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An integrative taxonomic study of Pavanelliella spp. (Monogenoidea, Dactylogyridae) with the description of a new species from the nasal cavities of an Amazon pimelodid catfish

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ABSTRACT

The present study is an integrative taxonomic analysis of *Pavanelliella* spp. (Monogenoidea, Dactylogyridae), and describes a new species from the nasal cavities of the Amazonian pimelodid catfish *Brachyplatystoma rousseauxii* (Siluriformes Pimelodidae) from the Tapajós River (Amazon Basin, Pará state, Brazil). *Pavanelliella jarii* sp. n. is characterized by the presence of 3–4 rings in the male copulatory organ, the absence of rings around the vaginal atrium and by its sinuous vaginal canal, which sometimes forms 0.5–1 rings in the distal portion. The sequencing of the small subunit ribosomal DNA (ssrDNA) and internal transcribed spacer 1 (ITS-1) of three species of *Pavanelliella*, *Vancleaveus cicinnus*, and an undetermined dactylogyrid allowed the phylogenetic reconstruction of these dactylogyrids. The analysis indicated that *P. jarii* sp. n. is closely related to *Pavanelliella takemotoi* and *Pavanelliella pavanellii*, which formed a sister clade to ancylodiscoidines parasites of siluriform fish from the Oriental and Afrotropical regions. The analysis also corroborated the non-monophyly of Ancyrocephalinae, revealing that ancylodiscoidines arose between ancyrocephalines lineages, in a sister relationship to pseudodactylogyrines + marine ancyrocephalines + ancyrocephalines parasites of afrotropical perciforms + dactylogyrines. Cladistical analysis indicates that the haptoral anchor/bar complex has been lost several times in the evolutionary history of Dactylogyridae. The analysis also indicated that *Dactylogyrus* is polyphyletic, as *Acolpenteron ureteroecetes* and *Dactylogyroides longicirrus* arose between the three lineages formed by *Dactylogyrus* spp.

1. Introduction

Catfish (Siluriformes) originate in freshwater environments and account for about 3100 species distributed worldwide [1], representing around one-third of all known freshwater fishes [2]. More than half (55%) of their richness is reported in the Neotropics, where some of the most primitive lineages of siluriforms are recognized [3,4]. Pimelodidae is one of 15 endemic families of catfishes from the Neotropical region [5] and currently accounts for approximately 134 known species [4]. Despite its diversity, only 25 species of pimelodids have been examined for monogenoidean parasites.

Forty-six species of monogenoids were reported parasitizing pimelodids [6]. Of the species found on the body surface, one was from *Phanerothecioides* Kritsky, Vianna and Boeger, 2007; one was from *Phanerothecium* Kritsky and Thatcher, 1977; and two were from *Scleroductus* Jara and Cone, 1989. Thirty-nine species were reported from the gills: three from *Urocleidoides* Mizelle and Price, 1964; one from *Amphocleithrum* Price and Gonzáles-Romero, 1969; 23 from *Demidospermus* Suriano, 1983; one from *Cosmetocleithrum* Kritsky, Thatcher and Boeger, 1986; three from *Vancleaveus* Kritsky, Thatcher and Boeger, 1986; one from *Unibarra* Suriano and Incorvaia, 1995; and six were from *Amelloblastella* Kritsky, Mendoza-Franco e Scholz, 2000. The

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remaining, three species of *Pavanelliella* Kritsky and Boeger, 1998, were reported in the nostrils.

Kritsky and Boeger [7] erected *Pavanelliella* to accommodate the new species *Pavanelliella pavanellii* Kritsky and Boeger, 1998, recovered from the nasal cavities of the pimelodid *Pseudoplatystoma corruscans* (Spix and Agassiz, 1829) (type host) from the Baía River in the state of Mato Grosso do Sul, Brazil (type locality), and *Calophysus macropterus* (Lichtenstein, 1819) from the Solimões River in the state of Amazonas, Brazil. The genus was characterized by a coiled male copulatory organ (MCO) with counterclockwise rings, an accessory piece non articulated with the MCO, overlapping gonads and the absence of haptoral bars, anchors and hooks 4A [7]. Kritsky and Mendoza-Franco [8] emended the diagnosis of the genus, and described *Pavanelliella scaphiocotylus* Kritsky and Mendoza-Franco, 2003, from the nostrils of the heptapterid catfish, *Rhamdia guatemalensis* (Günther, 1864) from Yucatán, Mexico. Aguiar et al. [9] described *Pavanelliella takemotoi* Aguiar, Ceccarelli and Luque, 2011, and *Pavanelliella laertei* Aguiar, Ceccarelli and Luque, 2011, of pimelodids from the Mogi Guaçu River in the state of São Paulo, Brazil.

The present study addresses the fauna of monogenoids taken from the nasal cavities of pimelodids (12 species) from the Amazon and Paraná Basins and describes one new species of *Pavanelliella* from the Amazon Basin. The molecular data confirmed the validity of these species and allowed the evolutionary history of *Pavanelliella* to be recovered, clarifying its relationship within Dactylogyridae Bychowsky, 1933.

2. Material and methods

2.1. Sample area, hosts collection and deposit

Pimelodid fish belonging to 12 species (Table 1) were caught with hooks, gill nets and/or cast nets in the Amazon and Parana basins, Brazil. In the Amazon basin, the fish were caught in the Tapajós River (sub-basin of the rivers Amazon, Tapajós, Juruena), in the municipality of Itaituba, in the state of Pará (4° 33′ 9.70″ S, 56° 17′ 59.60″ W) in September and October 2011 and June 2012, and in the municipality of Santarém, in the state of Pará (2° 20′ 2″ S, 54° 52′ 34″ W) in October 2014. In the Mogi Guaçu River (Paraná basin, sub-basin of the Rio Grande), in the municipal region of Pirassununga, in the state of São Paulo (21° 55′ 33″ S, 47° 22′ 7″ W), the collections were carried out in April 2012 and January 2016. Host scientific names were validated according to Britski et al. [10], Buckup et al. [11], Lundberg and Littmann [12] and Queiroz et al. [13]. Specimens and/or tissues of the hosts were deposited in the collection of the Fish Biology and Genetics Laboratory, of the Universidade Estadual Paulista Julio de Mesquita Filho (Julio de Mesquita Filho São Paulo State University) (LBP/UNESP), São Paulo, Brazil, and tissues were deposited in the attached collection of the collection of Platyhelminthes of the Adão José Cardoso Museum of Zoology of the State University of Campinas (ZUEC PLA/UNICAMP).

2.2. Sample collection, morphological study and deposit of the helminths

The nasal cavities of all the hosts were opened with dissection scissors, washed with heated water (~65 °C) in a Petri dish and the contents were examined under a stereo microscope during field research. The helminths were removed from the sediment using small probes and were fixed in 4% formalin for morphological study or in 95% ethanol for molecular study. To obtain sequences of Neotropical species distinct from *Pavanelliella* spp., the gills of *Phractocephalus hemioliopterus* (Bloch and Schneider, 1801) were removed and fixed in ethanol 95%. Specimens of Dactylogyridae gen. sp. and *Vancleaveus cicinnus* Kritsky, Thatcher and Boeger, 1986 were collected and fixed for molecular analysis in laboratory.

Some specimens were stained with Gomori's trichrome and mounted in Damar to determine the internal soft structures, while others were prepared with Gray and Wess's medium to study the sclerotized structures [14]. The measurements were taken in, micrometers, according to the procedures of Mizelle and Klucka [15]. The dimensions of the organs and other structures represented here were measured for the most part in the dorsoventral view, based on the analysis of digital images

Table 1
Pimelodid species examined for nasal cavities monogenoids, with number of specimens examined (n), locality of catch and fish size and weight.

