

Morphology and molecular characterization of *Demidospermus spirophallus* n. sp., *D. prolixus* n. sp. (Monogenea: Dactylogyridae) and a redescription of *D. anus* in siluriform catfish from Brazil

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(Received 29 September 2016; Accepted 26 February 2017; First published online 6 April 2017)

Abstract

The present study describes *Demidospermus spirophallus* n. sp. and *Demidospermus prolixus* n. sp. (Monogenea, Dactylogyridae) from the siluriform catfish *Loricaria prolixa* Isbrücker & Nijssen, 1978 (Siluriformes, Loricariidae) from the state of São Paulo, Brazil, supported by morphological and molecular data. In addition, notes on the circumscription of the genus with a redescription of *Demidospermus anus* are presented. *Demidospermus spirophallus* n. sp. differed from other congeners mainly because of the morphology of the male copulatory organ (MCO), which exhibited 2½ counterclockwise rings, a tubular accessory piece with one bifurcated end and a weakly sclerotized vagina with sinistral opening. *Demidospermus prolixus* n. sp. presents a counterclockwise-coiled MCO with 1½ rings, an ovate base, a non-articulated groove-like accessory piece serving as an MCO guide, two different hook shapes, inconspicuous tegumental annulations, a non-sclerotized vagina with sinistral opening and the absence of eyes or accessory eyespots. The present study provides, for the first time, molecular characterization data using the partial ribosomal gene (28S) of two new species of *Demidospermus* from Brazil (*D. spirophallus* n. sp. and *D. prolixus* n. sp.), and *Demidospermus anus* from *Loricariichthys platymetopon* Isbrücker & Nijssen, 1979 collected in the Upper Paraná River floodplain, Brazil. Additionally, a revision of the species composition of this genus and others that occur in catfish is proposed to elucidate problems with their circumscription. The Brazilian species of *Demidospermus* clustered together as sister taxa among Neotropical dactylogyrids from siluriforms. The morphological characterization of *D. spirophallus* n. sp. and *D. prolixus* n. sp., and the molecular data of the three species in the present study will extend knowledge about this monogenean genus from the Neotropical region, and provide new information for future phylogeny studies.

Introduction

The order Siluriformes is a monophyletic group popularly known as ‘catfish’, with about 35 families and over

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3000 valid living species distributed in freshwater, brackish and marine environments in every continent of the world (Nelson, 2006; Sullivan *et al.*, 2006; Eschmeyer *et al.*, 2016). Most of these species are widely distributed in Neotropical regions (around 50% of the total number of species), although a large minority (about 72 species) are endemic and have restricted geographic ranges, being known only from the original site and basins in which they were discovered (Burguess, 1989; Eschmeyer *et al.*, 2016; Froese & Pauly, 2016). Loricariidae is one of the most diverse families of this order in numbers of genera and species. This family includes seven subfamilies (Chiachio *et al.*, 2008) and 900 valid species, making this taxon the second most species-rich family of Neotropical freshwater fishes after Characidae (Roxo *et al.*, 2014). Many species are of considerable economic importance, being sold for use in aquariums or for human consumption, mainly by riverside populations.

In general, loricariids present high intraspecific phenotypic plasticity, resulting in a great variability of shapes and colour throughout their ontogenetic development. Our limited knowledge of their biological characteristics, together with the biogeographic patterns of distribution and high morphological variability of the loricariids, make studies on the group difficult (Reis *et al.*, 1990; Weber, 2003; Armbruster, 2004). Catfish are suitable hosts for a rich and diverse fauna of gill monogeneans, representing an interesting model for phylogenetic studies of monogeneans from the Neotropical region (Mendoza-Palmero *et al.*, 2012).

Monogeneans represent a diverse group of fish parasites, in terms of number of species, morphology and ecology (Poulin, 2002; Thatcher, 2006). These parasites are known to be highly host specific, parasitizing only one host species or a narrow group of closely related fishes (Thatcher, 2006). The host specificity shown by most monogeneans makes it easier to relate the ecological characteristics of the hosts and the diversity of their monogenean parasites, and to control the phylogenetic history of their associations (Poulin, 2002). Dactylogyridae is one of the most important and largest families among these parasites, occurring in different species of fishes from continental waters of South America (Thatcher, 2006). Approximately 379 nominal species belonging to 31 genera have been described in siluriform fishes, with an increasing number of new descriptions in recent years (Mendoza-Palmero *et al.*, 2012, 2015; Cohen *et al.*, 2013). Most of the descriptions are based on morphological data, and do not provide the molecular characterization of the species described. Molecular techniques have been used to support taxonomic and morphological descriptions of monogeneans, and provide ways of performing phylogenetic analysis based on different markers (e.g. 18S, internal transcribed spacer (ITS) and 28S rDNA regions of the rDNA gene, and cytochrome *c* oxidase (COI) and 16S mitochondrial genes) (Plaisance *et al.*, 2005; Simková *et al.*, 2006; Wu *et al.*, 2006, 2007, 2008; Fehlauer-Ale & Littlewood, 2011; Hahn *et al.*, 2011; Gilmore *et al.*, 2012; Sarabeev & Desdevises, 2014; Mendoza-Palmero *et al.*, 2015), and a means of elucidating the relationship between parasite species, their hosts and the relationship between them (Poulin, 2002; Braga *et al.*, 2014).

Demidospermus was erected to include *Demidospermus anus* Suriano, 1983 as the type species, collected from the gills of *Loricariichthys anus* (Valenciennes, 1835) (= *Loricaria anus*) from Argentina. Currently, *Demidospermus* represents one of the most speciose genera among dactylogyrids of freshwater siluriforms (Mendoza-Palmero *et al.*, 2012), with 29 valid species (including two new species described here) reported in a great variety of catfish species from South America (Mendoza-Palmero *et al.*, 2012). Mendoza-Palmero *et al.* (2015), in a study on the phylogeny of monogenean parasites on Neotropical catfishes, presented the first molecular data of *Demidospermus* species (*D. mortenthaleri* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012 and two other undescribed species, *Demidospermus* sp. 11 and *Demidospermus* sp. 23), parasites of *Brachyplatystoma juruense* (Boulenger, 1898) and *Brachyplatystoma vaillantii* (Valenciennes, 1840) (Pimelodidae), respectively, from Iquitos, Peru. The results of their analyses reveal that dactylogyrids from Neotropical catfishes do not represent a monophyletic group, and suggest that the genus *Demidospermus* is not natural, requiring revision of the species composition and phylogeny.

The aim of the present study was to describe two new species of *Demidospermus*, supported by morphological and molecular data, both gill parasites of *Loricaria prolixa* Isbrücker & Nijssen, 1978 from the Sapucaí-Mirim River, in the state of São Paulo, Brazil. Additionally, the molecular characterization of *D. anus* parasites of the gills of *Loricariichthys platymetopon* Isbrücker & Nijssen, 1979, from the floodplain of the Upper Paraná River, Brazil, and their phylogenetic relationship with other parasites of siluriform fishes are presented.

Materials and methods

Collection and examination of samples

Fifty specimens of *L. prolixa* were collected between March 2012 and December 2013 in the reservoirs of three small hydroelectric power plants (SHPP) (ANEEL, 2008): Palmeiras (20°32'57.33"S, 47°48'47.26"W), Anhanguera (20°29'38.38"S, 47°51'33.11"W) and Retiro (20°26'12.5"S, 47°53'18.59"W), all in the Sapucaí-Mirim River, near the municipalities of Guará and São Joaquim da Barra, in the state of São Paulo, Brazil. Additionally, five specimens of *L. platymetopon* were collected in June 2016 in the Upper Paraná River floodplain, near the municipality of Porto Rico, Brazil (20°43'S, 53°10'W) during the sampling of the Long-Term Ecological Research Program (Pesquisas Ecológicas de Longa Duração – PELD) of the Ichthyology and Aquaculture Research Center (Nupélia) of the State University of Maringá.

Fish were collected from 17.00 to 07.00 hours using a nylon monofilament gill net. The fish specimens were stored individually in plastic bags and placed in a Styrofoam box with ice for immediate transportation to the laboratory where they were measured (cm), weighed (g) and necropsied.

The gills were removed and analysed fresh or placed in vials containing hot water (about 60°C). They were then shaken and absolute ethanol was added (Thatcher, 2006). The monogeneans were collected using a stereomicroscope and some specimens were mounted in Gray

and Wess, Hoyer or GAP (glycerin and picric acid) medium to highlight the sclerotized structures, while others were stained with Gömöri's trichrome and mounted in Canada balsam for analysis of the internal organs (Kritsky *et al.*, 1986). Measurements (in micrometres) are presented as the mean, followed by the range and number of specimens measured (*n*) in parentheses.

Morphological and morphometrical analyses were performed using a computerized image analysis system with differential interference contrast (DIC) (Leica Application Suite, V3; Leica Microsystems, Wetzlar, Germany). The illustrations of the sclerotized structures were produced with the aid of a camera lucida mounted on a Leica DMLS microscope, with phase contrast optics.

Voucher specimens of the fish host were deposited in the Ichthyological Collection of the Limnology, Ichthyology and Aquaculture Research Center (NUP) of the State University of Maringá, state of Paraná, Brazil (NUP 15002). The holotype and paratypes of the proposed new species were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil, and voucher specimens were deposited in the zoological collection of the Amazonas National Research Institute, state of Amazonas, Brazil (INPA) and the Helminthological Collection of the Department of Parasitology, Institute of Biosciences, São Paulo State University – UNESP (CHIBB), in the municipality of Botucatu, São Paulo, Brazil.

