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Neotropical Monogenoidea 59. Polyonchoineans from *Characidium* spp. (Characiformes: Crenuchidae) from southern Brazil

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Abstract: Gyrodactylidae and Dactylogyridae (Monogenoidea) are described or reported from three species of *Characidium* Reinhardt (Crenuchidae), small species of Characiformes, from streams located in southern Brazilian states. *Gyrodactylus carolinae* sp. n. (Gyrodactylidae) is described from the body surface of *Characidium lanei* Travassos (type host), *C. pterostictum* Gomez, and *Characidium* sp. from streams in the states of Paraná and São Paulo. This new species closely resembles species of *Gyrodactylus* von Nordmann, 1832 described from species of Poeciliidae, from which it differs by the morphology of the hooks and nucleotide sequences of ITS1–5.8S–ITS2 rDNA. *Gyrodactylus inesperatus* sp. n. is described from the body surface of *Characidium* sp. from a stream in the State of São Paulo. The latter new species is characterised by lacking a shield on the superficial bar and by the morphology of the hooks, both unique characteristics for Neotropical species of *Gyrodactylus*. *Marumbius* gen. n. (Dactylogyridae) is proposed to accommodate two species, *M. dorsivaginatus* sp. n. from the gills of *Characidium pterostictum* (type host) and *C. lanei*, and *M. amplexus* sp. n. from the gills of *C. lanei* (all from the state of Paraná). Both species are characterised by having dorsal vagina, hook pairs 2–4, 6 and 7 composed by two subunits, hook pairs 1 and 5 lacking proximal subunit, and by the length of proximal subunits (when present) varying among hook pairs, completely or partially overlapping gonads, and male copulatory organ (MCO) represented by an incomplete coil of a sclerotized tube articulated to the accessory piece by a copulatory ligament. *Cacatuocotyle paranaensis* Boeger, Domingues et Kritsky, 1997 is reported from *C. lanei* at low prevalence in the Rio Marumbi (state of Paraná). The Monogenoidea that parasitize species of *Characidium* are members of several independent lineages, some of distant evolutionary relationships, suggesting a complex origin for this parasitic fauna.

Keywords: taxonomy, *Gyrodactylus*, *Marumbius* gen. n., *Gyrodactylus carolinae* sp. n., *Gyrodactylus inesperatus* sp. n., *Marumbius dorsivaginatus* sp. n., *Marumbius amplexus* sp. n., Neotropical region

Freshwater Monogenoidea is by far the best known group of metazoan fish parasites in Brazil and in the Neotropical region, with more than 400 species described from continental waters (Eiras et al. 2011). However, the diversity of the group is far from being well characterised. Most of what is known about the diversity of Monogenoidea in the Neotropics is derived from studies on medium- to relatively large-sized fish species. Small species, especially small species of Characiformes and Siluriformes, are likely parasitized by presently undescribed species of often-undisclosed higher categories.

Thus, in this study, species of Gyrodactylidae and Dactylogyridae are described or reported from three species of *Characidium* Reinhardt (Crenuchidae), small species of Characiformes from streams located in the southern states of São Paulo and Paraná. Two species of *Gyrodactylus* von Nordmann, 1832 (Gyrodactylidae) are described and *Marumbius* gen. n. (Dactylogyridae) is proposed to accommodate two new species. Previous to this study, only *Urocleidoides anops* Kritsky et Thatcher, 1974 and *Cacatuocotyle paranaensis* Boeger, Domingues et Kritsky, 1997 were known to parasitize species of *Characidium* from Colombia and Brazil, respectively (Kritsky and Thatcher 1974, Boeger et al. 1997).

MATERIALS AND METHODS

Host fish were captured from small coastal streams of the states of Paraná and São Paulo. Fish were captured with seine nets and electronarcosis, euthanized by pitting and placed immediately in hot water (about 65 °C). Parasite specimens were immediately preserved either in ethanol (70–80%) or 5% formalin for subsequent processing. Fish hosts were preserved in 70–80% ethanol. Vouchers of the host species (symbiotypes as defined by Brooks 1993) are deposited in the ichthyological collection of the Museu Capão da Imbuia, Curitiba, Brazil as follow: *Characidium lanei* Travassos (MHMCI 13012); *Characidium pterostictum* Gomez (MHMCI 13013); and the unidentified *Characidium* sp. (MHNCI 13026).

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Table 1. New and available sequences for *Gyrodactylus* spp.

	GenBank No.	USNPC No.	Reference
Gyrodactylus alexgusevi Zietara et Lumme, 2003	AY061979	-	Zietara and Lumme (2003)
Gyrodactylus arcuatoides Huyse, Malmberg et Volckaert, 2004	AY338429	-	Huyse et al. (2003)
Gyrodactylus arcuatus Bychowsky, 1933	JN703797	-	Hansen et al. (2012)
Gyrodactylus carolinae sp. n.	KF673399	HWML 49860	Present study
Gyrodactylus carolinae sp. n.	KF673400	USNPC 107218	Present study
Gyrodactylus carolinae sp. n.	KF673402	USNPC 107182	Present study
Gyrodactylus carolinae sp. n.	KF673403	-	Present study
Gyrodactylus carolinae sp. n.	K673401	USNPC 107217	Present study
Gyrodactylus gondae Huyse, Malmberg et Volckaert, 2004	AF328866	-	Zietara et al. (2002)
Gyrodactylus harengi Malmberg, 1957	AJ576064	-	Huyse and Malmberg (2004)
Gyrodactylus lotae Gusev, 1953	AY061978	-	Zietara and Lumme (2003)
Gyrodactylus lotae Gusev, 1953	EF446730	-	Zietara et al. (2008)
Gyrodactylus lotae Gusev, 1953	EF446731	-	Zietara et al. (2008)
Gyrodactylus pictae Cable, Oosterhout, Barson et Harris, 2005	AY692023	-	Cable et al. (2005)
Gyrodactylus turnbulli Harris, 1986	AJ001846	-	Cable et al. (1999)
Gyrodactylus turnbulli Harris, 1986	EF445942	-	unpublished