Fish species	n	Locality	Size (cm) ± SD and weight (g) ± SD
Leiarius marmoratus (Gill, 1870)	3	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	41 ± 20; 949 ± 827
Pimelodus blochii Valenciennes, 1840	2	Igarapé Bazim[a], Itaituba (04° 47′ 24.1″ N, 56° 46′ 40″ W)	17 ± 2; 53.5 ± 17
	1	Igarapé Tracuá[a], Itaituba (04° 29′ 1″1 N, 56° 17′ 2″ W)	
Pimelodus ornatus Kner, 1858	6	Lago do Varela[b], Itaituba (04° 44′ 40″ N, 56° 25′ 42″ W)	14 ± 3; 50 ± 20
Pinirampus pirinampu (Spix & Agassiz, 1829)	4	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	58 ± 12; 1833 ± 1232
Platynematichthys notatus (Jardine, 1841)	4	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	60 ± 13; 2469 ± 1380
Phractocephalus hemioliopterus (Bloch & Schneider, 1801)	4	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	56 ± 19; 2705 ± 3295
	7	Tapajós River, Santarém (02° 20′ 13″ N, 54° 52′ 53″ W)	
Brachyplatystoma rousseauxii (Castelnau, 1855)	9[c]	Igarapé Jari[a], Santarém (2° 20′ 24″ N, 54° 53′ 59″W)	46 ± 16; 1154 ± 1177
	2	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	
Pseudoplatystoma punctifer (Castelnau, 1855)	2[c]	Igarapé Jari[a], Santarém (2° 20′ 24″ N, 54° 53′ 59″W)	62 ± 18; 2371 ± 2611
	2[c]	Lagoon in the Jamanxim River[a], Itaituba (04° 45′ 19.2″ N, 56° 26′ 16.4″ W)	
	3[c]	Tapajós River, Santarém (2° 20′ 13″ N, 54° 52′ 53″ W)	
	5[c]	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	
Pseudoplatystoma tigrinum (Valenciennes, 1840)	2[c]	Igarapé Jari[a], Santarém (2° 20′ 24″ N, 54° 53′ 59″W)	58 ± 12; 1745 ± 1056
	3[c]	Igarapé Tracuá[a], Itaituba (04° 29′ 1″1 N, 56° 17′ 2″ W)	
	1[c]	Lagoon in the Jamanxim River[a], Itaituba (04° 45′ 192″ N, 56° 26′ 16.4″ W)	
	3[c]	Tapajós River, Santarém (2° 20′ 13″ N, 54° 52′ 53″ W)	
	1[c]	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	
Zungaro zungaro (Humboldt, 1821)	3[c]	Jamanxim River[a], Itaituba (05° 14′ 07″ N, 56° 25′ 49″ W)	97 ± 32; 14.419 ± 9749
	1	Tapajós River, Itaituba (04° 33′ 10″ N, 56° 17′ 60″ W)	
Pimelodus maculatus (Lacepède, 1803)	1[c]	Mogi-Guacu River[d], Pirassununga (21° 55′ 33″ S, 47° 22′ 7″ W)	26.5; 215 g
Pimelodus microstoma Steindachner, 1877	1[c]	Mogi-Guacu River[d], Pirassununga (21° 55′ 33″ S, 47° 22′ 7″ W)	13.5; 61 g

[a] Tapajós river's tributary.
[b] Lagoon linked to Tapajós river.
[c] Had specimens parasitized by *Pavanelliella* spp.+
[d] Parana River Basin.

processed using ImageJ 1.43 m software [16]. MCO length data provided by Aguiar et al. [9] were reviewed here using digital images processed in ImageJ 1.43 m. The lengths of curved or bent structures (anchors, bars, accessory piece) were measured by the straight-line distances between the extreme ends; while the length of the coiled male copulatory organ was obtained by measuring the entire organ. The average measurement is followed by the ranges and the number of specimens measured (n) is given in parentheses. Some specimens were observed using differential interference contrast (DIC) and phase contrast optics using an Axioplan 2 Zeiss microscope. Illustrations were prepared through a drawing tube attached on a Motic BA310 E microscope. The definitions of prevalence and mean intensity follow the definition of Bush et al. [17]. Type specimens and vouchers were deposited in the ZUEC PLA/UNICAMP and in the Helminthological Collection of the Museum of Zoology of the University of São Paulo (MZUSP), both in the state of São Paulo; and in the Invertebrate Collection of the National Institute of Amazonian Research (INPA), state of Amazonas, all in Brazil.

2.3. Molecular analysis of the parasites

A single specimen of each species was placed on a glass slide with a solution of proteinase K (20 mg/ml) diluted to 1:40 in a lysis buffer (pH 8, 10 mM Tris–Cl, 25 mM EDTA, 100 mM NaCl e 0.5% SDS) at 56 °C. The diagnostic structures of the helminth were observed and photographed for five to ten minutes with a Leica DM1000 microscopy. The digestion reaction was stopped by diluting the proteinase K with the lysis buffer. The helminth was transferred to a 1.5 ml tube with 5 μl lysis buffer and dried in an Eppendorf Vacufuge Concentrator 5301 (Hamburg, Germany) and the DNA was extracted using a DNeasy® Blood and Tissue Kit (Qiagen, USA), following the manufacturer's instructions. The concentration of the DNA was verified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA).

Polymerase chain reactions (PCRs) were conducted in two steps. Firstly, using the primers DAC18F1 (5′ – AATCAGCTATGGTTCC TTGGAT – 3′) (designed for this study) and IR8 (5′ – GCTA GCTGCGTTCTTCATCGA – 3′) [18], which anneal in the 116 position of the 18S rDNA and the 2440 position of ITS1, respectively. Posteriorly a nested PCR was carried out using the primers 930F (5′ – GCATGGAA-TAATGGAATAGG – 3′) [19] and IR8. The PCRs were performed in a ProFlex™ PCR System Thermal Cycler (Thermo Scientific Wilmington, USA) with a final volume of 25 μl and with the DreamTaq Green PCR Master Mix (2 ×) (Thermo Scientific Wilmington, USA), following the manufacturer's recommendations. A quantity of 0.2 mM of each primer and 4 μl of the extracted DNA was used in the reactions, with initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 90 s, and final elongation at 72 °C for 10 min. The nested PCRs were conducted with 1 μl of the product of the PCRs, diluted 1:1 in ultrapure water, with initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 60 s, and then final elongation at 72 °C for 7 min.

The amplicons were electrophoresed in 1.5% agarose gel (BioAmerica, Miami, FL, USA) in TBE buffer (0.045 M Tris-borate, 0.001 M EDTA, pH 8.0), stained with SYBR® Safe DNA Gel Stain (Thermo Scientific Wilmington, USA), analyzed in a K33-3333 (Kasvi, Paraná, Brasil) scanner and compared with the 1 kb Plus DNA Ladder (Invitrogen by Life Technologies, CA, USA). PCR products were purified using QIAquick PCR Purification Kit (Qiagen, USA) and sequencing was performed at the Human Genome Research Center (HGRC), of the University of São Paulo, with a BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., CA, USA) in an ABI 3730 DNA sequencing analyzer (Applied Biosystems), with the additional use of the primers 1200R (5′ – GGGCATCACAGACCTG – 3′) [19] and S1 (5′ – ATTCCGATAACGAACGAGACT – 3′) [20].

2.4. Phylogenetic inference

Contigs were edited using BioEdit 7.1.3.0 [21] and standard nucleotide BLAST searches were then conducted [22] to verify the similarity of the sequence of the present study with others sequences of monogenoids in the NCBI BioSystems database [23]. The alignment of the sequences was performed with the ClustalW algorithm Version 2 [24] implemented in SeaView Version 4 [25]. In order to estimate the evolutionary divergence between the sequences of *Pavanelliella* spp., a pairwise analysis was carried out comparing the number of substitutions of nucleotides between the sequences, and the number of different nucleotides without considering substitutions. These analysis were performed using the Maximum Composite Likelihood and the Number of Differences implemented in MEGA6 [26], treating gaps as complete deletions.

For phylogenetic reconstructions, 96 ssrDNA and ITS-1 sequences of dactylogyrids downloaded from the NCBI database [23] were used. The alignment of these sequences plus those of Dactylogyridae gen. sp., *V. cicinnus* and three of *Pavanelliella* spp. performed in this study were subjected to less stringent g-blocks [27]. Phylogenetic trees were generated from the sequence alignments using Bayesian Inference (BI) and Maximum Likelihood (ML).

BI analysis was carried out using the MrBayes version 3.2 software package [28]. Two independent Markov Chain Monte Carlo (MCMC) analyses were run for 5,000,000 generations in 5 chains of 1000,000. Tree sampling was set for 100th generation, MCMC diagnostic for every 1000th generation and the first 25% of the samples were discarded. The model was set as *lset nst = mixed and rates = invgamma*. ML analysis was done in the PhyML with Smart Model Selection [29,30]. The nodal support was validated by bootstrap with 1000 replicates. After phylogenetic inference using the diplectanid *Lamellodiscus echeneis* (Wagener, 1857) as an outgroup (not shown), the following analyses were run using the *Onchocleidus similis* Mueller, 1936 sequence as a functional outgroup [31]. The supports of the branches were categorized as weak, moderate, good or strong support as proposed by Yang et al. [32]. The zoogeographic regions considered in the phylogenetic tree are the same as those identified by Holt et al. [33].