Cohen & Kohn (2008) reported the occurrence of *D. anus* parasitizing *L. platymetopon* from the Itaipu Reservoir, in the state of Paraná, Brazil, and lent their material mounted on permanent slides for the analysis of the present study. Four paratypes of *Demidospermus paranaensis* Ferrari-Hoeninghaus, Bellay, Takemoto & Pavanelli, 2010 deposited at the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC 37255) were also examined. The scientific names of the hosts follow Froese & Pauly (2016).

Molecular analysis

To ensure that species separation was performed correctly and to confirm identification, each parasite specimen subjected to molecular analysis was mounted on a slide with glycerin and photographed. The same specimens were then used for molecular characterization. The total genomic DNA was extracted using the Qiagen Dneasy® Blood and Tissue Kit (Qiagen, California, USA), 30 µl for each sample, according to the manufacturer's protocol. Conventional polymerase chain reaction (PCR) amplifications were performed in 25-µl reaction mixes, containing 5 µl of DNA extract, 0.5 µl of each PCR primer using Ready-to-Go PCR beads (Pure Taq™ Ready-to-Go™ beads, GE Healthcare, Chicago, USA), a solution consisting of the stabilizer bovine serum albumin (BSA), deoxynucleoside triphosphates (dNTP: dATP, dCTP, dGTP and dTTP), ~2.5 units of puReTaq DNA polymerase and reaction buffer. The beads were reconstituted to a final volume of 25 µl and the concentration of each dNTP was 200 µM in 10 mM Tris–HCl (pH 9.0 at room temperature), 50 mM KCl and 1.5 mM MgCl₂. The thermocycling profile employed was: initial denaturation of DNA at 94°C for 3 min; followed by 34 cycles of amplification at 94°C for 30 s, 56°C for 30 s

and 72°C for 1.5 min; and a final extension at 72°C for 7 min (Mendoza-Palmero *et al.*, 2015). The primers used for amplification and sequencing were partial 28S rDNA (large subunit, LSU) fragments U178 (5'-GCA CCC GCT GAA YTT AAG-3') and L1642 (5'-CCA GCG CCA TCC ATT TTC A-3') (Lockyer *et al.*, 2003), and L1200R (5'-GCA TAG TTC ACC ATC TTT CGG-3') for sequencing (Littlewood *et al.*, 2000).

PCR products were run on agarose gels using GelRed and loading buffer, and purified using the QIAquick PCR Purification Kit (Qiagen). Automated sequencing was performed directly on the purified PCR products from specimens using BigDye v.3.1 Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, California, USA) for cycle sequencing. Sequences were run on an Applied Biosystems ABI 3500 DNA genetic analyser.

Phylogenetic analysis

For phylogenetic analysis, contiguous sequences were edited in Sequencher™ v.5.2.4 (Gene Codes, Ann Arbor, Michigan, USA) and were subjected to BLAST analysis (<http://blast.ncbi.nlm.nih.gov>) to confirm identity sequences. Newly generated sequences of partial 28S rDNA were aligned with related sequences obtained previously from dactylogyrids of catfishes retrieved from GenBank (table 1). *Dictyocotyle coeliaca* Nybelin, 1941 (AF348359) was used as an outgroup. The species, hosts and accession numbers used in this study are described in table 1.

Nucleic acid sequences were aligned using the Muscle program implemented in Geneious version 7.1.3 (Kearse *et al.*, 2012). All analyses were performed using only positions that were unambiguously alignable across all taxa. The phylogenetic analyses were performed including species of the Dactylogyridae, which were polarized using Monocotylidae as an outgroup.

The JModelTest 2.1.1 program (Posada, 2008) was used to select the most appropriate evolutionary model for Maximum Likelihood (ML) and Bayesian inference (BI). Prior to ML analysis the best-fitting models of nucleotide substitution were determined based on Akaike Information Criteria (AIC) using jModelTest 2.1.1 (Posada, 2008), with GTR+I+G for the partial LSU gene. The supports for ML were determined by performing 100 bootstrap replicates.

Bayesian inference (BI) was performed using the BEAST program (Drummond *et al.*, 2012) and run with a GTR+I+G nucleotide substitution model. Markov Chain Monte Carlo (MCMC) chains were run for 50 million generations and the log-likelihood scores plotted. Only the final 75% of trees were used to produce the consensus trees by setting the burn-in parameter at 5 million generations. Phylogenetic trees were generated and edited in FigTree v.1.3.1 (Rambaut, 2009).

Results

Demidospermus spirophallus n. sp.

Dactylogyridae Bychowsky, 1933; *Demidospermus* Suriano, 1983.

Table 1. Species of the Dactylogyridae of siluriforms used in the present study, with details of locality, host and GenBank accession numbers.

Parasite	Host	Locality	GenBank	Reference
Dactylogyridae				
Ancyrocephalinae				
<i>Ameloblastella chavarriai</i>	<i>Rhamdia quelen</i>	Catemaco Lake, Mexico	KP056251	Mendoza-Palmero <i>et al.</i> , 2015
<i>Ameloblastella edentensis</i> (= <i>Ameloblastella</i> sp. 16)	<i>Hypophthalmus edentatus</i>	Nanay River, Peru	KP056255	Mendoza-Palmero <i>et al.</i> , 2015
<i>Ameloblastella</i> sp. 23	<i>Hypophthalmus edentatus</i>	Nanay River, Peru	KP056233	Mendoza-Palmero <i>et al.</i> , 2015
<i>Aphanoblastella aurorae</i>	<i>Goeldiella eques</i>	Santa Clara, Peru	KP056239	Mendoza-Palmero <i>et al.</i> , 2015
<i>Aphanoblastella</i> sp. 3	<i>Goeldiella eques</i>	Nanay River, Peru	KP056238	Mendoza-Palmero <i>et al.</i> , 2015
<i>Cosmetocleithrum bifurcum</i> (= <i>Cosmetocleithrum</i> sp. 8)	<i>Hassar orestis</i>	Aquarium Momón River, Peru	KP056216	Mendoza-Palmero <i>et al.</i> , 2015
<i>Demidospermus anus</i> (1–2)	<i>Loricariichthys platymetopon</i>	Upper Paraná River floodplain	(1): KY766956 (2): KY766957	Present study
<i>Demidospermus spirophallus</i> n. sp.	<i>Loricaria prolixa</i>	Sapucaí-Mirim River, Brazil	KY766954	Present study
<i>Demidospermus mortenthaleri</i>	<i>Brachyplatystoma juruense</i>	Santa Clara, Peru	KP056245	Mendoza-Palmero <i>et al.</i> , 2015
<i>Demidospermus prolixus</i> n. sp.	<i>Loricaria prolixa</i>	Sapucaí-Mirim River, Brazil	KY766955	Present study
<i>Demidospermus</i> sp. 11	<i>Brachyplatystoma vaillantii</i>	Nanay River, Peru	KP056235	Mendoza-Palmero <i>et al.</i> , 2015
<i>Demidospermus</i> sp. 23	<i>Brachyplatystoma vaillantii</i>	Nanay River, Peru	KP056236	Mendoza-Palmero <i>et al.</i> , 2015
<i>Ligictaluridus pricei</i>	<i>Ameiurus nebulosus</i>	Moldau River, Czech Republic	AJ969939	Simková <i>et al.</i> , 2006
<i>Unibarra paranoplatensis</i>	<i>Aguarunichthys torosus</i>	Santa Clara, Peru	KP056219	Mendoza-Palmero <i>et al.</i> , 2015
<i>Vancleaveus januacaensis</i>	<i>Pterodoras granulosus</i>	Itaya River, Peru	KP056247	Mendoza-Palmero <i>et al.</i> , 2015
Ancylo-discoidinae				
<i>Bychowoskyella pseudobagri</i>	<i>Tachysurus fulvidraco</i>	Shaoguan, China	EF100541	Wu <i>et al.</i> , 2008
<i>Chauhanellus boegeri</i>	<i>Genidens genidens</i>	Antonina Bay, Brazil	KP056241	Mendoza-Palmero <i>et al.</i> , 2015
<i>Chauhanellus</i> sp.	<i>Genidens genidens</i>	Antonina Bay, Brazil	KP056242	Mendoza-Palmero <i>et al.</i> , 2015
<i>Quadriacanthus kobeensis</i>	<i>Clarias batrachus</i>	Guangzhou, China	AY841874	Ding & Liao, 2005 (unpublished)
<i>Schilbetrema</i> sp.	<i>Pareutropius debauwi</i>	Aquarium from Czech Republic, origin West Africa	KP056243	Mendoza-Palmero <i>et al.</i> , 2015
<i>Thaparocleidus asoti</i>	<i>Silurus asotus</i>	Rongchang, China	DQ157669	Wu <i>et al.</i> , 2006
<i>Thaparocleidus cochleavagina</i>	<i>Silurus asotus</i>	Rongchang, China	EF100547	Wu <i>et al.</i> , 2008
<i>Thaparocleidus infundibulovagina</i>	<i>Silurus asotus</i>	Rongchang, China	EF100548	Wu <i>et al.</i> , 2008
<i>Thaparocleidus magnicirrus</i>	<i>Silurus asotus</i>	Kaiping, China	EF100549	Wu <i>et al.</i> , 2008
<i>Thaparocleidus siluri</i>	<i>Silurus ganis</i>	Morava River, Czech Republic	AJ969940	Simková <i>et al.</i> , 2006
<i>Thaparocleidus</i> sp.	<i>Pangasius</i> sp.	Aquarium from Czech Republic, origin Asia	KP056249	Mendoza-Palmero <i>et al.</i> , 2015
<i>Thaparocleidus</i> sp.	<i>Pangasius</i> sp.	Aquarium from Czech Republic, origin Asia	KP056250	Mendoza-Palmero <i>et al.</i> , 2015
<i>Thaparocleidus</i> sp.	<i>Silurus asotus</i>	Chongqing, China	DQ157670	Wu <i>et al.</i> , 2006
<i>Thaparocleidus varicus</i>	<i>Silurus asotus</i>	Chongqing, China	DQ157668	Wu <i>et al.</i> , 2006
Dactylogyrinae				
Dactylogyridae gen. sp. 4	<i>Ageneiosus vittatus</i>	Nanay River, Peru	KP056218	Mendoza-Palmero <i>et al.</i> , 2015
Dactylogyridae gen. sp. 9	<i>Platynemachthys notatus</i>	Santa Clara, Peru	KP056220, 21, 23–24	Mendoza-Palmero <i>et al.</i> , 2015
Dactylogyridae gen. sp. 10	<i>Platynemachthys notatus</i>	Santa Clara, Peru	KP056225–26	Mendoza-Palmero <i>et al.</i> , 2015
Dactylogyridae gen. sp. 12	<i>Sorubim lima</i>	Iquitos-Belém, Peru	KP056228	Mendoza-Palmero <i>et al.</i> , 2015