In the laboratory, parasites were collected from the sediment using a small probe under a dissecting microscope. Gills were removed from the fish, shaken and parasites were collected as described above. Some specimens were stained with Gomori's trichrome and mounted in Canada balsam for study of their soft anatomy; other specimens were cleared and mounted in Hoyer's or Gray and Wess' media for study of their sclerotized structures (all solutions prepared as in Humason 1979). Illustrations were prepared with the aid of a camera lucida on an Olympus BX51 microscope equipped with phase contrast.

Measurements, all in micrometres, were made following the procedures of Mizelle and Klucka (1953) for Dactylogyridae and Kritsky et al. (1995) for Gyrodactylidae; the mean is followed by the range and the number of structures measured (n) in parentheses; body length includes that of the haptor (longitudinal axis of haptor added to that of body proper). Length of the male copulatory organ (MCO) of Dactylogyridae represents the distance between bars in respective figures. Numbering of the hook pairs follows that recommended by Mizelle (1936) and Mizelle and Price (1963). Haptoral terminology is that provided by Mizelle and Kritsky (1967) and Kritsky and Mizelle (1968). Prevalence values are presented as the percentage of parasitized hosts followed by the number of parasitized host/total number of sampled hosts between parentheses.

Individual specimens of a new species of *Gyrodactylus,* preserved in ethanol, were placed in glycerine on a microscope slide and the haptoral extremity was cut with a fine blade. The haptoral portion was mounted in Hoyer mounting medium (Humason 1979) for species identification and to serve as vouchers. DNA was extracted from the anterior portion of the worm using the DNAEasy tissue kit (Qiagen, Hilden, Germany). The primers ITS1 (5'-TTTCCGTAGGTGAACCT-3') and ITS2 (5'-TCCTCCGCTTAGTGATA-3'), of Cunningham (1997) were used to amplify and sequence the fragments ITS1, 5.8S and ITS 2.

The polymerase chain reaction (PCR) was performed with the following program: initial denaturation at 94 °C for 5 min followed by 40 cycles as follows: denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, and final extension 72 °C. PCR was achieved in 25 µl containing 10–30 ng of template DNA, 3 mM $MgCl_2$, 1× PCR-Buffer (Invitrogen, Carlsbad, CA, USA), 0.5 pmoles of each primer, 0.4 mM dNTP, and 1U Platinum Taq polymerase (Invitrogen) in a total volume of 25 µl. Confirmation of the amplification of the fragments by PCR was achieved through electrophoresis in a 1.5% agarose gel, subsequent staining in ethidium bromide and visualization under UV light. Amplicons were purified using the MinElute Purification kit (Qiagen). Sequences were obtained with the BigDye 3.1 chemistry in the 3130 DNA Analyser with the same program and primers used during amplification. Sequences were edited using Geneious 5.0.4 (created by Biomatters; available from http://www.geneious.com/).

Alignment of the sequences was obtained using the algorithm MUSCLE (Edgar 2004) within Geneious 5.0.4. Distance analysis (Neighbor Joining) was performed with the software MEGA version 5 (Tamura et al. 2011) using the model Maximum Composite Likelihood (Nei and Kumar 2004) removing ambiguous positions for each sequence pair and bootstrap support of 1 000 repetitions. Sequences included in the distance analysis were the sequences produced in this study, sequences of Neotropical and non-Neotropical species that systematically clustered together in the distance analysis of NCBI-BLAST (Altschul et al. 1997). Further, sequences of arbitrarily chosen Neotropical species and sequences of other non-Neotropical species presenting morphological similarity to the new species were selected from GenBank (Table 1).

The resulting phylogram from the neighbor-joining analysis is intended to represent intra- and interspecific distances but not phylogenetic relationships. This decision is based on the fact that, among other things, alignment of fragments of ITS 1 and ITS 2 is often difficult due to abundant indel regions (see Álvarez and Wendel 2003). Thus, except for the included 5.8S rDNA region, this fragment has limited reliability in phylogenetic reconstructions without consideration of homologies of its secondary structure among included taxa (Coleman 2007), especially if distant taxa are considered.

Type specimens and vouchers were deposited in the United States National Parasite Collection (USNPC), Beltsville, USA; University of Nebraska State Museum, Harold W. Manter Laboratory (HWML), Lincoln, USA; Helminthological Collection of the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (IPCAS); and the Parasitological Collection of the In-

stituto Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC), as indicated in the respective species accounts.