3. Results

3.1. Quantitative descriptors of Pavanelliella spp. parasites from the nasal cavities of pimelodid catfishes

Of the 70 fish analyzed, which belonged to 12 species, infestation by at least one species of *Pavanelliella* was observed in the nasal cavities of specimens of *Brachyplatystoma rousseauxii* (Castelnau, 1855), *Pseudoplatystoma punctifer* (Castelnau, 1855), *Pseudoplatystoma tigrinum* (Valenciennes, 1840), *Zungaro zungaro* (Humboldt, 1821), *Pimelodus maculatus* (Lacepède, 1803) and *Pimelodus microstoma* Steindachner, 1877 (Tables 1 and 2). Morphologic data and the quantitative descriptors concerning each *Pavanelliella* species are shown in Table 2. The description of the new *Pavanelliella* species, based on morphologic and molecular data (ssrDNA partial + ITS-1 sequencing), is presented below. Molecular data of *P. pavanellii*, *P. takemotoi* and of Dactylogyridae gen. sp. and *V. cicinnus* are also provided.

3.2. Morphological and molecular taxonomy

3.2.1. Taxonomic summary

Class: Monogenoidea Bychowsky, 1937
Subclass: Polyonchoinea Bychowsky, 1937
Order: Dactylogyridea Bychowsky, 1937
Dactylogyridae Bychowsky, 1933
Pavanelliella Kritsky and Boeger, 1998

Table 2
Measurements and epidemiologic data about *Pavanelliella* species, parasites of catfish from Brazil.

	Pavanelliella pavanellii					*Pavanelliella scaphiocotylus*	*Pavanelliella takemotoi*	*Pavanelliella laertei*	*Pavanelliella jarii* sp. n.
	Pseudoplatystoma corruscans, *Calophysus macropterus*[7]	*Pimelodus maculatus*[9]	*Pseudoplatystoma punctifer* Present study	*Pseudoplatystoma tigrinum* Present study	*Zungaro. Zungaro* Present study	*Rhamdia guatemalensis*[8]	*Pimelodus maculatus* [9]	*Pimelodus microstoma*[9]	*Brachyplatystoma rousseauxii* Present study
BL	424 (306–514; n = 22)	340 (250–451; n = 7)	395 (351–450; n = 9)	340 (230–443; n = 10)	359 (331–405; n = 5)	518 (384–602; n = 12)	426 (246–580; n = 19)	334 (274–374; n = 7)	565 (428–787; n = 11)
BW	124 (82–162; n = 21)	102 (69–126; n = 7)	117 (84–166; n = 9)	95 (61–144; n = 10)	111 (91–134; n = 5)	80 (69–92; n = 12)	140 (86–240; n = 19)	135 (92–159; n = 4)	141 (108–185; n = 8)
PH	24 (19–28; n = 22)	19 (13–26; n = 4)	24 (20–28; n = 8)	22 (14–40; n = 10)	28 (19–40; n = 5)	30 (26–33; n = 11)	23 (14–36; n = 22)	25 (20–30; n = 5)	30 (24–34; n = 8)
HaL	–	60 (48–77; n = 7)	74 (52–97; n = 9)	69 (47–95; n = 10)	78 (64–91; n = 5)	108 (84–135; n = 11)	59 (46–69; n = 15)	51 (42–66; n = 5)	109 (80–140; n = 8)
HaW	19 (15–20; n = 18)	88 (87–120; n = 7)	94 (351–450; n = 8)	84 (56–139; n = 9)	96 (90–109; n = 5)	122 (102–140; n = 11)	82 (51–122; n = 15)	70 (60–79; n = 2)	129 (109–141; n = 6)
HL	18 (17–19; n = 25)	12 (8–14; n = 7)	17 (14–19; n = 8)	16 (14–18; n = 9)	18 (13–21; n = 5)	14 (13–15; n = 16)	16 (13–21; n = 96)	14 (11–17; n = 23)	18 (13–22; n = 20)
MCOL	161 (140–175; n = 7)	121 (103–149; n = 4)[a]	181 (137–220; n = 9)	170 (100–234; n = 10)	136 (92–173; n = 5)	–	241 (161–305; n = 7)[1]	237 (177–316; n = 4)[1]	294 (250–363; n = 12)
FRD	24 (21–28; n = 30)	19 (13–24; n = 7)	27 (22–35; n = 9)	23 (18–29; n = 9)	24 (19–28; n = 5)	19 (16–23; n = 9)	19 (16–27; n = 29)	18 (17–20; n = 6)	25 (18–34; n = 12)
APL	44 (35–52; n = 15)	27 (18–48; n = 4)	34 (22–41; n = 10)	32 (23–39; n = 9)	36 (21–44; n = 5)	27 (24–29; n = 6)	27 (21–40; n = 25)	20 (18–21; n = 3)	29 (24–34; n = 12)
TL	53, 54; n = 2	35, 39; n = 2	53 (45–66; n = 4)	48 (36–66; n = 8)	42 (40–44; n = 2)		67 (42–104; n = 5)	30 (20–43; n = 3)	58 (35–97; n = 5)
TW	22, 24; n = 2	11, 14; n = 2	31 (21–49; n = 3)	19 (8–27; n = 8)	12 (11–13; n = 2)	–	20 (19–27; n = 5)	17 (8–29; n = 3)	26 (18–34; n = 3)
GL	67 (46–78; n = 5)	55 (40–77; n = 4)	76 (63–104; n = 5)	68 (28–104; n = 8)	68 (62–78; n = 4)	60 (51–72; n = 9)	64 (45–104; n = 7)	57 (38–86; n = 3)	90 (62–149; n = 7)
GW	31 (22–36; n = 5)	16 (9–23; n = 4)	29 (22–36; n = 3)	30 (13–492; n = 8)	19 (15–21; n = 2)	27 (21–35; n = 8)	18 (6–30; n = 7)	18 (10–29; n = 3)	29 (25–34; n = 4)
Egg	–	–	55 × 74	–	–	––	–	–	–
P%	–	14	67	61	50	–	35	14	82
MAI	–	0.2	11	20	1.5	–	2.3	1.5	6
MII	–	1.25	16.5	32	3	–	0.8	0.2	5

Body long (BL), Body wide (BW), Pharynx diameter (PH), Haptor long (HaL), Haptor wide (HaW), Hook long (HL), Male copulatory organ long (MCOL), First ring diameter (FRD), Accessory piece long (APL), Accessory piece largest space occupied in the body (APLS), Testis long (TL), Germarium long (GL), Germarium wide (GW). Prevalence (P%), Mean abundance of infection (MAI), Mean intensity of infection (MII).

[a] These data were performed in present study.

Species: *Pavanelliella jarii* sp. n

Type host: *Brachyplatystoma rousseauxii* (Castelnau, 1855) (Siluriformes, Pimelodidae). Portuguese popular name "dourada".

Site of infection: Nasal cavities.

Type locality: Igarapé Jari, Tapajós river (Amazonas basin), Santarém, state of Pará, Brazil Igarapé Jari (2° 20′ 24″ N, 54° 53′ 59″W).

Prevalence of infection: 9 of 11 (82%).

Mean intensity of infection: 6.

Mean abundance of infection: 5.

Specimens deposited: Holotype (ZUEC PLA 73), paratypes (ZUEC PLA 74–78, MZUSP 7951a–b, 7952, INPA 743–746).

GenBank accession number: MF398302.

Comparative measures: Table 2.

Etymology: The specific name is a reference to the locality, where the parasite was recovered, in the community of Jari do Socorro, Santarém, Pará, Brazil.