Continued

Table 1. (Cont.)

Parasite	Host	Locality	GenBank	Reference
Dactylogyridae gen. sp. 13	<i>Hypopthalmus edentatus</i>	Nanay River, Peru	KP056229-30	Mendoza-Palmero <i>et al.</i> , 2015
Dactylogyridae gen. sp. 18	<i>Pseudoplatystoma fasciatum</i>	Santa Clara, Peru	KP056231	Mendoza-Palmero <i>et al.</i> , 2015
Dactylogyridae gen. sp. 23	<i>Platysilurus mucosus</i>	Santa Clara, Peru	KP056232	Mendoza-Palmero <i>et al.</i> , 2015
Dactylogyridae gen. sp. 26	<i>Platynematchthys notatus</i>	Santa Clara, Peru	KP056234	Mendoza-Palmero <i>et al.</i> , 2015
Monocotylidae				
<i>Dictyocotyle coeliaca</i> (= <i>Decacotyle hymmae</i>)	<i>Aetobatus narinari</i>	Heron Island, Australia	AF348359	Chisholm <i>et al.</i> , 2001

Description

Based on 13 specimens mounted in Gray and Wess and 10 specimens stained with Gömöri trichrome and mounted in Canada balsam (fig. 1a–i). Body elongated, fusiform, 880 (670–1225; $n = 8$) long, divisible into cephalic region, trunk, short peduncle and haptor. Greatest width 125 (89–177; $n = 12$) at level of testis. Two terminal and two bilateral cephalic lobes, moderately developed. Three bilateral pairs of head organs; cephalic glands in the pharyngeal region. Eyes and accessory eyespot granules absent. Pharynx subspherical, 43 (33–53; $n = 12$) long, 45 (24–60; $n = 12$) wide. Oesophagus short, intestinal caecum confluent posterior to gonads. Counterclockwise-coiled male copulatory organ (MCO), 212 (193–230; $n = 11$) long, with 2½ rings, proximal ring 21 (18–22; $n = 10$) in diameter. Sheath-like accessory piece serving as guide for the MCO, with one of the ends bifurcated, 53 (49–57; $n = 5$) long. Gonads in tandem, ovary pretesticular. Testis elongated 254 ($n = 1$) long and 150 ($n = 1$) wide; between the intestinal caeca. Vas deferens conspicuous, looping around left intestinal caecum. Seminal vesicle a distal dilatation of vas deferens, with great variation of forms. Oviduct, ootype, uterus not observed. Ovary 52 (40–65; $n = 4$) long, 59 (21–86; $n = 4$) wide. Egg oval with a small posterior filament. Seminal receptacle anterior to ovary. Sinistral vagina aperture weakly sclerotized, curved and tubular, opening into subspherical seminal receptacle through a weakly sclerotized duct. Vitelline follicles densely scattered throughout trunk, absent in region of reproductive organs. Haptor 106 (63–135; $n = 10$) long and 136 (84–206; $n = 10$) wide, with two pairs of anchors and two bars. Anchors with well-developed superficial root, short deep root, short and straight shaft, and elongated straight point. Dorsal anchor 32 (29–37; $n = 11$) long (fig. 2a), base 26 (24–28; $n = 11$) wide (fig. 2b) and root 16 (13–19; $n = 11$) wide (fig. 2c). Ventral anchor 28 (25–31; $n = 12$) long; short, deep root; root 18 (20–25; $n = 12$) wide and base 24 (20–26; $n = 12$) wide. Bars with medial narrowing in the base and variable aperture and distance between ends. Dorsal bar ‘V’ wide open-shaped, articulate, with variable aperture of angulation, distance between ends 58 (23–76; $n = 11$) (fig. 2d), 81 (73–99; $n = 11$) total length (fig. 2e) and 24 (14–38; $n = 11$) height. Ventral bar ‘V’ shaped with ends laterally directed, with an evident narrowing in the middle and varying aperture of angulation; 89 (78–102; $n = 12$) total length, distance between ends 61 (24–97; $n = 11$) and 29 (14–37; $n = 11$) height. Seven pairs of hooks, similar in form and size,

with distribution as proposed by Mizelle (1936), each with a recurved point, delicate throughout; protruding thumb, and filamentous hooklet loop approaching three-quarters of shank length. Shanks of hooks were 8 (6–9; $n = 11$) long, and hooklets 4 (3–5; $n = 11$) long.

Taxonomic summary

Type host. *Loricaria prolixa* Isbrücker & Nijssen, 1978 (Siluriformes, Loricariidae).

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

Site of infection. Gills.

Infection rate. Prevalence: 98.0%; mean intensity of infection: 92.5 ± 10.5 (9.0–400.0); mean abundance: 90.7 ± 10.5 (0–400.0).

Specimens deposited. Holotype CHIOC (38611a); paratypes CHIOC (38611b–d); vouchers INPA (705 a–d) and CHIBB (226L–232L).

Etymology. The specific epithet is from the Latin and is derived from the coiled disposition of the male copulatory organ (MCO) (*spiro* = anything wound up or coiled + *phallus* = penis).

Remarks

Demidospermus spirophallus n. sp. was included in the genus proposed by Suriano (1983) based on agreement with the amended generic characteristics of the diagnosis presented by Kritsky & Gutiérrez (1998), such as the tubular MCO with a sheath-like accessory piece, sinistral vagina, the absence of eyes or accessory granules, gonads in tandem, and ventral and dorsal bars shaped in either ‘U’ or ‘V’ forms. In *D. spirophallus* n. sp., the bars showed varied opening angles, depending on the mounting of the permanent slides.

This new species differs from its congeners mainly due to the morphology, diameter and number of rings of the counterclockwise-coiled male copulatory organ (MCO); the accessory piece, the shape of the vagina and the presence of a weakly sclerotized duct that composes the seminal receptacle. The sinistral vagina is tubular, curved,