RESULTS

Class Monogenoidea Bychowsky, 1937

Subclass Polyonchoinea Bychowsky, 1937

Order Gyrodactylidea Bychowsky, 1937

Family Gyrodactylidae van Beneden et Hesse, 1863

Gyrodactylus carolinae sp. n. Figs. 1–5, 30, 31, 33

Description (based on 27 specimens – all from the State of Paraná): Body elongate, 431 (n = 1) long, 73 (n = 1) wide. Cephalic glands, head organs, spike sensilla conspicuous. Cephalic glands anterolateral, lateral, posterolateral to pharynx. Excretory vesicles, pores lateral to oesophagus. Pharynx composed of two tandem bulbs; distal pharyngeal bulb muscular, 32 (n = 1) wide; digitiform projections of distal pharyngeal bulb not observed; proximal pharyngeal bulb glandular, 38 (n = 1) wide.

MCO 18 (n = 1) wide, armed with one spine, one row of 4–6 spinelets; spinelets similar in shape and size, each with wide, truncate base. Testis ovate 25 (n = 1) wide, posterior to germarium. Germarium ovate, 14 (n = 1) long, 21 (n = 1) wide. Uterus with up to two generations of embryos. Large syncytial glandular mass overlapping distal midlength of caeca.

Anchor 55 (50–67; n = 25) long; shaft straight, recurved point 18 (16–20; n = 16); deep root poorly developed, knob-like; superficial root elongate. Superficial bar 10 (9–12; n = 3) long, 49 (45–51; n = 3) wide, with two robust, elongate anterolateral projections; shield subtriangular, striated. Deep bar constricted near midlength and at attachment of deep bar to anchor. Hooklet with straight shaft, point recurved; heel convex; toe slightly pointed, depressed; shelf convex; hook shank proximally bulbous; hook 25 (23–28; n = 15) long; hooklet 6 (6–8; n = 25) long. Long unicellular glands in peduncle with ducts directed to haptoral region.

Type host: *Characidium lanei* Travassos.

Other host: *Characidium pterostictum* Gomez and *Characidium* sp.

Site of infection: Body surface.

Type locality: Rio Marumbi, municipality of Morretes, Paraná, Brazil (25°29'27"S; 45°49'67"W), in March (holotype collected on 18 March 2010) and August 2010 and May 2011.

Other localities: Rio do Nunes, municipality of Antonina, Paraná, Brazil (25°20'37"S; 48°45'59"W), in May 2005; Rio das Conchas (24°9'24"S; 48°18'4"W), in November 2003, Rio das Almas (24°8'51"S; 48°20'52"W), Ribeirão Bonito, São Paulo, Brazil, in November 2003.

Prevalence: *Characidium pterostictum* 28% (19/67); *C. lanei* 23% (18/79); (not available for *Characidium* sp.).

Specimens studied: Holotype (CHIOC 37880); 25 paratypes (CHIOC 37875–37879, 37900; USNPC 107182–107189, 107217, 107218; HWML 49859–49862; IPCAS M – 541).

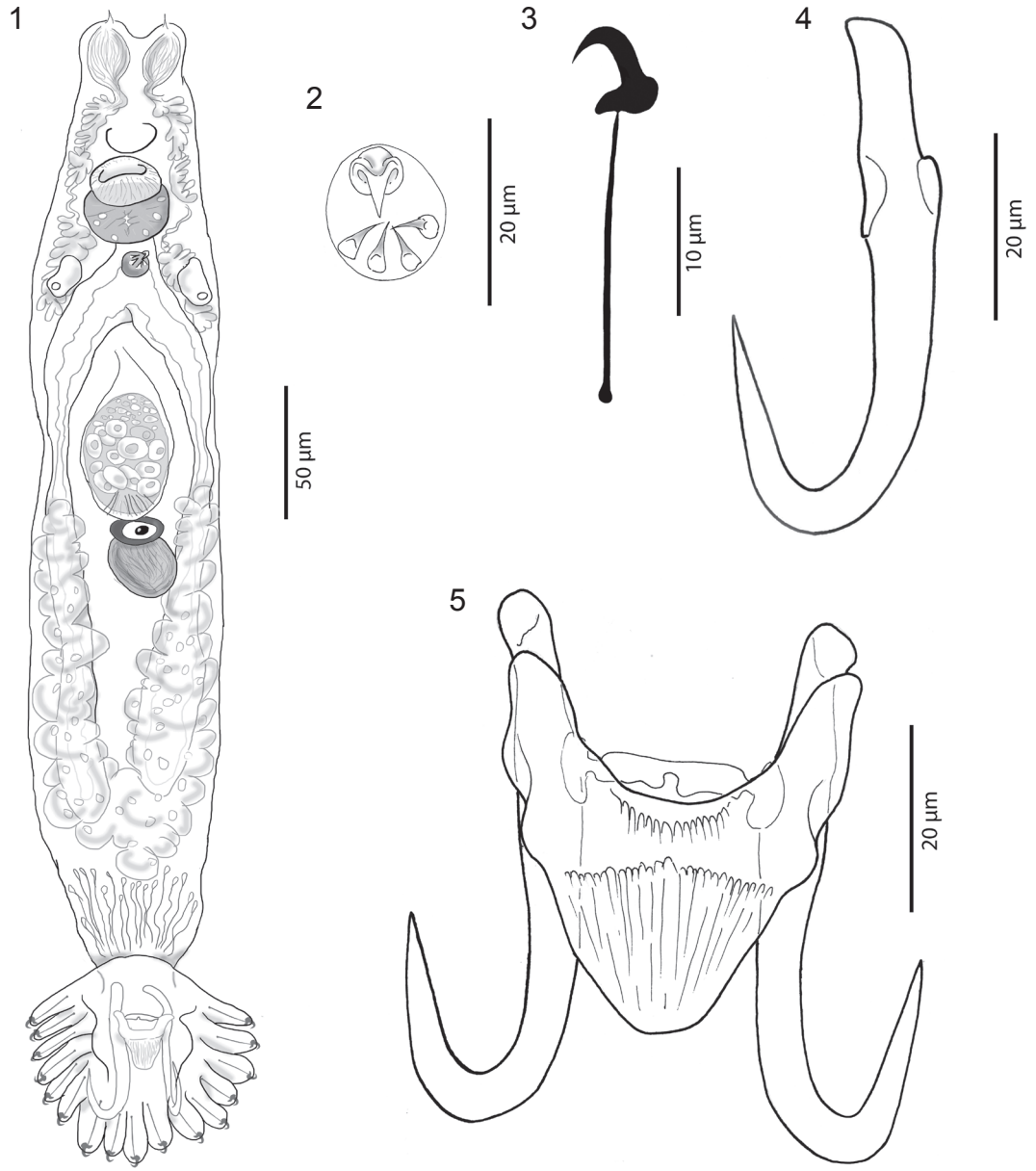
Etymology: The species is named after Carolina Souza Nascimento, a wonderful girl that left us unexpectedly at the age of 21, before fulfilling all her potential as a student of Monogenoidea.