Description (based on 13 specimens: 6 stained with Gomori's trichrome and 7 mounted in Gray and Wess's medium). Body 565 (428–787; n = 11) long, fusiform, tapering posteriorly, peduncle absent; greatest width of trunk 141 (108–185; n = 8) at level of vaginal pore. Cephalic margin tapered; poorly developed terminal lobes; three bilateral pairs, cup-shaped head organs; cephalic glands unicellular, latero-posterior to pharynx, some near to intestinal bifurcation. Eyes 4, equal, equidistant, 3 eyes sometimes present (2 anterior and 1 anterior or 1 anterior and 2 posterior), as well as just few accessory granules in cephalic region. Pharynx subspherical 30 (24–34; n = 8) in diameter; esophagus moderately long; bifurcate intestinal caeca confluent in posterior trunk after germarium. Haptor 129 (109–141; n = 6) wide, 109 (80–140; n = 8) long, sub circular, suction cup like, margin of haptor delicate, armed with 14 marginally distributed hooks. Hooks 18 (13–22; n = 20) long, tapering proximal and distally, with an erected thumb and a delicate point; filamentous hook loop with about 2/3 of shank length. Gonads overlapping. Testis 58 (35–97; n = 5) long, 26 (18–34; n = 3) wide, elongate piriform, dorsal to germarium; vas deferens looping left intestinal cecum; seminal vesicle a dilatation of vas deferens, sigmoid, lying to midline in anterior trunk. Prostatic reservoir single, saccate. Genital pore opening ventrally, in midline of body, bellow of the anterior bifurcation of the caeca, anterior to MCO. Genital atrium not muscular. Copulatory complex comprising MCO and accessory piece. MCO sclerotized, 294 (250–363; n = 12) long, tubular, coiled; with 4, but rarely 3 counterclockwise rings, 25 (18–34; n = 12) proximal ring diameter; and with a conical base surrounded by 3 sclerotized flaps (2 proximal and 1 distal). Accessory piece 29 (24–34; n = 12) total length, non-articulated with MCO, comprising a sheath enclosing the distal portion of MCO, distally partite in two parts, one of them enveloping the distal portion of the MCO, the other, shorter and resembling a drop of liquid. Germarium elongate 90 (62–149; n = 7) long, 29 (25–34; n = 4) wide; uterus observed, but oviduct and ootype, not. Vagina sinistral, with sclerotized atrium cup-shaped and without a pre-atrium; vaginal canal sclerotized, sinuous, forming a 0.5–1 loop in distal portion, which expands to open inside the vaginal atrium; seminal receptacle subspherical, overlapping germarium partially. Vitellaria dense, absent in the region of the reproductive organs. Eggs not observed.

Molecular data: partial ssrDNA and ITS-1 sequencing resulted in 929 base pairs (bp) for *P. jarii* sp. n., of which 514 bp are from ssrDNA and 415 bp from ITS-1. These sequences did not match any of the others available in the NCBI database in a BLASTn search. Estimates of evolutionary divergence showed that *Pavanelliella jarii* sp. n. was the most divergent species of the genus (Table 3).

Remarks: *Pavanelliella jarii* sp. n. (Figs. 1 and 3) can be distinguished from its congeners by the morphology of the male copulatory organ and vaginal canal. The new species possesses a MCO with 4 rings preceding the accessory piece, differing from *P. pavanellii* (2 rings), *P. laertei* (3 rings), *P. takemotoi* (5 rings) and *P. scaphiocotylus* (6 rings) [7,9] (Figs. 2 and 3). *Pavanelliella jarii* sp. n. has a sinuous vaginal canal, which distally forms 0.5–1 loop and expands to open into the vaginal atrium, whereas the remaining species of *Pavanelliella* have a vaginal canal that forms a greater number of loops (*P. pavanellii* = 1–3 loops; *P. laertei* = 2–3; *P. takemotoi* 2–5; *P. scaphiocotylus* = 4–5) [7,9].

Pavanelliella scaphiocotylus, *P. takemotoi* and *P. laertei* have a vaginal canal that forms loops with a greater diameter than the width of the vaginal atrium [8,9], resembling *P. jarii* sp. n. (Fig. 3), whereas *P. pavanellii* possesses loops that are smaller than the vaginal atrium [7,9] (Fig. 2). The accessory piece of *P. jarii* sp. n. is distally bipartite, and quite different from the accessory pieces of *P. scaphiocotylus*, which have a single distal portion, although it does resemble that of *P. takemotoi* and *P. laertei*, which can also have a bipartite accessory piece

Table 3
Estimates of evolutionary divergence between four sequences of ssrDNA partial and ITS-1 of three species of Pavanelliella. The upper triangle shows the number of base differences among the sequences. In the lower triangle is the difference in relative frequency rate of nucleotide substitutions.

	1	2	3	4
1 *Pavanelliella jarii* sp. n. (*Brachyplatystoma rosseauxii*)		79	80	82
2 *Pavanelliella pavannellii* (*Pseudoplatystoma punctifer*)	14.4		1	28
3 *Pavanelliella pavannellii* (*Pseudoplatystoma tigrinum*)	14.6	0.1		29
4 *Pavanelliella takemotoi* (*Pimelodus maculatus*)	14.9	4.6	4.8	

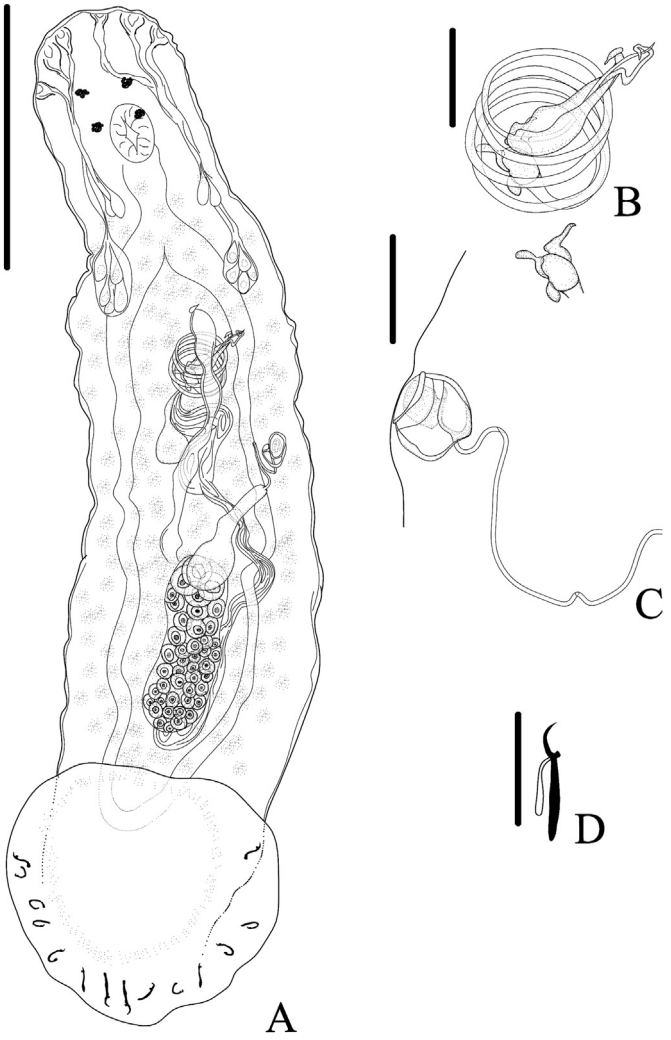

Fig. 1. *Pavanelliella jarii* sp. n. A: whole composite drawn. B: copulatory complex. C: vagina in dorsal view. D: Hook. Scale bar = 150 μm (A), 20 μm (B–D).