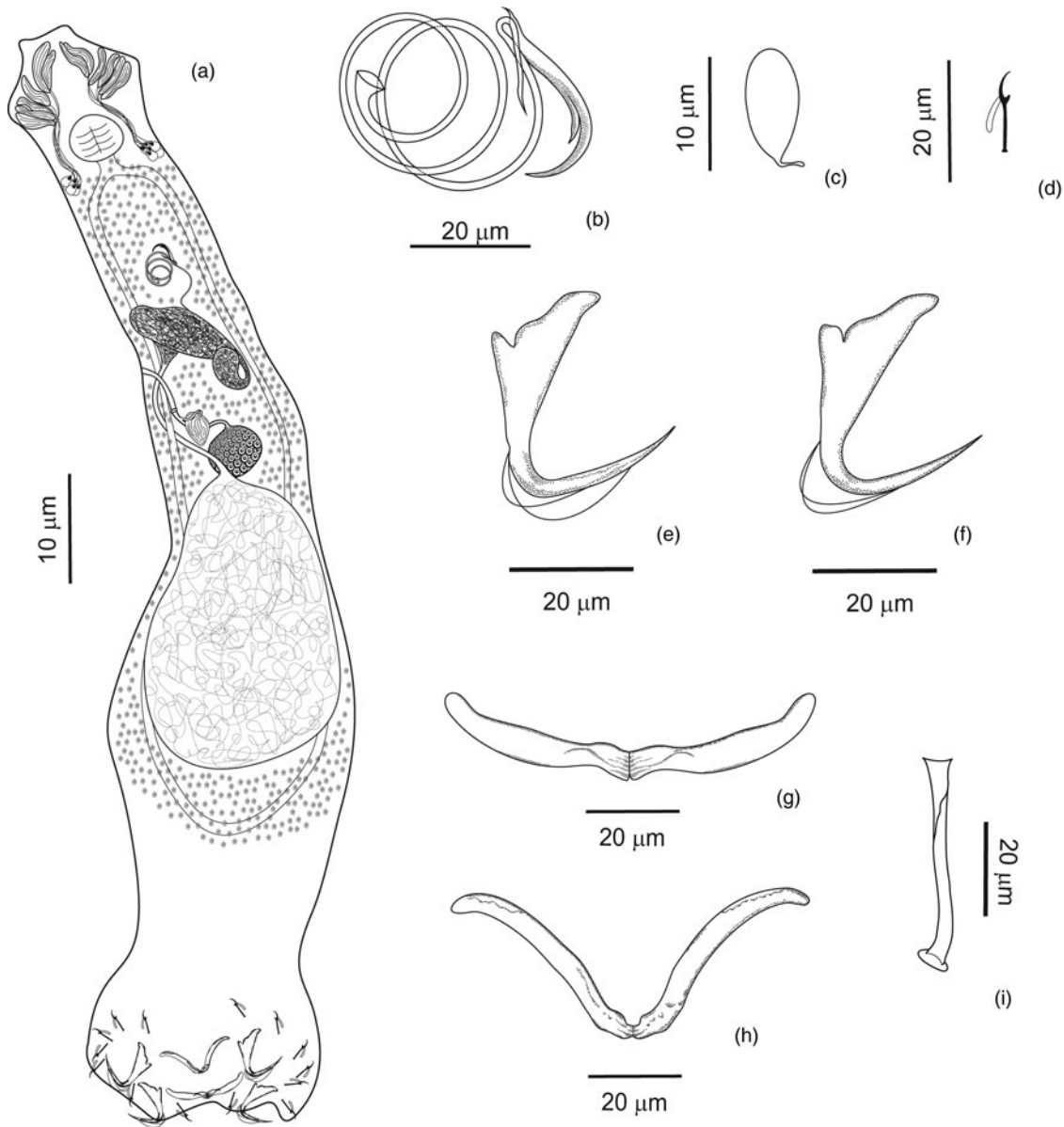


Fig. 1. Morphology of *Demidospermus spirophallus* n. sp., showing: (a) entire body, dorsal view (composite); (b) copulatory complex, ventral view; (c) egg; (d) hook; (e) dorsal anchor; (f) ventral anchor; (g) dorsal bar; (h) ventral bar; and (i) vagina, dorsal view.

delicate, weakly sclerotized, opening into a small seminal receptacle with a pouch-like shape, throughout a weakly sclerotized duct, versus the non-sclerotized vagina described in other species in this genus, such as *D. luckyi* (Kritsky, Thatcher & Boeger, 1987) (= *Omothecium luckyi*), *D. pinirampi* (Kritsky, Thatcher & Boeger, 1987) (= *Omothecium pinirampi*), *D. ceccarellii* Cepeda & Luque, 2010 and *D. brachyplatystomae* Cepeda & Luque, 2010. The presence of the sclerotized duct as part of the seminal receptacle has never been described before in *Demidospermus* specimens.

The MCO of this new species has 2½ rings and the accessory piece is tubular, with one bifurcated end, while the accessory piece of the type species *D. anus* is composed of only one sclerotized piece, the number of the cirrus rings

is lower (1½) and the diameter of the rings are greater than in *D. spirophallus* n. sp. The species *D. paranaensis*, described parasitizing a loricariid from Brazil, also exhibits a counterclockwise-coiled MCO, although the cirrus is composed of just 1½ rings, long and elliptical shaped, and its tegument presents conspicuous annulations throughout the trunk and peduncle (inconspicuous in *D. spirophallus* n. sp.). Although some species of the same genus may present the cirrus as a sigmoid tube (*D. centromochli* Mendoza-Franco & Scholz, 2009), C-shaped (*D. ichthyocercus* Monteiro, Kritsky & Brasil-Sato, 2010) or a short and robust tube (*D. brevicirrus* Mendoza-Palmero, Scholz, Mendoza-Franco & Kuchta, 2012), the majority have a counterclockwise-coiled MCO.

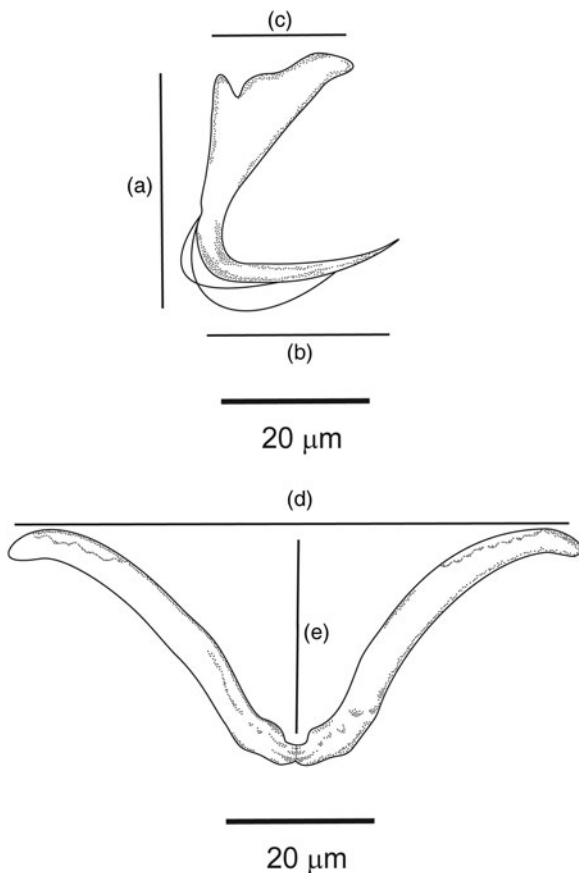


Fig. 2. Anchor and bar of *Demidospermus spirophallus* n. sp., showing: (a) length of superficial root to base; (b) width of base; (c) root width; (d) distance between ends; and (e) bar length.

Demidospermus spirophallus n. sp., as well as *D. paranaensis*, *D. anus*, *D. ichthyocercus* and *D. centromochli*, present no differences in terms of the morphology of the haptor hooks, unlike some of its congeners (e.g. *D. macropteri* Mendoza-Franco & Scholz, 2009, *D. peruvianus* Mendoza-Palmero & Scholz, 2011, *D. curvovaginatus* Mendoza-Palmero & Scholz, 2011, *D. striatus* Mendoza-Palmero & Scholz, 2011, *D. lebedevi* (Kritsky & Thatcher, 1976), *D. brevicirrus*, *D. mortenthaleri* and *D. pinirampi*).

Although *D. lebedevi* also shows a cirrus composed of circular rings, it has 2–3 rings with a greater diameter (72 (56–83)) than those of *D. spirophallus* n. sp. Furthermore, *D. lebedevi* has eyes and an accessory eyespot, which are absent in the new species.

Demidospermus prolixus n. sp.

Description

Based on two specimens mounted in Gray and Wess, five in Hoyer and one stained with Gömöri trichrome and mounted in Canada balsam (fig. 3a–h). Body elongated, fusiform, 747 (416–987; $n = 7$) long, divisible into cephalic region, trunk, short peduncle and haptor.

Greatest width 119 (109–130; $n = 7$) at level of testis. Two terminal and two bilateral cephalic lobes, moderately developed. Three bilateral pairs of head organs; cephalic glands in the pharyngeal region. Eyes and accessory eyespot granules absent. Pharynx subspherical, 37 (34–48; $n = 7$) long; 31 (25–58; $n = 7$) wide. Oesophagus short, intestinal caecum confluent posterior to gonads. Counterclockwise-coiled male copulatory organ (MCO), 223 (210–234; $n = 7$) long, and $1\frac{1}{2}$ rings, 66 (60–74; $n = 7$) long and 27 (20–31; $n = 7$) wide. Accessory piece straight and strongly sclerotized, groove-like, 36 (32–38, $n = 7$) long. Gonads in tandem, ovary pretesticular. Testis elongated 138 (113–164; $n = 2$) long and 64 (54–74; $n = 2$) wide; between the intestinal caeca. Vas deferens conspicuous, looping around left intestinal caecum. Seminal vesicle a distal dilatation of vas deferens, with great variation in forms. Oviduct, ootype, uterus not observed. Egg oval, 106 (100–112; $n = 2$) long and 52 (49–54; $n = 2$) wide, with a posterior filament, 52 (45–58; $n = 2$) long. Seminal receptacle not observed. Sinistral vagina aperture non-sclerotized. Vitelline follicles densely scattered throughout trunk, below the pharynx and anteriorly to the peduncle. Haptor 78 (60–103; $n = 7$) long and 170 (152–205; $n = 7$) wide, slightly subtrapezoidal, with two pairs of anchors and two bars. Anchors with well-developed superficial root; short, deep root; short and curved shaft. Ventral anchor 36 (34–38; $n = 5$) long; root 15 (13–16; $n = 7$) wide and base 19 (16–22; $n = 7$) wide. Dorsal anchor 36 (34–39; $n = 5$) long; root 15 (13–16; $n = 5$) wide, and base 21 (20–24; $n = 6$) wide. Bars with medial narrowing in the base and variable aperture and distance between ends. Ventral bar 'V' wide open-shaped, articulate, with variable aperture of angulation (conspicuously more open than the dorsal bar), total length 85 (83–86; $n = 5$), distance between ends 73 (71–80; $n = 5$) and 19 (11–26; $n = 5$) height. Dorsal bar 'V' shaped, with an evident narrowing in the middle and varying aperture of angulation; 72 (70–73; $n = 5$) total length, distance between ends 39 (31–47; $n = 5$) and height 30 (28–33; $n = 5$). Seven pairs of hooks, dissimilar in terms of the shank size: hook pairs 1–6 are similar (shanks 9 (8–10; $n = 15$) long and hooklets 5 (4–6; $n = 15$) long), but hook pair 7 presents a longer shank (shanks 13 (10–14; $n = 15$) long and hooklets 6 (5–7; $n = 15$) long). The hook distribution is as proposed by Mizelle (1936), each with a recurved point, delicate throughout; protruding thumb and filamentous hooklet loop approaching half the shank length.

