associated with the base of the MCO. Accessory piece non-articulated with the MCO base, bifurcate, pincer-shaped, with one branch bifurcated at distal portion, serving as guide for distal portion of MCO; articulation process absent (Fig. 2B). Testis spherical, 25 (23-27; n=2) long, 17 (16–18; n=2) wide. Seminal vesicle sigmoid (observed only in paratypes). Prostatic reservoir elongate to bacilliform (Fig. 2A). Vaginal pore sinistral-ventral with opening marginal, vaginal vestibule slightly sclerotized, vaginal canal sclerotized, elongated, comprising proximal portion loop before entering seminal receptacle (Fig. 2A, C). Germarium ovate, 35 (24-46; n=2) long, 20 (16-24; n=2) wide (Fig. 2A). Seminal receptacle spherical (Fig. 2A). Eggs, Mehlis' glands, ootype, and uterus not observed. Peduncle short and broad (Fig. 2A). Haptor subhexagonal, 56 (38–77; n=8) long, 94 (71–118; n=8) wide (Fig. 2A). Dissimilar anchors with well-developed roots (Figs. 2G–H). Ventral anchor, inner 33 (25–40; n=10) long, outer 48 (31–56; n=10) long, base 11 (7–14; n=10) long; with superficial root well developed, subtriangular, distal portion slightly tapered; deep root developed, elongated

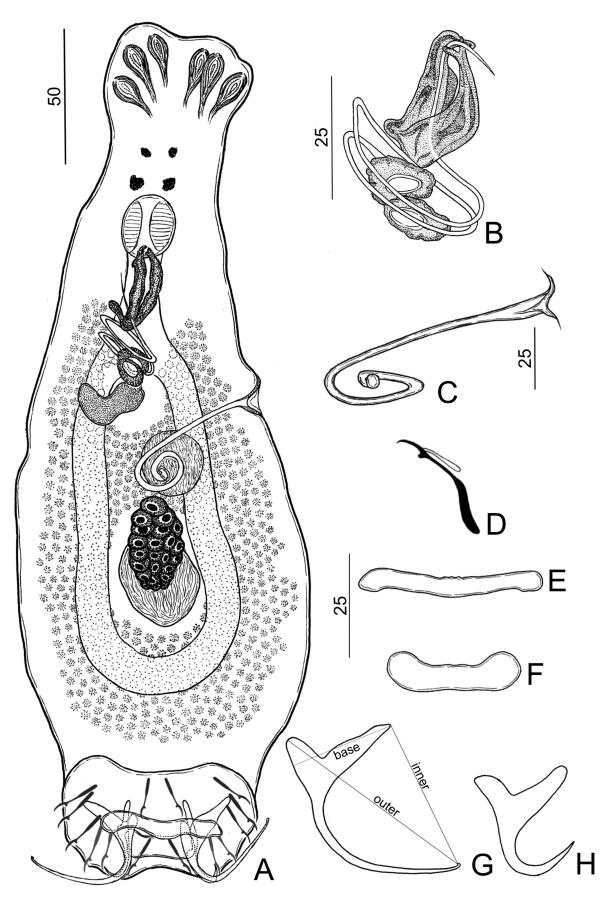


FIGURE 2. *Diaphorocleidus forficata* **sp. n. A.** Holotype, whole-body (ventral); **B.** MCO; **C.** Vagina; **D.** Hooks; **E.** Ventral bar; **F.** Dorsal bar; **G.** Ventral anchor; **H.** Dorsal anchor. Scales: **A** = 50μm; **B**–**H** = 25μm.

and with rounded surface; shaft elongated and evenly curved on the axis; point short and slightly curved; shaft/point exceeding the level of the superficial root (Fig. 2G). Dorsal anchor, inner 21 (19–26; n=7) long, outer 29 (25–33; n=7) long, base 10 (8–12; n=7) long, with superficial root well developed and elongated; deep root, developed, and conical; shaft short and curved on the axis; point elongated, extending to the end of the superficial root (Fig. 2H). Ventral bar rod-shaped, slightly arced, with terminations curved in posterior direction 56 (44–72; n=8) long, 6 (5–8; n=8) wide, (Fig. 2E). Dorsal bar robust, smaller than the ventral bar, with ends slightly rounded or truncated, 37 (28–50; n=9) long, 7 (6–10; n=9) wide (Fig. 2F). Hooks similar in shape and size, 25 (23–27; n=7) long, shank with proximal dilatation comprising approximately $\frac{1}{2}$ of the shank length, slightly curved shaft, point slightly curved, erect thumb, hook filament delicate, extending to the beginning of the dilation (Fig. 2D).