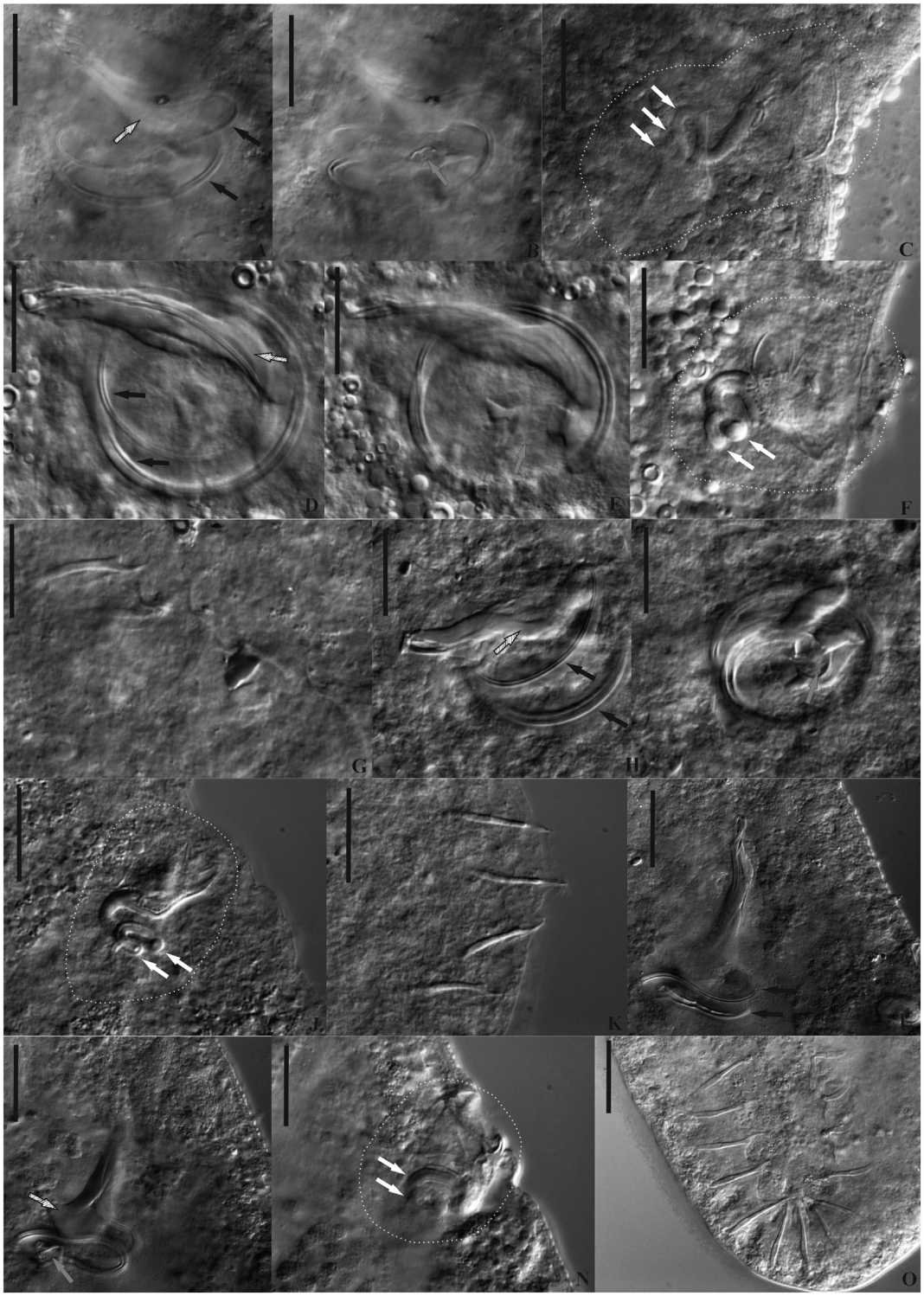

Fig. 2. *Pavanelliella pavanellii* parasite of: *Pimelodus maculatus*. A: copulatory complex ventral view (CHIOC37560). B: dorsal view C: vagina (CHIOC37559). *Pseudoplatystoma punctifer*. D copulatory complex in ventral view (PTJ1403-2). E: dorsal view. F: vagina G: hook (PTJ11118-1). *Pseudoplatystoma tigrinum.* H copulatory complex in ventral view (PTJ1187-7). I: dorsal view. J: vagina (PTJ1130-49). K: hook. *Zungaro zungaro*. L: copulatory complex in ventral view (PTJ1156-1). M: dorsal view. N: vagina. O: hook. Arrows: striped (accessory piece), black (MCO - male copulatory organ), gray (base of MCO) and white (vaginal canal). Scale bar = 20 μm.

[7–9]. While the accessory piece in *P. takemotoi* is simple, in *P. laertei* and *P. jarii* sp. n. one of the parts of this piece is slender, longer, and surrounds the distal portion of the MCO, while the other part is shorter. However, while in *P. jarii* sp. n. the shorter part resembles a drop of liquid, in *P. laertei* it sometimes resembles a hook. Additionally, the new species can also be differentiated from its congeners by having generally larger measurements (Table 2).

In terms of molecular analysis, *P. jarii* sp. n. exhibited differences of

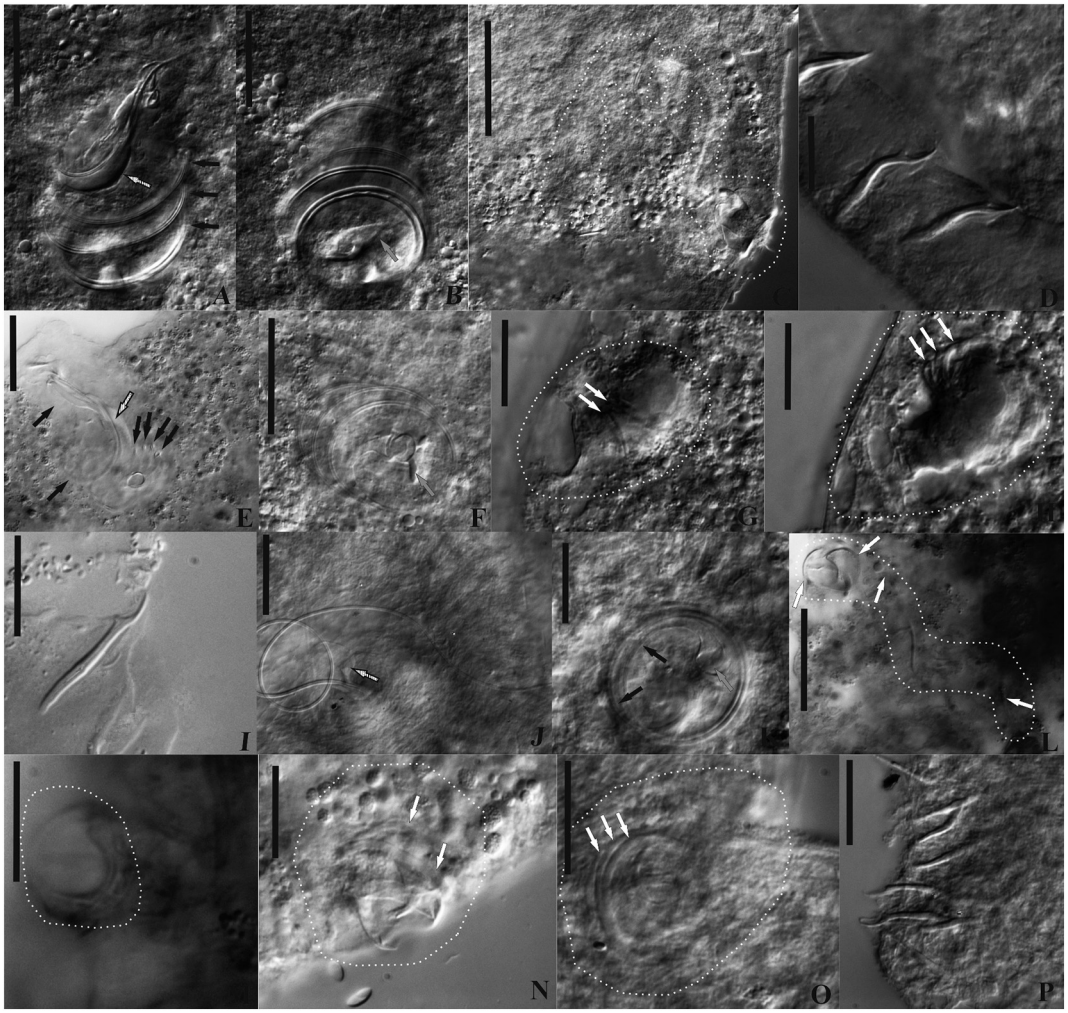

Fig. 3. *Pavanelliella jarii* sp. n. A: copulatory complex in ventral view (PTJ1413-3). B: dorsal view. C: vagina (PTJ1412-4). D: hook (PTJ1414-2). *Pavanelliella takemotoi.* E: copulatory complex in lateral view (CHIOC37571). F: dorsal view (CHIOC37576b). G: vagina in dorsal view (CHIOC37582b). H: vagina in ventral view. I: hook. *Pavanelliella laertei.* J: copulatory complex in ventral view (CHIOC37563). K: dorsal view. L: vagina in ventral view (CHIOC37562). M: vagina in dorsal view. N: vagina (CHIOC37566). O: vagina (PMG1202). P: hook (CHIOC37564). Scale bar = 20 μm (A–C, E–L, N–P), 10 μm (D, M).

14.4% and 14.6% with *P. pavannellii* and 14.9% with *P. takemotoi* (Table 3) in estimates of evolutionary divergence. These molecular analyses of *Pavanelliella* spp. also support the erection of the new taxon, in addition to the morphologic differences.

Pavanelliela pavanellii Kritsky and Boeger, 1998

Pavanelliela pavanellii: Kritsky and Boeger [7]: 160–163, Figs. 1–4 (descr); Aguiar et al. [9]: 216–217, Figs. 16–18 (citat); Cohen et al. [6]: 58, Fig. 269 (citat).

Type host: *Pseudoplatystoma corruscans* (Spix and Agassiz, 1829).

Type locality: Baia River, Mato Grosso do Sul, Brazil.

Site of infection: Nasal cavities.