Taxonomic summary

Type host. *Loricaria prolixa* Isbrücker & Nijssen, 1978 (Siluriformes, Loricariidae).

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

Site of infection. Gills.

Infection rate. Prevalence: 98.0%; mean intensity of infection: 169.4 ± 19.3 (16.0–732.0); and mean abundance: 166.0 ± 19.2 (0–732.0).

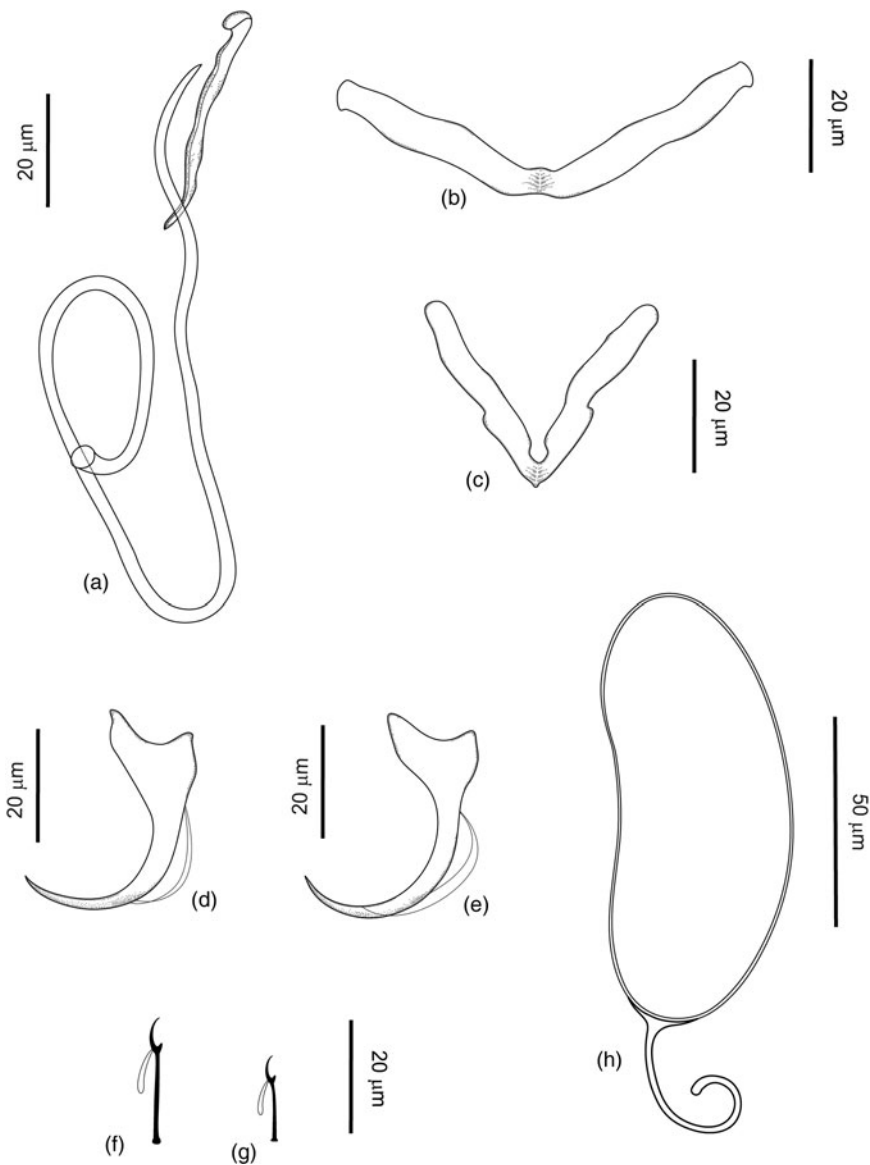


Fig. 3. Morphology of *Demidospermus prolixus* n. sp., showing: (a) copulatory complex, ventral view; (b) ventral bar; (c) dorsal bar; (d) dorsal anchor; (e) ventral anchor; (f) hook pair 7; (g) shape of hook pairs 1–6; (h) egg.

Specimens deposited. Holotype CHIOC (38609a); paratype CHIOC (38609b–c; 38610); vouchers INPA (704 a–e) and CHIBB (233L–236L).

Etymology. The specific epithet is derived from the name of the type host (*Loricaria prolixa*).

Remarks

Demidospermus prolixus n. sp., as well as *D. spirophallus* n. sp., was included in this genus based on agreement with the amended generic characteristics of the diagnosis presented by Kritsky & Gutiérrez (1998), previously cited. The morphology of the copulatory complex of this new

species is very similar to that of *D. paranaensis* described by Ferrari-Hoeinghaus *et al.* (2010) from *L. platymetopon*, as the presence of a longer, counterclockwise-coiled MCO, with an ovate base and non-articulated groove-like accessory piece serving as a MCO guide. However, the MCO and the accessory piece are more robust and larger than those observed in *D. paranaensis*.

This new species differs from other congeners mainly due to the morphology of the bars (dorsal bar with conspicuous protuberances), two different hook shapes (fig. 3a–g), and inconspicuous tegumental annulations (conspicuous in *D. paranaensis*). *Demidospermus prolixus* n. sp. shares the presence of a non-sclerotized vagina with other congeners, such as *D. pinirampi*, *D. luckyi*,

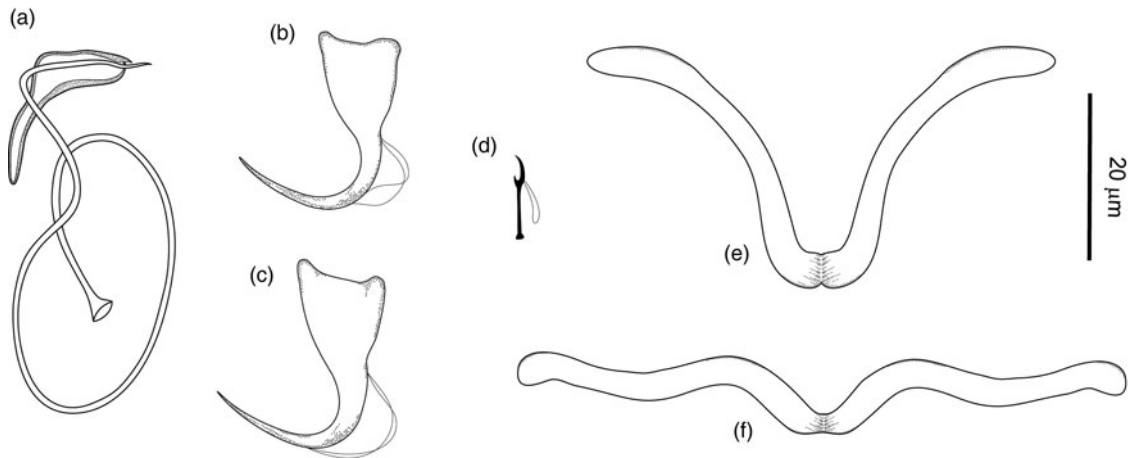


Fig. 4. Morphology of *Demidospermus anus*, dorsal view: (a) copulatory complex; (b) ventral anchor; (c) dorsal anchor; (d) hook; (e) dorsal bar; and (f) ventral bar.

D. ceccarellii and *D. brachyplatystomae*, and has no eyes or accessory eyespots, like *D. anus*, *D. paranaensis*, *D. macropteri* and *D. spirophallus* n. sp. However, other congeners have subspherical eyespots and/or scattered granules.