Remarks. *Gyrodactylus carolinae* sp. n. is clearly a member of a wider group of species from the Neotropical region composed by *G. bullatarudis* Turnbull, 1956; *G. costaricensis* Kritsky et Fritts, 1970; *G. jarocho* Rubio-Godoy, Paladini, García-Vásquez, Shinn, 2010; *G. milleri* Harris et Cable, 2000; *G. pictae* Cable, Oosterhout, Barson et Harris, 2005; *G. poeciliae* Harris et Cable, 2000; *G. rasini* Lucký, 1973, *G. turnbulli* Harris, 1986; and *G. xalapensis* Rubio-Godoy, Paladini, García-Vásquez, Shinn, 2010, as suggested by the comparative morphology of hard parts and, whenever known, by the pattern in the armature of the male copulatory organ (MCO).

Except for the new species, all remaining species are parasites of poeciliid fish (Cyprinodontiformes). The anchor of these species usually presents superficial bar with pronounced anterolateral projections. The armature of the MCO, whenever described, has just a single row of spinelets, usually in small numbers (4–6), and a large spine. In general, these characters are reminiscent of the species placed in Malmberg's (1970) subgenus *Mesonephrotus*, which includes marine, brackish and freshwater species from other biogeographic realms.

Among the Neotropical species, *G. carolinae* most closely resembles *G. bullatarudis* by presenting great similarity in the morphology of all hard parts, including the armature of the MCO (see Kritsky and Fritts 1970). Both species depict a small number of spinelets on the MCO, deep bar with a notch near midlength and superficial bar with a shield tapering distally. The distinct morphology of the hooks, however, allows their differentiation (see Fig. 6). The hooks of *G. carolinae* present a more robust shaft, longer point, more delicate base of the hooklet and rounder heel.

The new species differs from the remaining members of the above-mentioned group of morphologically similar Neotropical species especially by the morphology of the hook (see Fig. 6). The point of *G. carolinae* is relatively longer and the base of the hooklet is more delicate than those of *G. costaricensis*, *G. poeciliae* and *G. bullatarudis*. The new species also differs from *G. pictae*, *G. milleri* and *G. turnbulli* by having a more robust base and shaft, and a point forming a more acute angle with the shaft. Further, the hooks of *G. jarocho*, *G. xalapensis* and *G. rasini* present a conspicuously wavy tip of the point (see Richards et al. 2000, Rubio-Godoy et al. 2010), whereas the point of *G. carolinae* is straight, as in the remaining species of this group.



Figs. 1–5. *Gyrodactylus carolinae* sp. n. from *Characidium pterostictum*. **Fig. 1.** Holotype (ventral view). **Fig. 2.** Male copulatory organ. **Fig. 3.** Hook. **Fig. 4.** Ventral anchor. **Fig. 5.** Anchor-bars complex.

Further support to the identity of the new species is derived from the pairwise comparison of ITS1–5.8S–ITS2 rDNA sequences. Sequences of specimens of *G. carolinae* cluster in a single group and present intraspecific distances much shorter than the interspecific genetic distances observed among studied sequences (Table 2; Fig. 6). Individuals of a same species should cluster in a single clade depicting small differences (distance) in nucleotide composition – supporting monophyly of the species proposed/evaluated – whereas similar groups (other species) should present greater genetic distances among them – revealing the identity and individuality of distinct species. Under these premises, molecular analyses intended to test the proposal or recognition of species would necessarily incorporate more than one individual. For cytochrome oxidase I, e.g. the Fish Barcode of Life Initiative suggests between 5–25 individuals of each species, depending on the species distribution (Steinke and Hanner 2011).