Others records: *Calophysus macropterus* (Lichtenstein, 1819), Solimões River, in Manaus, state of Amazonas, Brazil [7]; *Pimelodus maculatus* (Lacepède, 1803), Mogi-Guaçu River, Pirassununga, state of São Paulo, Brazil [9].

Material studied: vouchers CHIOC37559 and CHIOC37560.

Present records: *Pseudoplatystoma punctifer* (Castelnau, 1855) (LBP 12822, 15,017), ZUEC PLA 79, Lagoon in the Jamanxim River (04° 45′ 19.2″ N, 56° 26′ 16″ W), September 2011, ZUEC PLA 80, Tapajós River (04° 33′ 10″ N, 56° 17′ 60″ W), June 2012, Itaituba, in the state of Pará, Brazil; ZUEC PLA 81–83, MZUSP 7953–7954 and INPA 737–738, Igarapé Jari, tributary of Tapajós river (Amazon basin), Santarém, in the state of Pará, Brazil (2°20′23.76″S, 54°53′59.28″W), October 2014; *Pseudoplatystoma tigrinum* (Valenciennes, 1840) (LBP 12823), ZUEC PLA 87–88, MZUSP 7957a–b and INPA 739, 741, Igarapé Tracuá, tributary of Tapajós river (Amazon basin), Santarém, in the state of Pará, Brazil (04° 29′ 1″1 N, 56° 17′ 2″ W), September 2011; ZUEC PLA 86, 89, MZUSP 7958 and INPA 742, Igarapé Jari, tributary of Tapajós river (Amazon basin), Santarém, in the state of Pará, Brazil (2° 20′ 24″ N, 54° 53′ 59″W), October 2014; *Zungaro zungaro* (Humboldt, 1821) (LBP 12821), ZUEC PLA 84–85, MZUSP 7955–7956 and INPA 740, Jamanxim River, tributary of Tapajós river (Amazon basin), Itaituba, in the state of Pará, Brazil (05° 14′ 07″ N, 56° 25′ 49″ W), September 2011.

GenBank accession number: MF398303 (*P. punctifer*), MF398304 (*P. tigrinum*).

Comparative measurements: Table 2.

Molecular data: partial ssrDNA and ITS-1 sequencing resulted in 727 bp and 777 bp for *P. pavanellii* from *Ps. punctifer* and *Ps. tiginum*, respectively. The ssrDNA sequences in both species host were 516 bp. The ITS-1 sequences were 211 bp and 261 bp 415 from *Ps. punctifer* and *P. tiginum*, respectively. BLASTn search found that these sequences did not match any of the others available in the NCBI database. The estimates of evolutionary divergence showed that populations of *P. pavanellii* diverged 0.1% from one another (Table 3).

Remarks: comparative analysis of the specimens of *Pavanelliella* parasitizing the nostrils of *Ps. punctifer*, *Ps. tigrinum* and *Z. zungaro* from

the Tapajós River indicated that they are conspecific with *P. pavanellii*. Such specimens share general aspects of the morphology of the MCO such as 1–2 rings preceding the accessory piece; the morphology of the vaginal atrium, which is cup-shaped; and the presence of 1–3 loops in the distal part of the vaginal canal, which has a smaller diameter than the width of the vaginal atrium (Fig. 2). Despite this, the specimens studied here differed morphometrically according to the host species in which they were recovered, and from previously studied specimens [7,9]. Moreover, in the specimens of *P. pavanellii* associated with pimelodids from the Tapajós River, two loops in the distal portions of the vaginal canal were constantly observed, whereas Aguiar et al. [9] observed 1–2 loops when examining the type series of *P. pavanellii* and 2–3 in specimens of *P. pavanellii* from the Mogi Guaçu River in São Paulo. However, the molecular data provided here (Table 3) revealed low divergence between populations of *P. pavanellii* parasites of *Ps. punctifer* and *Ps. tigrinum*, suggesting that morphometric differences may be the result of the type of host colonized.

Pavanelliella takemotoi Aguiar, Ceccarelli and Luque, 2011

Pavanelliella takemotoi: Aguiar et al. [9]: 214–215, Figs. 1–8 (descr); Cohen et al. [6]: 58, Fig. 270 (citat).

Type host: *Pimelodus maculatus* (Lacepède, 1803).

Type locality: Mogi-Guaçu River, Pirassununga, in the state of São Paulo, Brazil.

Material studied: paratypes CHIOC37568a, CHIOC37571, CHIOC37576b, CHIOC37578 and CHIOC37582a–b.

Present record: *Pimelodus maculatus* Lacepède, 1803 ZUEC PLA 90–91, Mogi Guaçu River, Pirassununga São Paulo, Brazil (21° 55′ 33″ S, 47° 22′ 7″ W), January 2016.

GenBank accession number: MF398305.

Comparative measurements: Table 2.

Molecular data: partial ssrDNA and ITS-1 sequencing resulted in 912 bp for *P. takemotoi*. The ssrDNA sequence was 516 bp and the ITS-1 sequence 396 bp. These sequences did not match any other available in the NCBI database in a BLASTn search. The estimates of evolutionary divergence showed that *P. takemotoi* was closest to *P. pavanellii* (Table 3).

Remarks: the specimens of *Pavanelliella* parasites of *Pi. maculatus* examined here are clearly conspecific with *P. takemotoi*, mainly due to sharing the general morphology of the MCO, with 2–5 rings preceding the accessory piece; of the vaginal atrium, which was boot-shaped; and by the presence of 2–5 loops in the distal portion of the vaginal canal surrounding the vaginal atrium (Fig. 3). However, Aguiar et al. [9] did not find that the rings around the vaginal atrium of *P. takemotoi* were formed by the distal portion of the vaginal canal, which was observed in the present study through the proteinase K method. Furthermore, the molecular divergences observed between *P. takemotoi* and *P. jarii* (14.9%) and between *P. takemotoi* and *P. pavanellii* (4.6–4.8%) (Table 3) support the validity of *P. takemotoi*.

Pavanelliella laertei Aguiar, Ceccarelli and Luque, 2011

Pavanelliella laertei: Aguiar et al. [9]: 216–217, Figs. 9–15 (descr); Cohen et al. [6]: 58, Fig. 268 (citat).

Type host: *Pimelodus microstoma* Steindachner, 1877.

Type locality: Mogi-Guaçu River, Pirassununga, in the state of São Paulo, Brazil.

Material studied: paratypes CHIOC37562–64 and CHIOC37566.

Present record: *Pimelodus microstoma* Steindachner, 1877 ZUEC PLA 92–93, Mogi Guaçu River, Pirassununga São Paulo, Brazil (21° 55′ 33″ S, 47° 22′ 7″ W), April 2012.

Comparative measurements: Table 2.

Remarks: the specimens analyzed in the present study were characterized by a MCO with 2–3 rings preceding the accessory piece; a cup-shaped vaginal atrium; loops with a larger diameter than the width of the vaginal atrium in the distal, or less frequently in the medial portion of the vaginal canal (Fig. 3). Such characters confirmed that these specimens are conspecific with *P. laertei*. However, Aguiar et al. [9] erred in stating that this species does not have a vaginal atrium. The present study revealed that this species has a cup-shaped vaginal atrium, which is proportionally smaller than the vaginal atriums of *P. pavanellii*, *P. scaphiocotylus* and *P. jarii* sp. n., and generally surrounded by loops formed by the vaginal canal (Fig. 3). *Pavanelliella laertei* is morphologically closer to *P. scaphiocotylus*, *P. takemotoi* and *P. jarii* sp. n. from which it can be distinguished by the number of MCO rings; the number of loops in the vaginal canal; and by the morphology of the vaginal atrium, as can be seen in the taxonomic key provided below.

Dichotomous key for species of *Pavanelliella*

1. Smaller number of rings in the MCO preceding the accessory piece = 1; minimum number of loops of the vaginal canal = 1..... *Pavanelliella pavanellii*.

Smaller number of rings in the MCO preceding the accessory piece = 2; minimum number of loops of the vaginal canal = 2..... 2.

Smaller number of rings in the MCO preceding the accessory piece = 3; minimum number of loops of the vaginal canal = 0.5..... *Pavanelliella jarii* sp. n.

Smaller number of rings in the MCO preceding the accessory piece = 5; minimum number of loops of the vaginal canal = 4..... *Pavanelliella scaphiocotylus*.

2(1). Vaginal atrium cup-shaped; maximum number of loops of the vaginal canal = 3; hooks with erected thumb..... *Pavanelliella laertei*.