Demidospermus anus Suriano, 1983

Redescription

Characteristics based on two specimens mounted in Gray and Wess, and six specimens mounted in Hoyer (fig. 4a–f). Body elongated, fusiform, 356 (319–392; $n = 3$) long, divisible into cephalic region, trunk, short peduncle and haptor. Greatest width 65 (55–75; $n = 3$) at level of testis. Two terminal and two bilateral cephalic lobes, moderately developed. Three bilateral pairs of head organs; cephalic glands in the pharyngeal region. Eyes and accessory eyespot granules absent. Pharynx subspherical, 28 (24–32; $n = 4$) long. Oesophagus short, intestinal caecum confluent posterior to gonads. Counterclockwise-coiled male copulatory organ (MCO), 152 (143–156; $n = 5$) long, curved tube, with variable diameter and loop number (1–1½ rings), depending on the mounting slide. Sheath-like accessory piece serving as guide for the MCO, 31 (30–33; $n = 5$) long. Gonads intercaecal, tandem; ovary pretesticular. Vas deferens conspicuous, looping around left intestinal caecum; seminal vesicle a distal dilatation of vas deferens. Oviduct, ootype, uterus not observed. Sinistral vagina aperture weakly sclerotized, curved and tubular. Vitelline follicles densely scattered throughout trunk, absent in region of reproductive organs. Haptor 48 (46–52; $n = 3$) long and 71 (69–73; $n = 3$) wide, with two pairs of anchors and two bars. Anchors with short superficial root, deep root, short and straight shaft. Ventral and dorsal anchors similar, 20 (19–21; $n = 4$) long; base 15 (14–16; $n = 4$) wide; and root 11 (10–12; $n = 4$) wide. Dorsal bar 'V' shaped, wide, open-shaped, articulate, with medial narrowing in the base and variable aperture and distance between ends, 60 (50–70; $n = 3$) total length. Ventral bar 'V' shaped, more open than the dorsal

bar, with an evident narrowing in the middle; 65 (60–70; $n = 3$) total length. Seven pairs of hooks, similar in form and size, 11 (10–12; $n = 8$) total length, with distribution as proposed by Mizelle (1936), each with a recurved point, delicate throughout; protruding thumb and filamentous hooklet loop approaching three-quarters of shank length.

Taxonomic summary

Type host. *Loricariichthys anus* (Valenciennes, 1835) (= *Loricaria anus*) (Siluriformes, Loricariidae).

Type locality. Laguna de Chascomús (35°37'S, 58°04'W), Province of Buenos Aires, Argentina.

Site of infection. Gills.

Material studied (present study). Eight specimens from *Loricariichthys platymetopon* Isbrücker & Nijssen, 1979 (Siluriformes, Loricariidae), collected in June 2016 in the Upper Paraná River floodplain, near the municipality of Porto Rico, Paraná, Brazil (20°43'S, 53°10'W).

Specimens deposited. Vouchers CHIOC (38608 a–e), INPA (703 a–d) and CHIBB (237L–243L).

Additional hosts. *Loricariichthys platymetopon* from the reservoir of Itaipu Hydroelectric Power Station, Paraná, Brazil (Cohen & Kohn, 2008).

Remarks

Demidospermus was proposed by Suriano (1983) with *D. anus* as the type species. However, as described by the authors, the holotype, paratypes or vouchers of the type species were never deposited at the Helminthological Section of the Museo de La Plata, Argentina. The scarcity of information about this material, its incomplete description and the absence of sequences of the type species

make studies of this monogenean genus difficult, and represent a problem in its circumscription.

Ferrari-Hoeninghaus *et al.* (2010) described the species *D. paranaensis* from *L. platymetopon* collected in the Upper Paraná River floodplain, which is the same reservoir and the same host species from which the specimens of *D. anus* analysed in the present study were collected. In another study, Cohen & Kohn (2008) reported, for the first time, the occurrence of *D. anus* in *L. platymetopon* from the reservoir of the Itaipu Hydroelectric Power Station, Brazil. Ferrari-Hoeninghaus *et al.* (2010) considered that *D. paranaensis* differed from *D. anus* mainly based on the presence of annulations in the tegument (present in *D. paranaensis* and not described in *D. anus*), the size of the haptor pieces and the morphology of the MCO. However, analysing the paratypes of *D. paranaensis* deposited in the CHIOC and the specimens analysed by Cohen & Kohn (2008) described as *D. anus*, no differences were found between the morphology and measurements of the specimens. In both cases, all the specimens had annulations in the tegument, an absence of eyespots, and similar morphologies and measurements of the cirrus (124 (123–125; $n = 10$) total length), accessory piece (18 (17–19; $n = 5$) and haptor pieces (fig. 4a–f and fig. 5a–f). Moreover, the diameter of the cirrus ring varies greatly, depending on the mounting and compression. Considering that in both cases the gills were removed and fixed in 1:4000 formalin solution, the annulations may be a consequence of the technique used for fixation prior to analysis.

These annulations were not evident in the specimens of *D. anus* analysed in the present study or those analysed by Suriano (1983) and, in both cases, the material was analysed from frozen fish and *in vivo*, respectively. In general, the morphologies of *D. paranaensis* and *D. anus* analysed in the present study are very similar (figs 4 and 5), including the presence of partially encapsulated sperm, as described by Suriano (1983). However, differences in the morphology of the anchors, and the measurements of

the cirrus and accessory piece (greater in *D. anus* than *D. paranaensis*) were observed. Careful future morphological and molecular studies are required on these species.

Molecular characterization

The new partial LSU sequence length varied from 1483 bp for *D. spirophallus* n. sp., to 1281 bp for *D. prolixus* n. sp., 684 bp for *D. anus* (1) and 918 bp for *D. anus* (2). An unambiguous alignment including Dactylogyridae from the Siluriformes available in GenBank spanned 480 positions. A total of 39 monogenean specimens of siluriforms from the Neotropical, Palaearctic, Ethiopian and Oriental regions were used in the analyses (table 1). The ML and BI analyses yielded similar branching topology, with supported values in the most basal and terminal nodes. Tree main clades, A, B, C and D, were observed and corroborated the findings of Mendoza-Palmero *et al.* (2015) (fig. 6), with a few changes. Clade A comprises parasites of siluriforms from the Neotropical region and *Ligictaluridus pricei* (Mueller, 1936) a parasite of ictalurid fish from the Czech Republic, introduced to Europe with its host. Clade B consists of all *Thaparocleidus* spp. and *Aphanoblastella* spp., forming a polytomic clade related to clades A and B, with strong branching support. Clade C consists of all Dactylogyridae gen. spp. and *Demidospermus* spp. from the Neotropical region, and *Bychowskyella pseudobagri* Achmerow, 1952, and *Quadriacanthus kobeensis* Ha Ky, 1968 from Oriental regions such as China. *Demidospermus spirophallus* n. sp. and *D. prolixus* n. sp. parasites of *L. prolixa*, and specimens of *D. anus* parasites of *L. platymetopon* clustered together as sister taxa in a separate clade within the main clade C, with high supporting values. Finally, clade D consisted of dactylogyrids from the Neotropical and Ethiopian regions (*Chauhanellus* spp. from Brazil and *Schilbetrema* sp. from West Africa). The position of clade D with the

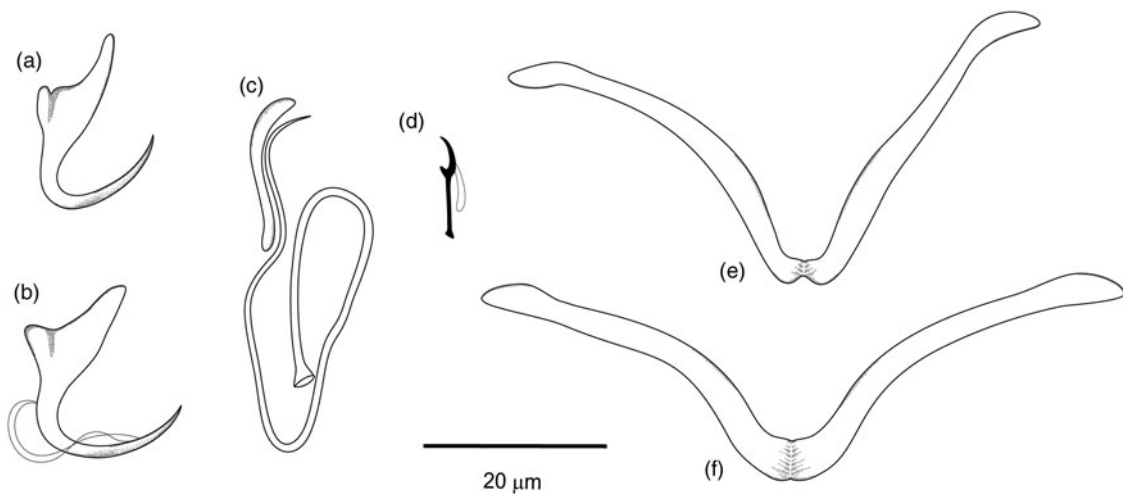


Fig. 5. Morphology of *Demidospermus paranaensis*, dorsal view: (a) ventral anchor; (b) dorsal anchor; (c) copulatory complex; (d) hook; (e) dorsal bar; and (f) ventral bar.