Structures	Bonito*	Ν	Bragança	Ν	
Body					
Length	310 (221–460)	10	298 (276–317)	3	
Width	174 (92–323)	10	169 (161–173)	3	
Haptor					
Length	56 (38–77)	8	64 (62–70)	2	
Width	94 (71–118)	8	132 (124–141)	2	
Pharynx					
Length	21 (19–24)	3	_	_	
Width	19 (18–21)	3	_	_	
MCO					
Length	189 (164–216)	6	203 (190–216)	2	
Ventral bar					
Length	56 (44–72)	8	51 (45–57)	2	
Width	6 (5-8)	8	6 (6)	2	
Dorsal bar					
Length	37 (28–50)	9	34 (27–41)	2	
Width	7 (6–10)	9	9 (8–10)	2	
Ventral anchor					
Outer	48 (31–56)	10	53 (51–57)	3	
Inner	33 (25–40)	10	34 (33–35)	3	
Base	11 (7–14)	10	10 (9–11)	3	
Dorsal anchor					
Outer	29 (25–33)	7	32 (31–36)	3	
Inner	21 (19–26)	7	22 (21–23)	3	
Base	10 (8–12)	7	9 (6–13)	3	
Germarium					
Length	35 (24–46)	2	_	_	
Width	20 (16–24)	2	_	_	
Testis					
Length	25 (23–27)	2	_	_	
Width	17 (16–18)	2	_	_	
Hooks					
Pairs 1–7	25 (23–27)	7	26 (25–27)	2	

TABLE 3. Comparative measurements (μ m) of the specimens of *Diaphorocleidus forficata* **sp. n.** of the nasal cavities of *B. melanurus* from two localities in the Pará, Brazil.

MCO = male copulatory organ.

*Type locality.

Remarks: *Diaphorocleidus forficata* **sp. n.** exhibits morphological similarities with the other species of the genus such as the MCO tubular with counterclockwise rings; accessory piece non-articulated with the base of the MCO; a vaginal pore submarginal and sinistral; overlapping gonads; and hooks similar in shape having shank comprising two subunits with proximal dilation (sensu Jogunoori *et al.* 2014). However, the new species can be easily distinguished from the congeners by having a MCO with two counterclockwise rings; an accessory piece with a bifurcated branch at the distal end; a vaginal canal elongated, with one loop in the proximal region before connecting to the seminal receptacle; dissimilar ventral and dorsal anchors; ventral anchor with shaft/point exceeding the level of its superficial root.

Currently, only two species of dactylogyrids are reported from Iguanodectidae fishes: *Jainus robustus* Mizelle, Kritsky & Crane, 1968 and *Diaphorocleidus affinis* (Mizelle, Kritsky & Crane, 1968) from the gills of *Bryconops affinis* (Gunther). *Diaphorocleidus forficata* **sp. n.** is easily distinguishable from *D. affinis* mainly by the morphology of the ventral and dorsal bars (dorsal bar V-shaped and ventral bar U-shaped with enlarged ends in *D. affinis*), while *D. forficata* **sp. n.** has a rod-shaped ventral bar and robust dorsal bar, which is smaller than the ventral bar, with ends slightly rounded or truncated.

Molecular data and phylogenetic inference

The ML and BI phylogenetics analysis based on partial 28S rDNA sequences converged with similar topologies, which differed only in the posterior probabilities (P) and boostraps (B) values (Fig. 3). The tree topology recovered two main clades (Clades A and B). Clade A (BI, P = 0.95) is divided into subclades A1–A5. Subclade A1 (BI, P = 1; ML, B = 81) comprises monopisthocotylan parasites of pimelodid catfishes (*Unibarra paranoplatensis* Suriano & Incorvaia, 1995 and *Ameloblastella edentesis* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016), heptapterids (*Ameloblastella chavarriai* Kritsky, Mendoza-Franco & Scholz, 2000) and doradids (*Vancleavus janauacaensis* Kritsky, Thatcher & Boeger, 1986). Subclade A2 (BI, P = 0.88; ML, B = 55) comprises monopisthocotyls of characiforms and siluriforms. The subclade A3 (BI, P = 0.77) is divided into two further subclades (subclades A4 and A5), where the subclade A4 (BI, P = 1; ML, B = 95) comprises species *Heteropriapulus* spp., *Trinigyrus anthus* Franceschini, Acosta, Zago, Müller & Da Silva, 2020 and *Unilatus unilatus* Mizelle & Kritsky, 1967 from loricariid catfishes. Subclade A5 (BI, P = 99; ML, B = 73) comprises *Urocleidoides* spp. and *Diaphorocleidus* spp. from characiforms. The clade formed by *Diaphorocleidus* spp. is well supported (BI, P = 1; ML, B = 100). *Diaphorocleidus forficata* **sp. n.** appears as a sister taxon of the clade formed by *D. neotropicalis* Zago, Franceschini, Abdallah, Müller, Azevedo, da Silva, 2021 and *D. magnus* Zago, Franceschini, Abdallah, Müller, Azevedo, da Silva, 2021 and *D. magnus* Zago, Franceschini, Abdallah, Müller, Azevedo, da Silva, 2021 (BI, P = 73; ML, B = 52) (see Fig. 3).