This, however, is not a widespread practice and molecular support for new species of Gyrodactylidae is often based solely on phylogenic hypotheses or an analysis of genetic distances using a single specimen for each putative OTU (Organizational Taxonomic Unit) (e.g. Cable et al. 2005, Paladini et al. 2011), or simply incorporates a description of the nucleotide composition of a fragment of ribosomal DNA (e.g. Vaughan et al. 2010) Undoubtedly, these procedures provide information on the phylogenetic position of the specimens under study and some support

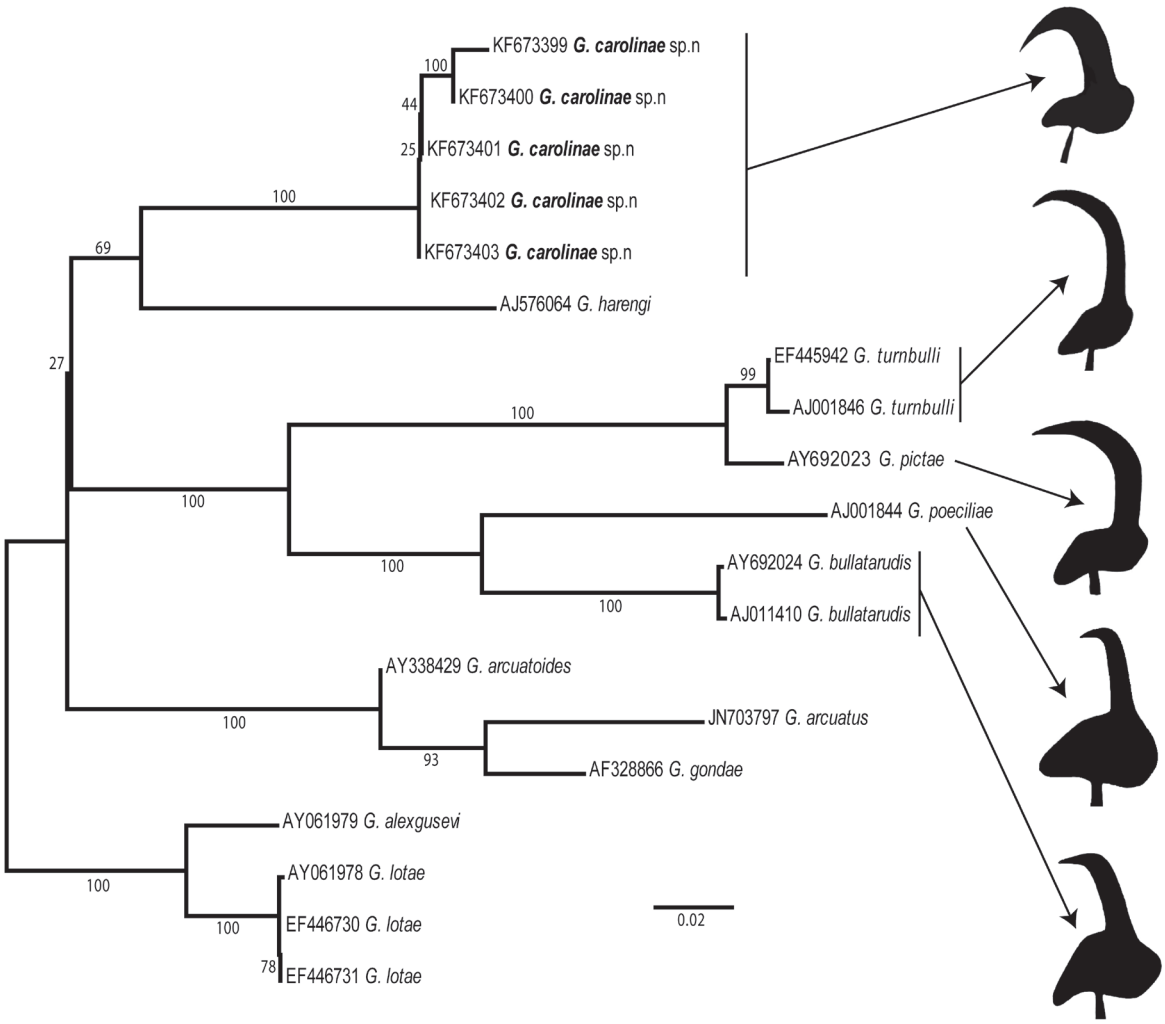


Fig. 6. Neighbor-joining phylogram of selected species of *Gyrodactylus* based on the Maximum Composite Likelihood (Nei and Kumar 2004) model for ITS1–5.8 S–ITS2 rDNA to represent intra- and interspecific distances. Bootstrap values (n = 1 000) are presented below each respective branch if above 50. Profile of hooklets of selected species is presented (not to the same scale).

for the proposal of a new species, but they do not satisfy the criteria of identity and monophyly mentioned above.

Gyrodactylus inesperatus sp. n. Figs. 7–11, 34

Description (based on 11 specimens): Body elongate, 421 (388–474; n = 3) long, 71 (62–86; n = 3) wide. Cephalic glands, head organs, spike sensilla conspicuous. Cephalic glands anterolateral, lateral, posterolateral to pharynx. Large excretory vesicles lateral to oesophagus. Pharynx composed of two tandem bulbs; distal pharyngeal bulb muscular 30 (26–35; n = 3) wide; digitiform projections of distal pharyngeal bulb not observed; proximal pharyngeal bulb glandular 31–36 (n = 2) wide.

MCO 11 (n = 1) wide, armed with one spine, one row of four large and four small spinelets; each spinelet with wide, truncate base. Testis ovate 17–25 (n = 2) wide, posterior to germarium. Germarium ovate, 22–23 (n = 2) long, 24–25 (n = 2) wide. Uterus with up to two generations of embryos. Large syncytial mass overlapping distal extremity of caeca; unicellular glands distributed in limited region posterior to testis.