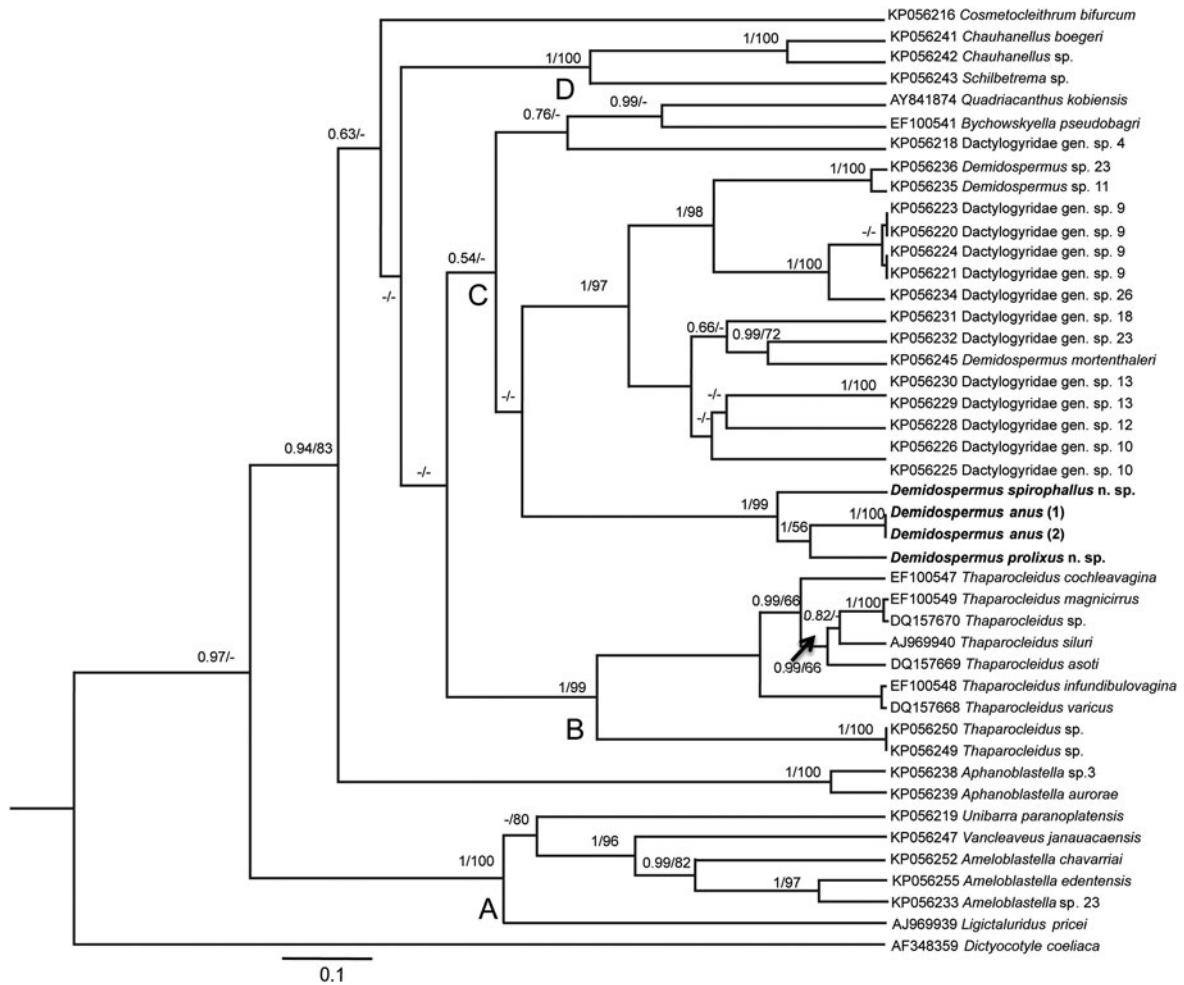


Fig. 6. Bayesian topology based on partial 28S ribosomal DNA sequences of Dactylogyridae from siluriforms. GenBank accession numbers precede species names. Support values are above nodes as follows: posterior probabilities for Bayesian analyses and bootstrap for maximum likelihood analyses. Dashes represent branches not supported by analyses (posterior probabilities <0.50 and bootstrap scores <50). Arrow indicates support values above the node. Branch length scale bar indicates number of substitutions per site. The species *Dictyocotyle coeliaca* (AF348359) was used as an outgroup.

species *Chauhanellus* sp., *Chauhanellus boegeri* Domingues and Fehlaue, 2006, and *Schilbetrema* sp. was not supported, as observed by Mendoza-Palmero *et al.* (2015). The non-identified Dactylogyridae gen. sp. 4 is allocated differently from that in the study by Mendoza-Palmero *et al.* (2015), within the *Bychowskyella pseudobagri* and *Quadriacanthus kobiensis* clade, with high Bayesian support and low bootstrap. *Cosmetocleithrum bifurcum* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016 (= *Cosmethocleithrum* sp. 8) was not resolved, forming a polytomy.

Discussion

In addition to the problems regarding the circumscription of the genus *Demidospermus* due to its incomplete description and the absence of deposited material of the type

species, a number of morphological characteristics described in the description of specimens of this genus vary considerably, such as the position of the vaginal opening (sinistral versus dextral; anterior versus medial opening); sclerotized versus non-sclerotized vagina; presence versus absence of eyespots or scattered granules; hook shape (similar morphology versus variable morphology), counterclockwise-coiled versus clockwise MCO. This represents a problem for the accurate identification of the specimens based on morphology, as some characteristics may overlap with those observed in other genera, and perhaps reinforces the possibility that this group is not natural.

The closeness of the phylogenetic relationships of host species has a strong influence on the distribution of monogenean parasites, to the extent that the majority of hosts are susceptible to a specific group of these parasites (Thatcher, 2006; Braga *et al.*, 2014). Neotropical

Table 2. Species of the genus *Demidospermus* described in the present study with details of locality, host type and family.

Species	Type host	Host family	Reference	Locality
<i>Demidospermus anus</i>	<i>Loricariichthys anus</i> (Valenciennes, 1835) (= <i>Loricaria anus</i>)	Loricariidae	Suriano (1983)	Argentina
<i>Demidospermus luckyi</i> (= <i>Omothecium luckyi</i>)	<i>Pinirampus pinirampu</i> (Spix & Agassiz, 1829)	Pimelodidae	Kritsky <i>et al.</i> (1987); Kritsky & Gutiérrez (1998)	Brazil
<i>Demidospermus pinirampi</i> (= <i>Omothecium pinirampi</i>)	<i>Pinirampus pinirampu</i>	Pimelodidae	Kritsky <i>et al.</i> (1987); Kritsky & Gutiérrez (1998)	Brazil
<i>Demidospermus valenciennesi</i>	<i>Parapimelodus valenciennis</i> (Lütken, 1874) (= <i>Parapimelodus valenciennesi</i>)	Pimelodidae	Gutiérrez & Suriano (1992)	Argentina
<i>Demidospermus paravalenciennesi</i>	<i>Synodontis clarias</i> (Linnaeus, 1758) (= <i>Pimelodus clarias</i>) ¹	Pimelodidae	Gutiérrez & Suriano (1992)	Argentina
<i>Demidospermus uncusvalidus</i>	<i>Synodontis clarias</i> ¹ and <i>Thachelyopterus galeatus</i> (Linnaeus, 1766) (= <i>Parauchenipterus galeatus</i>)	Pimelodidae and Auchenipteridae	Gutiérrez & Suriano (1992)	Argentina
<i>Demidospermus bidiverticulatum</i> (= <i>Paramphocleithrium bidiverticulatum</i> Suriano and Incorvaia, 1995)	<i>Synodontis clarias</i> ¹ and <i>Pimelodus albicans</i> (Valenciennes, 1840)	Pimelodidae	Suriano & Incorvaia (1995); Kritsky & Gutiérrez (1998)	Argentina
<i>Demidospermus armostus</i>	<i>Synodontis clarias</i> ¹ and <i>Pimelodus albicans</i>	Pimelodidae	Kritsky & Gutiérrez (1998)	Argentina
<i>Demidospermus corcinus</i>	<i>Bergiaria westermanni</i> (Lütken, 1874) (= <i>Iheringichthys westermanni</i>)	Pimelodidae	Kritsky & Gutiérrez (1998)	Argentina
<i>Demidospermus idolus</i>	<i>Pimelodus albicans</i>	Pimelodidae	Kritsky & Gutiérrez (1998)	Argentina
<i>Demidospermus leptosynophallus</i>	<i>Bergiaria westermanni</i>	Pimelodidae	Kritsky & Gutiérrez (1998)	Argentina
<i>Demidospermus majusculus</i>	<i>Pimelodus albicans</i>	Pimelodidae	Kritsky & Gutiérrez (1998)	Argentina
<i>Demidospermus labrosi</i> (= <i>Demidospermus corcinus</i>)*	<i>Iheringichthys labrosus</i> (Lütken, 1874)	Pimelodidae	França <i>et al.</i> (2003)	Brazil
<i>Demidospermus mandi</i> (= <i>Demidospermus leptosynophallus</i>)*	<i>Iheringichthys labrosus</i>	Pimelodidae	França <i>et al.</i> (2003)	Brazil
<i>Demidospermus macropteri</i>	<i>Calophysus macropterus</i> (Lichtenstein, 1819)	Pimelodidae	Mendoza-Franco & Scholz (2009)	Peru
<i>Demidospermus centromochli</i>	<i>Centromochlus heckelii</i> (De Filippi, 1853)	Auchenipteridae	Mendoza-Franco & Scholz (2009)	Peru
<i>Demidospermus osteomystax</i>	<i>Auchenipterus osteomystax</i> (Miranda Ribeiro, 1918)	Auchenipteridae	Tavernari <i>et al.</i> (2010)	Brazil
<i>Demidospermus ceccarelli</i>	<i>Brachyplatystoma filamentosum</i> (Lichtenstein, 1819)	Pimelodidae	Cepeda & Luque (2010)	Brazil
<i>Demidospermus brachyplatystomae</i>	<i>Brachyplatystoma filamentosum</i>	Pimelodidae	Cepeda & Luque (2010)	Brazil
<i>Demidospermus araguaiaensis</i>	<i>Brachyplatystoma filamentosum</i>	Pimelodidae	Cepeda & Luque (2010)	Brazil
<i>Demidospermus ichthyocercus</i>	<i>Pimelodus maculatus</i> Lacépède, 1803	Pimelodidae	Monteiro <i>et al.</i> (2010)	Brazil
<i>Demidospermus paranaensis</i>	<i>Loricariichthys platymetopon</i> Isbrücker & Nijssen, 1979	Loricariidae	Ferrari-Hoeninghaus <i>et al.</i> (2010)	Brazil
<i>Demidospermus peruvianus</i>	<i>Pimelodus ornatus</i> Kner, 1858	Pimelodidae	Mendoza-Palmero & Scholz (2011)	Peru
<i>Demidospermus curvovaginatus</i>	<i>Pimelodus</i> sp. Lacépède, 1803	Pimelodidae	Mendoza-Palmero & Scholz (2011)	Peru
<i>Demidospermus striatus</i>	<i>Pimelodus</i> sp.	Pimelodidae	Mendoza-Palmero & Scholz (2011)	Peru
<i>Demidospermus lebedevi</i> (= <i>Urocleidoides lebedevi</i>)	<i>Pimelodus grosskopfii</i> Steindachner, 1879	Pimelodidae	Kritsky & Thatcher (1976); Mendoza-Palmero & Scholz (2011)	Colombia
<i>Demidospermus annulus</i>	<i>Parapimelodus valenciennis</i>	Pimelodidae	Marcotegui & Martorelli (2011)	Argentina
<i>Demidospermus mortenthaleri</i>	<i>Brachyplatystoma juruense</i> (Boulenger, 1898)	Pimelodidae	Mendoza-Palmero <i>et al.</i> (2012)	Peru
<i>Demidospermus brevicirrus</i>	<i>Pimelodus</i> sp.	Pimelodidae	Mendoza-Palmero <i>et al.</i> (2012)	Peru
<i>Demidospermus prolixus</i> n. sp.	<i>Loricaria prolixa</i> Isbrücker & Nijssen, 1978	Loricariidae	Present study	Brazil