	1	2	3	4	5	6	7	8	9
1. U. indianensis - OK482868									
2. U. tenuis - MT556797	10.9								
3. U. parodoni - OK482867	10.5	2.7							
4. U. paradoxus - MT556795	18.2	19.9	19.4						
5. U. digitabulum - MT556796	16.9	19.9	19	8.7					
6. U. sinus - MT556799	17.6	19.4	19	9.4	6.9				
7. D. forficata sp. n.	17.4	19.4	18.7	18.7	18.7	18.9			
8. D. magnus - MZ408903	16.5	18.5	18	16.9	18.4	18.9	11.6		
9. D. neotropicalis - MZ408906	15.6	18	17.1	18.7	18.4	19.1	12	8.4	
10. D. neotropicalis - MZ408909	15.6	18	17.1	18.7	18.4	19.1	12	8.4	0

TABLE 4. Pairwise genetic identities of 28S rDNA sequences selected between *Urocleidoides* spp. and *Diaphorocleidus* spp.

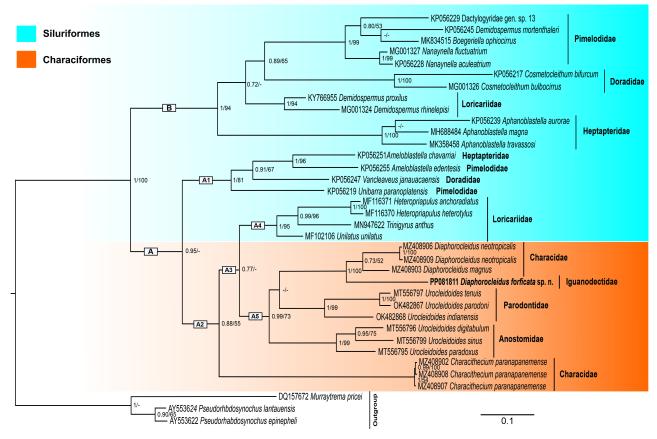


FIGURE 3. Bayesian topology based on partial 28S rDNA (622 bp long) sequences of dactylogyrids from the Neotropical region. The species newly sequenced for the present study is in bold. Species name precedes the GenBank sequence ID. Bootstrap support values and posterior probabilities are given between the branches (bootstrap values <50 and posterior probabilities <0.70 not reported). The branch length scale bar indicates the number of substitutions per site.

The Clade B (BI, P = 1; ML, B = 94) comprises monopisthocotylan parasites of pimelodid catfishes (*Nanayella* spp., *Boegeriella ophiocirrus* (Mendoza-Palmero, Mendoza-Franco, Acosta & Scholz, 2019) = *Walteriella ophiocirrus*), Dactylogyridae gen. sp. 13., *Demidospermus mortenthaleri* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012), doradids (*Cosmetocleithrum* spp.); loricariids (*Demidospermus* spp.) and heptapterids (*Aphanoblastella* spp.).

The genetic divergence was estimated (28S rDNA) among all Monopisthocotyla included in the phylogenetic analysis. Here, we present information on genetic divergence only between species of *Urocleidoides* and *Diaphorocleidus*, as they are closely related (Clade A5, Fig. 3). The genetic divergence between *D. forficata* **sp. n.** and specimens of *Urocleidoides* spp. varied between 17.4 and 19.4%. On the other, hand, the genetic divergence among the new species, *D. neotropicalis* and *D. magnus*, was 12% and 11.6%, respectively (see Table 4).

Discussion

The Characiformes group includes around 1,700 species of continental fish found in South America, which accounts for approximately 33% of all fish species in the continent (Reis *et al.* 2016). However, only 79 species of characiform fish have been examined for monopisthocotyls in their nasal cavities (Domingues & Boeger 2005; Cohen *et al.* 2013; Rossin *et al.* 2019; Soares *et al.* 2019). In this study, *Telethecium tiquira* and *Diaphorocleidus forficata* are reported parasitizing the nasal cavities of *B. melanurus* (Iguanodectidae: Characiformes), contributing to the knowledge of the diversity of helminth parasites from the Neotropical region.