Vaginal atrium boot-shaped; maximum number of loops of the vaginal canal = 5; hooks with straight thumb..... *Pavanelliella takemotoi*.

Additional SSU rDNA ITS1 sequences provided

Dactylogyridae gen. sp. ZUEC PLA 141–144, MZUSP 7959a–b, 7960a–b (GenBank accession number: MF398306), and *V. cicinnus* ZUEC PLA 145–154, MZUSP 7961a–k, 7962a–b, 7963a–l (GenBank accession number: MF398307), parasites from the gills of *Ph. hemioliopterus* (LBP 12883, 15,062, 15,064), Igarapé Jari, Tapajós river (Sub-Basin Amazonas, Tapajós, Juruena), Santarém, state of Pará, Brazil (2°20′23.76″S, 54°53′59.28″W), in October 2014.

3.3. Phylogenetic inference

The phylogenetic inference was based on 754 characters for 102 operational taxonomic units (OUT). This matrix was subjected to both phylogenetic inferences, automatically setting the best evolutionary model of nucleotide substitution as the general time reversible (GTR), with the submodel G + I + F for the ML and the submodel 121,324 for the BI. The BI resulted in a 50% majority-rule consensus tree for 37,501 samples from 75,002 trees read. In the BI tree (Fig. 4), eleven lineages can be distinguished. *Ancyrocephalus* spp. appears as sister lineage to all the remaining dactylogyrids. *Ligictaluridus pricei* (Mueller, 1936) appeared as a sister group to the remaining nine lineages (A–I), five of which (A–B, D–F), had members allocated in the Ancyrocephalinae, and these results were strongly supported by at least the PP.

Lineage A, represented by *V. cicinnus* and Dactylogyridae gen. sp. is basal to the lineages B—I, and in the lineage of *Pavanelliella*, despite the moderate value of PP (0.7) of one internal branch, it is suggested that *P. jarii* sp. n. was sister to the two other *Pavanelliella* species, with a closer relationship to *P. takemotoi*, (Fig. 4). Lineage C was composed of two clades of ancylodiscoines, the smallest of which, composed of *Schilbetrema* sp., *Quadriacanthus* sp. and two *Thaparocleidus* species and was sister to the larger clade composed exclusively of *Thaparocleidus*. These ancylodiscoines arose as sister group to the large clade composed by the D–F and G–I lineages (Fig. 4). In lineage D, there were two pseudodactylogyrines, which are sisters to the other ancyrocephalin lineages (E + F). The dactylogyrines were composed by three lineages (G–I), and with the exception of *Acolpenteron ureteroecetes* Fischthal and

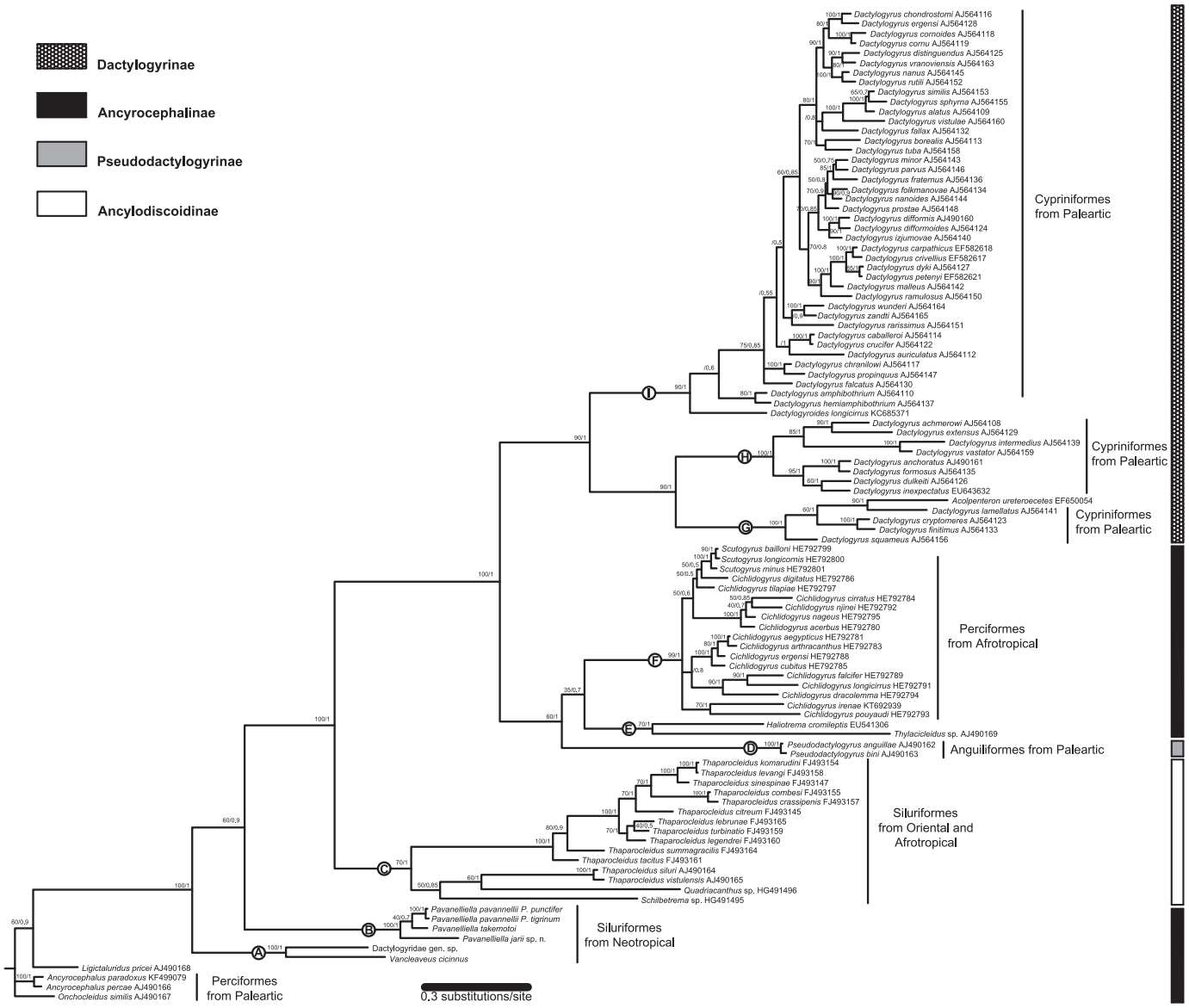

Fig. 4. Phylogenetic reconstruction of *Pavanelliella* spp. based on SSU rDNA partial and ITS1 gene sequence. The tree is a 50% majority-rule consensus tree, as suggested by Bayesian Inference. Nodes are supported by 1000 replicates of Bootstrapping from Maximum Likelihood and by Posterior Probability from Bayesian Inference.

Allison, 1940, and *Dactylogyroides longicirrus* (Tripathi, 1959), which appeared respectively in the G and I lineages, (Fig. 4), they were mostly represented of *Dactylogyrus* species.

ML and BI analysis converged in a similar tree topology (Fig. 4) and differed between one another only in relationships within lineages F and I, leading us to present only a BI tree. In the ML tree (not shown), all relationships within the F lineage were resolved and resulted in five clades, with some differences in comparison with the BI tree (Fig. 4). In the ML tree, there was a resolved clade with *Cichlidogyrus dracolemma* Řehulková, Mendlová and Šimková, 2013, *Cichlidogyrus longicirrus* Paperna, 1965 + *Cichlidogyrus falciferi* Dossou and Birgi, 1984, arising as a sister group for the remaining four clades of the F lineage. Nevertheless, most of the incongruences between the ML and BI trees are within the I lineage. In one of these incongruences, the ML tree revealed that *Dactylogyrus fallax* Wagener, 1857, was not a sister group to the clade composed of *Dactylogyrus vistulae* Prost, 1957, *Dactylogyrus alatus* Linstow, 1878, *Dactylogyrus sphyrna* Linstow, 1878, and *Dactylogyrus similis* Wagener, 1909, and that this last clade had a sister relationship with the remaining eleven species of *Dactylogyrus* of this group. The relationship of *Dactylogyrus propinquus* Bychowsky, 1931 + *Dactylogyrus chranilowi* Bychowsky, 1931, was resolved in the ML tree, in which this clade diverged as a sister group to *Dactylogyrus zandti* Bychowsky, 1933 + *Dactylogyrus wunderi* Bychowsky, 1931. Yet, *Dactylogyrus auriculatus* (Nordmann, 1832), emerged in the ML tree in a sister relationship with *Dactylogyrus falcatus* (Wedl, 1857), between all the *Dactylogyrus* spp. of this lineage (I) and the clade composed of *D. longicirrus*, *Dactylogyrus amphibothrium* Wagener, 1857, and *Dactylogyrus hemiamphibothrium* Ergens, 1956. However, in the ML tree, *D. longicirrus* appeared to be a sister to *Dactylogyrus amphibothrium* Wagener, 1857 + *Dactylogyrus hemiamphibothrium* Ergens, 1956, in a sister clade to the remaining species of this lineage.