Anchor 40–43 (n = 2) long; straight shaft; deep root poorly developed, knob-like; superficial root short, robust; articulation of superficial bar expanded, subovate. Superficial bar 10–15 (n = 2) long, 7 (n = 2) wide, with two short anterolateral projections; shield absent. Deep bar rod-shaped. Hooklet with straight, robust shaft, point short about 90° to shaft; heel subrectangular; toe slightly erected, blunt; shelf short, straight; shank tapering distally, 17 (16–18; n = 4) long; hooklet 7 (n = 4) long. Long unicellular glands in peduncle with ducts directed to haptoral region.

Type host: *Characidium* sp.

Type locality: Rio das Almas, Ribeirão Bonito, São Paulo, Brazil (24°8'52"S; 48°20'52"W), in November 2003 (holotype collected on 8 November 2003).

Site of infection: Body surface.

Prevalence: 100% (2/2).

Specimens studied: Holotype (CHIOC 37869); 10 para-

Table 2. Pairwise genetic distances between selected species of *Gyrodactylus* based on the fragment ITS 1-5.8S-ITS2 rDNA. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood (Nei and Kumar 2004) model. All ambiguous positions were removed for each sequence pair.

	KF673399 ***G. carolinae*** sp. n.	KF673400 ***G. carolinae*** sp. n.	KF673401 ***G. carolinae*** sp. n.	KF673402 ***G. carolinae*** sp. n.	KF673403 ***G. carolinae*** sp. n.	AY061979 ***G. alexgusevi***	AY061978 ***G. lotae***	EF446730 ***G. lotae***	EF446731 ***G. lotae***	AJ576064 ***G. harengi***	JN703797 ***G. arcuatus***	AF328866 ***G. gondae***	AY338429 ***G. arcuatoides***	AY692023 ***G. pictae***	EF445942 ***G. turnbulli***	AJ001846 ***G. turnbulli***	AJ001844 ***G. poeciliae***	AY692024 ***G. bullatarudis***
KF673400 ***G. carolinae*** sp. n.	0.008																	
KF673401 ***G. carolinae*** sp. n.	0.016	0.008																
KF673402 ***G. carolinae*** sp. n.	0.016	0.008	0.000															
KF673403 ***G. carolinae*** sp. n.	0.016	0.008	0.000	0.000														
AY061979 ***G. alexgusevi***	0.202	0.191	0.185	0.185	0.185													
AY061978 ***G. lotae***	0.203	0.191	0.186	0.186	0.186	0.050												
EF446730 ***G. lotae***	0.203	0.191	0.186	0.186	0.186	0.048	0.001											
EF446731 ***G. lotae***	0.203	0.191	0.186	0.186	0.186	0.048	0.001	0.000										
AJ576064 ***G. harengi***	0.188	0.175	0.164	0.164	0.164	0.177	0.172	0.171	0.171									
JN703797 ***G. arcuatus***	0.295	0.273	0.262	0.262	0.262	0.241	0.258	0.256	0.256	0.261								
AF328866 ***G. gondae***	0.249	0.231	0.227	0.226	0.226	0.227	0.229	0.227	0.227	0.241	0.083							
AY338429 ***G. arcuatoides***	0.178	0.156	0.156	0.155	0.155	0.151	0.148	0.146	0.146	0.171	0.080	0.016						
AY692023 ***G. pictae***	0.301	0.285	0.282	0.281	0.281	0.268	0.279	0.279	0.279	0.310	0.333	0.315	0.233					
EF445942 ***G. turnbulli***	0.290	0.274	0.271	0.270	0.270	0.275	0.281	0.281	0.281	0.304	0.328	0.315	0.230	0.025				
AJ001846 ***G. turnbulli***	0.295	0.279	0.277	0.276	0.276	0.281	0.287	0.287	0.287	0.307	0.333	0.320	0.233	0.031	0.005			
AJ001844 ***G. poeciliae***	0.305	0.278	0.284	0.284	0.284	0.284	0.291	0.291	0.291	0.334	0.361	0.347	0.280	0.274	0.273	0.281		
AY692024 ***G. bullatarudis***	0.272	0.262	0.260	0.259	0.259	0.246	0.264	0.264	0.264	0.300	0.338	0.335	0.254	0.235	0.234	0.236	0.153	
AJ011410 ***G. bullatarudis***	0.272	0.263	0.261	0.260	0.260	0.249	0.265	0.265	0.265	0.301	0.338	0.333	0.250	0.238	0.238	0.240	0.154	0.003

types (CHIOC 37870–37874; USNPC 107190; HWML 49863; IPCAS M – 542).

Etymology: The specific name refers to the fact that the species unexpectedly (= *inesperatus* in Latin) lacks a shield, a structure widely common among viviparous species of Gyrodactylidae.

Remarks. Contrary to all other species of Neotropical freshwater *Gyrodactylus*, *G. inesperatus* sp. n. lacks a shield on the superficial bar. The absence of the shield in several species of *Gyrodactylus* may be in fact result of incomplete descriptions due to the fact that the shield may become greatly transparent during slide preparation or even 'shrink' due to interaction with mounting medium. Indeed, shrinking of the shield has been observed in specimens of *Gyrodactylus* sp. from *Astyanax* sp. (Characidae) mounted in Hoyer's mounting medium (W. Boeger – unpublished data). Other species, however, appear to actually lack the shield and this absence may be both by retention of a primitive character state (as observed in the oviparous Gyrodactylidae; Boeger et al. 1994) or by secondary loss.