Considering the two new species described here, we increase the knowledge of the species diversity of *Diaphorocleidus* (13 species) and *Telethecium* (3 species). The *Diaphorocleidus* species are reported parasitizing

the gills, nasal cavities and body surface of six characiform families (Acestrorhynchidae, Bryconidae, Characidae, Curimatidae, Hemiodontidae and Iguanodectidae) (Mendoza-Franco *et al.* 2019; Zago *et al.* 2021). *Diaphorocleidus* spp. are characterized mainly with counterclockwise rings in the MCO, an accessory piece that is not articulated with the MCO, a sinistral vagina, and hooks with proximal shank dilatation (Jogunoori *et al.* 2014). The phylogenetic analysis presented in this study, also supports the proposal of the *D. forficata*. Zago *et al.* (2021) performed a phylogenetic analysis based on the 28S rDNA and COI mtDNA gene sequences including two species *Diaphorocleidus* (*D. magnus* e *D. neotropicalis*). As a result, these authors recovered a monophyletic clade for these species. Based on our phylogenetic analysis using 28S rDNA gene sequences, we found that *D. forficata* is closely related to a clade formed by *D. neotropicalis* and *D. magnus*, supporting the hypothesis of Zago *et al.* (2021) (Figure 3).

According to Zago *et al.* (2021), the occurrence of *Diaphorocleidus* spp. in six characiform fish families may be related to the phylogenetic relationships established among the hosts. Due to the diversification of this fish lineage having occurred in the Neotropical continent, the phylogenetic contiguity observed in Characiformes probably allowed the parasite species to reach hosts belonging to phylogenetically distant lineages (see Braga *et al.* 2015). This pattern has already been evidenced in other lineages of Neotropical dactylogyrids (e.g., *Anacanthorus* spp.) and their Characiformes hosts (see Santos-Neto *et al.* 2019). In this context, the phylogenetic contiguity observed among the neotropical characiforms could be contributing to a greater reach of the *Diaphorocleidus* spp. in host characiform families.

Telethecium was proposed to accommodate two species: T. nasalis and T. paniculum from the nasal cavities of the arowana, Osteoglossum bicirrhosum (Cuvier) (Osteoglossiformes) and the yellowfin river pellona, Pellona falvipinnis (Valenciennes) (Clupeiformes), respectively. Kritsky et al. (1996) suggested that Telethecium resembles Kritskyia Kohn, 1990 and Pavanelliella, by sharing a MCO with counterclockwise rings, a sinistral vagina, and the lack of the anchor/bar complex. Aguiar et al. (2017) reported specimens of a dactylogyrid which resembles Telethecium and Pavanelliella (i.e., lacking anchor/bar complex) parasitizing the nasal cavities of ctenolucids fishes (Characiformes) from Tapajós River, Brazil; however, it was not described in their study. Aguiar et al. (2017) also proposed a phylogenetic hypothesis for some species of Pavanelliella, which allows inferring that the anchor/bar complex was independently and secondarily lost in the evolutionary history of Dactylogyridae.

According to Braga et al. (2015), most Neotropical monopisthocotylan lineages display a strong association with specific fish orders or families, highlighting a significant positive correlation between the evolutionary history of Neotropical fishes and their monopisthocotylan parasites. The occurrence of Telethecium species in Osteoglossiformes, freshwater Clupeiformes, and Characiformes (present study), representing distinct evolutionary lineages, further strengthens this connection, suggesting that host phylogenetic relationships play a key role in shaping monopisthocotylan parasite diversity. Betancur et al. (2017) proposed a phylogenetic analysis of some groups of bony fishes based on molecular data. This analysis indicates that Osteoglossiformes represents one of the most basal groups within the Teleostei, having Otomorpha (Clupeiformes, Alepocephaliformes, Gonorynchiformes, Cypriniformes, Characiformes, Gymnotiformes and Siluriformes) and Euteleostei as sister groups. However, considering the hypothesis of common evolutionary history between *Telethecium* spp. and their hosts, sorting events (i.e., extinction, 'missing the boat') (Page & Charleston 1998) may be necessary to explain the absence of *Telethecium* spp. on several otomorph orders, and euteleosts. In addition, we cannot discount the influence of geographic distribution, as well as environmental and biological attributes on the occurrence of *Telethecium* spp. on their hosts. Concerning the relevance of these host-parasite associations, we believe that a more extensive study, based on denser sampling for monopisthocotylan parasites from nasal cavities of neotropical fishes and cladistics studies will shed light on our knowledge of the main diversification and coevolutionary processes associated in this host-parasite system.

The present study represents the first record of monopisthocotyls for *B. melanurus*. This study integrated molecular and morphological data to support the proposal of *D. forficata*, and the phylogenetic analyses (28S rDNA) of this study showed that the new species is closely related with *D. neotropicalis* and *D. magnus* forming a monophyletic group. Additionally, the diagnosis of *Telethecium* was amended to accommodate the new species *Telethecium tiquira*. The data obtained here increase the knowledge about the diversity of monopisthocotyls from the nasal cavities of Neotropical freshwater fishes for 26 species.

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