Continued

Table 2. (Cont.)

Species	Type host	Host family	Reference	Locality
<i>Demidospermus spirophallus</i> n. sp.	<i>Loricaria prolixa</i> Isbrücker & Nijssen, 1978	Loricariidae	Present study	Brazil

*Junior synonyms of *Demidospermus leptosynophallus* and *Demidospermus corcinus*, respectively (invalid species).

¹According to Monteiro *et al.* (2010), the fish identified as *Synodontis clarias* (= *Pimelodus clarias*) is in fact *Pimelodus maculatus*.

siluriforms, specifically, do not represent a monophyletic assemblage, and include descendants of four independent monophyletic clades (Sullivan *et al.*, 2006; Braga *et al.*, 2014). The origin of their monogenean fauna is unclear, reflecting the differences of the fish lineages that make up this order (Braga *et al.*, 2014). The reports of monogeneans of *Demidospermus* described until now (29 species) are limited to freshwater siluriforms from the Neotropics (table 2), belonging to the Loricariidae, Pimelodidae and Auchenipteridae families, distributed from the Peruvian Amazon to Argentina. These fish families are not phylogenetically closely related (see Sullivan *et al.*, 2006, and references therein; Mendoza-Palmero & Scholz, 2011) and the occurrence of some species of *Demidospermus* parasitizing these fishes, along with recently reported molecular data about these monogeneans (see Mendoza-Palmero *et al.*, 2015), suggest the need to review the species composition of this genus and others that occur in catfishes, for the correct generic assignment of these species.

Ten species belonging to *Demidospermus* have been recorded in catfishes from Brazil, and other species have been described in fishes collected in Argentina (11 species), Peru (Peruvian Amazon) (7 species) and Colombia (1 species). Among these, only four species of *Demidospermus* were described specifically from loricariid fishes (table 2), including the type species *D. anus*. The loricariid recorded as hosts to *Demidospermus* spp. are *L. anus* (= *Loricaria anus*), which was reported as a host to the type species *D. anus* (Suriano, 1983); *L. platymetopon*, a host of *D. paranaensis* (Ferrari-Hoeninghaus *et al.*, 2010) and *D. anus* (Cohen & Kohn, 2008); *Loricariichthys castaneus* (Castelnau, 1855), parasitized with *Demidospermus* sp. (Azevedo *et al.*, 2010), and *L. prolixa*, a host of the two new species described in this study.

Mendoza-Palmero *et al.* (2015), in a study on the phylogeny of Neotropical monogeneans from catfishes (pimelodids), suggested a possible polyphyly of the group, based on the subdivision of the monogeneans into three main clades (A, B and C) within Dactylogyridae. In addition, the authors presented the first molecular data of *Demidospermus* species (*D. mortenthaleri* and two other undescribed species – *Demidospermus* sp. 11 and *Demidospermus* sp. 23), parasites of the pimelodids *B. juruense* and *B. vaillantii*, respectively, from Iquitos, Peru. However, observing their phylogenetic results and carefully analysing the morphology of *D. mortenthaleri* (see Mendoza-Palmero *et al.*, 2012), and other species of *Demidospermus* described parasitizing *Brachyplatystoma filamentosum* (Lichtenstein, 1819) from Brazil (Cepeda & Luque, 2010) (*D. ceccarelli*, *D. brachyplatystomae* and *D. araguaiaensis*), we hypothesize that these species do not in fact belong to this genus, and so should be included in a new genus. Although these species resemble each

other, they show conspicuous differences in terms of the diagnostic characteristics of the *Demidospermus* genus (e.g. bar shapes). The same may have occurred with the undescribed species, namely *Demidospermus* sp. 11 and *Demidospermus* sp. 23, but the absence of a morphological description for these species (Mendoza-Palmero *et al.*, 2015) limits conclusions at the present time. No sequences of either *Demidospermus* specimens from Brazil or monogenean parasites from loricariid fishes have been performed until now. The present study therefore provides, for the first time, data regarding the molecular characterization of *Demidospermus* species parasitizing loricariid fishes, supporting future investigations and phylogenetic studies.

Demidospermus spirophallus n. sp., *D. prolixus* n. sp. and *D. anus* clustered together within the most diverse clade, the Neotropical region (clade C, see Mendoza-Palmero *et al.*, 2015), and were closely related to the monogeneans previously described in the Ancylo-discoidinae subfamily. These three species of *Demidospermus* from Brazilian loricariids did not group together with other *Demidospermus* species parasites of pimelodids from Peru (*Demidospermus* sp. 23, *Demidospermus* sp. 11 and *D. mortenthaleri*), exhibiting a distant relationship, as described previously by Mendoza-Palmero *et al.* (2015), reinforcing the fact that this genus is not natural. Based on the results of the morphological and molecular data presented in this study, we suggest that, in fact, lineages of dactylogyrids from loricariids belong to the *Demidospermus* genus, and an erection of two new genera should be proposed to accommodate parasite species of pimelodids from Peru (one to accommodate the species *Demidospermus* sp. 23 and *Demidospermus* sp. 11, and another for *D. mortenthaleri*). However, future morphological and phylogenetic analyses with the inclusion of new sequences of dactylogyrids identified as belonging to the *Demidospermus* genus from other siluriform fish may clarify the host associations, and confirm whether this dactylogyrid possesses specificity for parasitizing loricariid fishes or if, in fact, they can be distributed among siluriform fishes in general.

Acknowledgements

We are most grateful to Edmir Daniel Carvalho (*in memoriam*), professor and researcher at the Universidade Estadual Paulista, Botucatu Campus, São Paulo, Brazil, who dedicated his life to the study of ecology and environmental impacts on rivers and reservoirs caused by anthropic actions, and actively participated in the development of this project. We would like to thank Sandro Geraldo de Castro Britto and Diogo Freitas Souza for logistical support in the collection of hosts;

Aline Gouveia de Souza Lins for help in the analysis of parasites; and Cláudio Henrique Zawadzki, Walter Antonio Pereira Boeger, Rodney Kozłowski de Azevedo and Vanessa Doro Abdallah Kozłowski for taxonomic suggestions. We are also most grateful to Simone Chincz Cohen and Anna Kohn (Oswaldo Cruz Foundation – FIOCRUZ) for allowing us to examine some specimens of *Demidospermus* in their care.

Financial support

The authors would like to thank CAPES (Coordination for the Improvement of Higher Education Personnel) and FAPESP (São Paulo Research Foundation) (L.F. – Process: 2012/07850-7) for financial and scientific support, and the post-graduate scholarships granted to M.I.M. (grant number AUX-PE-PNP 3005/2010); and the Young Researcher Program PROPE-UNESP 02/2016 and New Doctor Program PROPE-UNESP 03/2016 for the scholarships granted to M.I.M. and L.F., respectively. R.J.S. is supported by CNPq (307808/2014-9) and CNPq-PROTAX (440496/2015-2)/FAPESP 2016/50377-1. We would also like to thank FUNEP (Teaching and Extension Research Support Foundation) (Process: 1.01852/2011), Duke Energy and CELAN (Central Elétrica Anhanguera) for financial and logistical support.

Conflict of interest

None.

Ethical standards

Specimens of *L. prolixa* were collected under Permanent License for the Collection of Zoological Material – SISBio 13794-1.

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