Thus, the absence of the shield should be confirmed always by additional methods, such as scanning electron microscopy or by adequate slide specimen preparation for light microscopy. The absence of the shield in *G. inesperatus* was confirmed in all specimens stained with Gomori's trichrome and mounted in Canada balsam (see Kritsky et al. 1978 for the method). Gomori's trichrome is known to stain some haptoral structures, resulting in a more conspicuous shield that can be easily observed under optical light microscopy. Other options are any of several similar techniques developed for light or confocal microscopy (e.g. Kritsky et al. 1978, Richards and Chubb 1995, García-Vásquez et al. 2012).

In addition, the hook morphology of *G. inesperatus* is distinct from that of any other Neotropical species and hooks present a robust, straight shaft, a short robust point forming about 90° with the shaft and a subrectangular heel and toe.

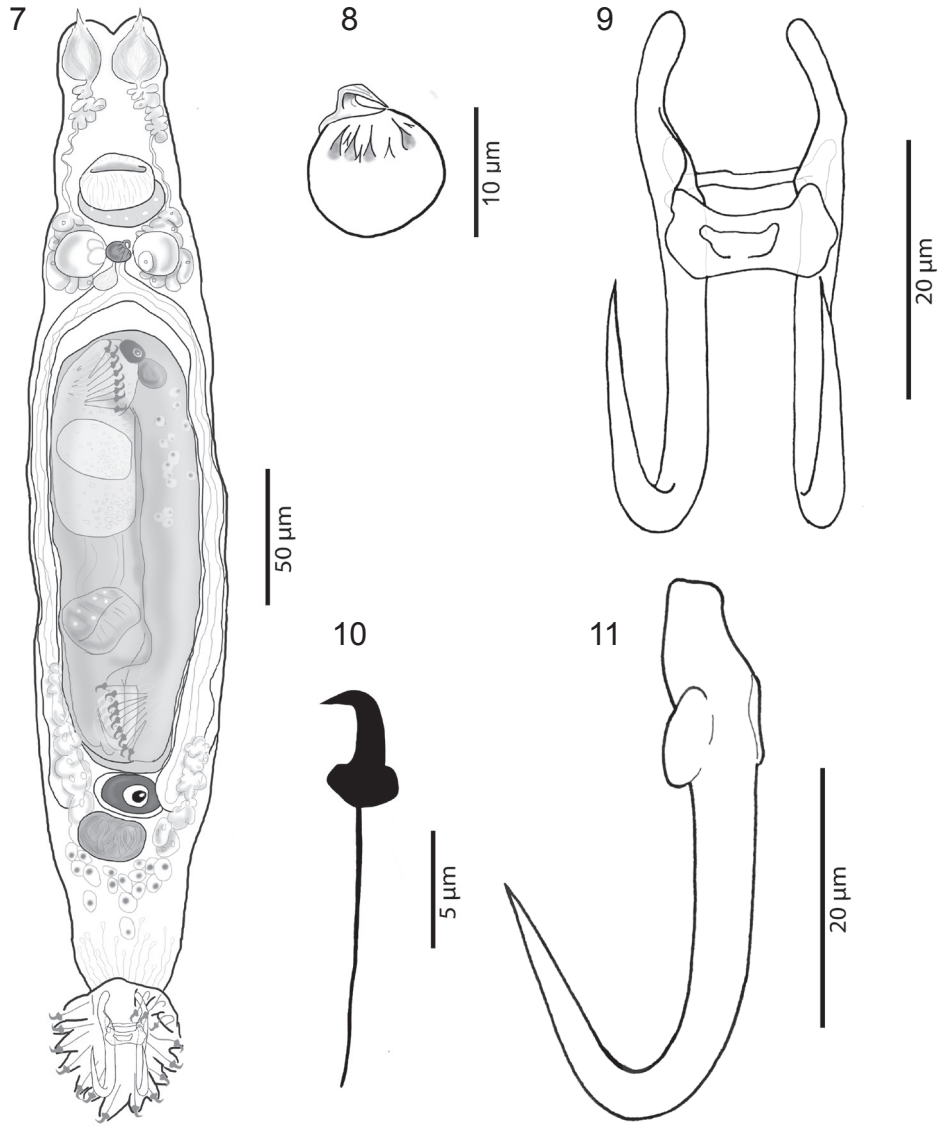
Order Dactylogyridea Bychowsky, 1937

Family Dactylogyridae Bychowsky, 1933

Subfamily Ancyrocephalinae Bychowsky, 1937

Marumbius gen. n.

Diagnosis: Body fusiform, slightly flattened dorsoventrally, comprising body proper (cephalic region, trunk, peduncle) and haptor. Tegument smooth. Four cephalic



Figs. 7–11. *Gyrodactylus inesperatus* sp. n. from *Characidium* sp. **Fig. 7.** Holotype (ventral view). **Fig. 8.** Male copulatory organ. **Fig. 9.** Anchor-bars complex. **Fig. 10.** Hook. **Fig. 11.** Ventral anchor.

lobes; pairs of bilateral head organs; cephalic glands unicellular, lateral or posterolateral to pharynx. Eyespots four; granules small, ovate. Mouth subterminal, midventral, prepharyngeal; pharynx muscular, glandular; oesophagus short or inconspicuous; intestinal caeca two, confluent posterior to gonads, lacking diverticula.