4. Discussion

Other authors have described a number of interesting models encompassing dactylogyrids and their different lineages from teleost hosts when investigating issues relating to evolutionary aspects [34–41]. The system composed of dactylogyrids parasites of Neotropical siluriforms also offers an opportune way to test such historical association hypotheses. Firstly, because the dactylogyrids and the catfishes are both presumed to constitute natural groups [2,12,18,42–46]. Additionally,

some lineages of dactylogyrids are known to occur exclusively in the Neotropical region, like most of their catfish hosts. However, the lineages of the Neotropical dactylogyrids have only recently been scrutinized with empirical tests, while phylogenetic inferences regarding the dactylogyrid parasites of Neotropical catfish have been made even more recently [42,47–53].

Until now only four species of *Pavanelliella* have been identified, all from the Neotropics [7–9], suggesting a low diversity for this genus. The present study, however, describes the fifth species of *Pavanelliella* (*P. jarii* sp. n.) based on morphology and molecular data. Some authors have suggested a much greater diversity than is currently imagined for dactylogyrid parasites of Neotropical catfish [9,42,54,55], and probably we can also hope new descriptions of species for *Pavanelliella*. In the Neotropical region, there are more than 1500 catfish species, and at least pimelodids and heptapterids (~300 spp.) are potential hosts to *Pavanelliella* spp. Furthermore, we provide six new ssrDNA partial + ITS-1 sequences, four of the *Pavanelliella* species, one of Dactylogyridae gen. sp. and another of *V. cicinnus*, all parasitizing pimelodids. These data enhance the molecular data previously provided for Neotropical dactylogyrids, such as LSU rDNA by Mendoza-Palmero et al. [42], Franceschini et al. [52] and Acosta et al. [53], total ssrDNA by Müller et al. [51], and COI by Gasques et al. [48].

Species of the *Pavanelliella* have fewer morphologic characters for comparison with each other [9], as they do not possess the haptoral bar/anchor complex [8,9] that is synapomorphic of Dactylogyrinea Bychowsky, 1937 [43,56–58]. However, within Dactylogyrinea, as well as *Pavanelliella*, the anchor/bar complex has not been observed in five other genera of dactylogyrids [59–63] and two of pseudomurraytrematids [64,65]. Malmberg [66] proposed to group all members of Dacylogyrinea without an anchor/bar complex in the "Ananchorea", although this proposal has not been accepted [67]. In any organism group it is hard to determine if a character (*e.g.* the absence of anchor/bar complex in monogenoids) is homologous or homoplasious, unless it has been scrutinized under phylogenetic hypothesis [56,68]. The present phylogenetic study, based on the positions of the *Pavanelliella* lineage and of *A. ureteroecetes*, allowed the inference that the anchor/bar complex was independently and secondarily lost in the evolutionary history of Dactylogyridae.

Between the dactylogyrids in which the anchor/bar complex was not observed are included species of *Kritskyia* Kohn, 1990 and *Telethecium* Kritsky, Van Every and Boeger, 1996. Unfortunately, the absence of sequences in the NCBI database did not allow the evolutionary history of such genera to be reconstructed, which would have been interesting due to their morphological closeness to *Pavanelliella* [7]. In addition to these genera is a dactylogyrid which is morphologically close to *Telethecium* and *Pavanelliella*, found parasitizing the nostrils of ctenolucids from the Tapajós River (unpublished data), and which also lacks the anchor/bar complex. A future, more comprehensive study of these dactylogyrids should include these genera in its analysis to elucidate the relationship between them.

The results obtained here converge with previous morphological and molecular analyses by using different markers (*i.e.* 18S rDNA, 28S rDNA, ITS1, 16S, as well as combinations of the same), which indicated the non-monophyly of the Ancyrocephalinae [18,40,42,43,45,46,69–72]. However, while the basal paraphyly of the freshwater ancyrocephalines recovered in the present study (Fig. 4) may be an artefact caused by the outgroup selected, all the adjacent relationships remained the same as those verified in the first inference made to functionally select the outgroup. In the majority of the previous molecular phylogenetic inferences, the overall recovered topology, as in the present study, encompassed a group composed of freshwater ancyrocephalines, which was a sister group to the ancylodiscoidines and had an unresolved relationship with the pseudodactylogyrines, dactylogyrines and the marine lineages of ancyrocephalines (*e.g. Ligophorus* Euzet and Suriano, 1977, *Euryhaliotrema* Kritsky and Boeger, 2002 and *Aliatrema* and *Thylacicleidus* Wheeler and Klassen, 1988) [18,42,45,46,70,72].

The absence of consensus on the relationship between Dactylogyrinae Bychowsky, 1933, Pseudactylogyrinae Ogawa, 1986, and the lineages of marine ancyrocephalines is pervaded by concurrent hypotheses based, in almost every case, and also in our, on recovered relationships, which lack strong branch support. Nevertheless, the hypothesis provided in the present study (Fig. 4) regarding these groups corroborates the most frequent hypothesis, and suggests that dactylogyrines are a sister group to pseudodactylogyrines + marine ancyrocephalines + ancyrocephalines parasites of Afrotropical cichlids from brackish water and freshwater (mostly *Cichlidogyrus* spp. and *Scutogyrus* spp.) [46,70,72]. However Plaisance et al. [45] and Mendoza-Palmero et al. [42] recovered a similar topology, through which they hypothesized that Dactylogyrinae + Pseudodactylogyrinae form a sister group to the lineages of marine ancyrocephalines + afrotropical ancyrocephalines. Ancyrocephalines parasites of Afrotropical cichlids arising between lineages of marine ancyrocephalines have been frequently reported [18,46,70,72], and may have an evolutionary history linked to secondary dispersion to freshwater environments and the colonization of cichlids, as previously indicated [34].

Synonymy, as well as splitting genera based on phylogenetic inferences, has been proposed as a way to organize some of the groups within Dactylogyridae. Among the genera evaluated in the present study, it is inferred that at least two require revision. The relationships observed between species of *Cichlidogyrus* and *Scutogyrus* were uncertain, agreeing with the data that reveal conflicts within these genera [34,39,40,72,73], although there is no doubt about their close phylogenetic relationship, morphological character sharing, and evolutionary biology [34,39,40,42,72–80]. However, there remains no consensus about whether *Scutogyrus* is monophyletic [34,73] as there is regarding the unnatural origin of *Cichlidogyrus*[40,42,72,73]. The results depicted here allow only the inference that *Cichlidogyrus* is not monophyletic.

Several studies have identified the monophyly of *Dactylogyrus* [46,81–83], and the phylogenetic analyses (BI and ML) of the present study showed similar results to evaluations previously carried out for this genus [36,46], where it was possible to observe three main lineages composed mostly of parasites of cypriniforms from the Palearctic region. However, in the present study *A. ureteroecetes*, a parasite of a perciform, appears in the G lineage in a sister relationship along with *Dactlogyrus lamellatus* Achmerow, 1952, while in the I lineage, *Dactylogyroides longicirrus* (Tripathi, 1959), a parasite of a cypriniform, arises as a sister group to the large clade composed of *Dactylogyrus* spp. Although the present phylogenetic proposal provides strong evidence that *Dactylogyrus* is not monophyletic, it is necessary to expand the database with sequences of other markers, especially with the addition of members of other subfamilies anteriorly recognized as circumscribed in this family [43], and other species of the genera *Acolpenteron* Fischthal and Allison, 1940, *Pseudoacolpenteron* Bychowsky and Gussev, 1955 and *Pellucidhaptor* Price and Mizelle, 1964 [46], which can provide new insights.

The present study revealed the relationship of Neotropical lineages of ancyrocephalines from freshwater environments with other dactylogyrids through sequencing of the partial 18S rDNA and the ITS1. The evolutionary relationships hypothesized that *Pavanelliella* is monophyletic and, in spite of its phylogenetic affinities to Dactylogyridae gen. sp. and *V. cicinnus*, the early-diverging branch in which these monogenoids arose suggests that Neotropical ancyrocephalines colonized catfish more than once in their evolutionary history.

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