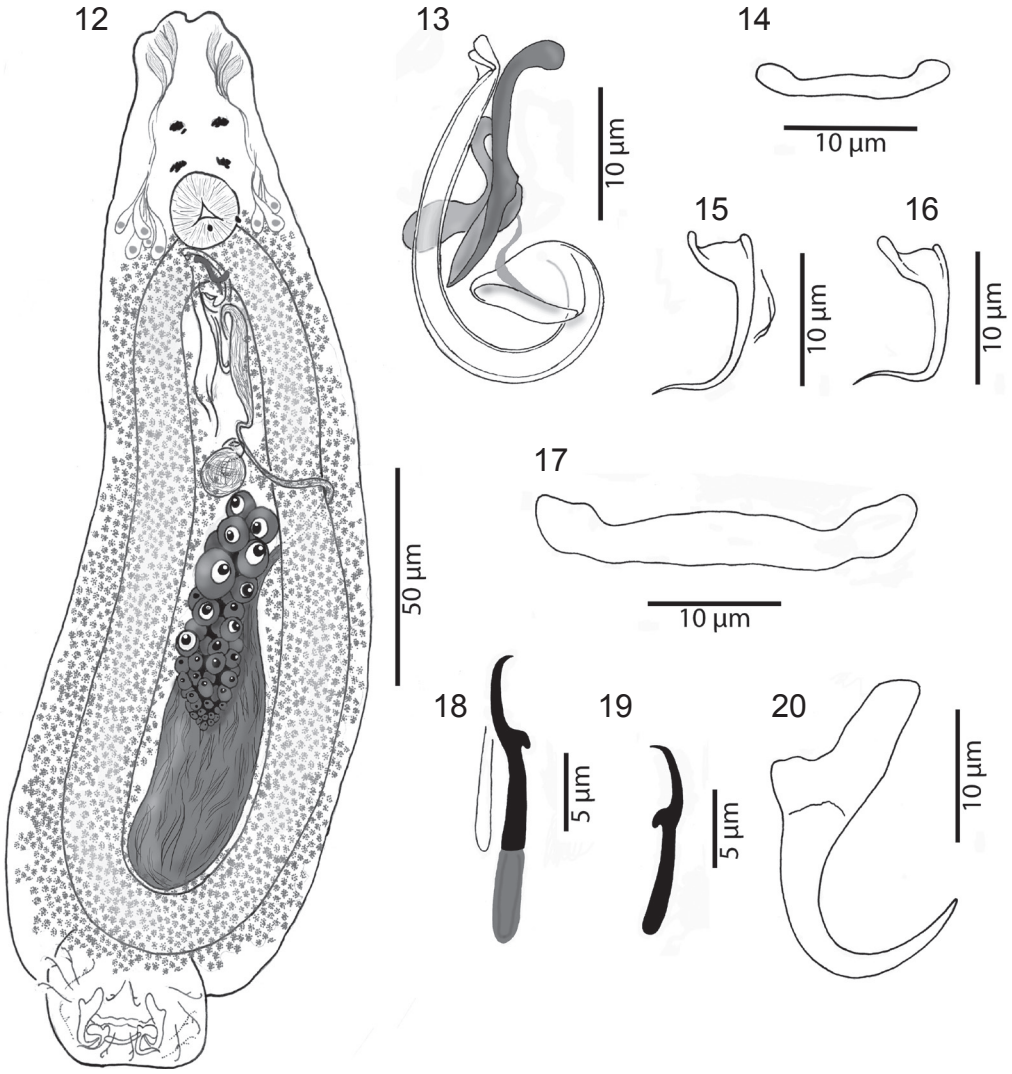
Common genital pore midventral at level of intestinal bifurcation. Gonads intercaecal, completely or partially overlapping; germarium ventral to testis. Vas deferens looping left intestinal caecum; seminal vesicle a simple dilation of vas deferens. Copulatory complex comprising MCO and accessory piece. MCO sclerotized, tubular. Accessory piece serving as guide for distal portion of MCO, articulated to MCO base by copulatory ligament. Seminal receptacle pregermarial; vaginal pore single, dorsal, intercaecal; vaginal duct intercaecal; vagina non-sclerotized or distally sclerotized; vaginal opening sclerotized or non-sclerotized. Vitellaria in trunk, absent from regions of other reproductive organs. Peduncle short to inconspicuous. Haptor armed with dorsal and ventral anchor/bar complexes, seven pairs of similar hooks. Hook distribution ancyrocephaline (see Mizelle 1936); shank of hook pairs 1, 5 comprising a single subunit; shank of hook pairs 2–4, 6, 7 comprising 2 subunits; length of proximal subunits varies according to hook pair. Parasites of the gills of Neotropical Crenuchidae (Teleostei).

Type species: *Marumbius dorsivaginatus* sp. n.

Other species: *Marumbius amplexus* sp. n.

Etymology: The generic name makes reference to the river from which the type species was collected, the Rio Marumbi.

Remarks: *Marumbius* gen. n. is proposed to accommodate species of Neotropical Dactylogyridae presenting the combined diagnostic characteristics: dorsal vagina,



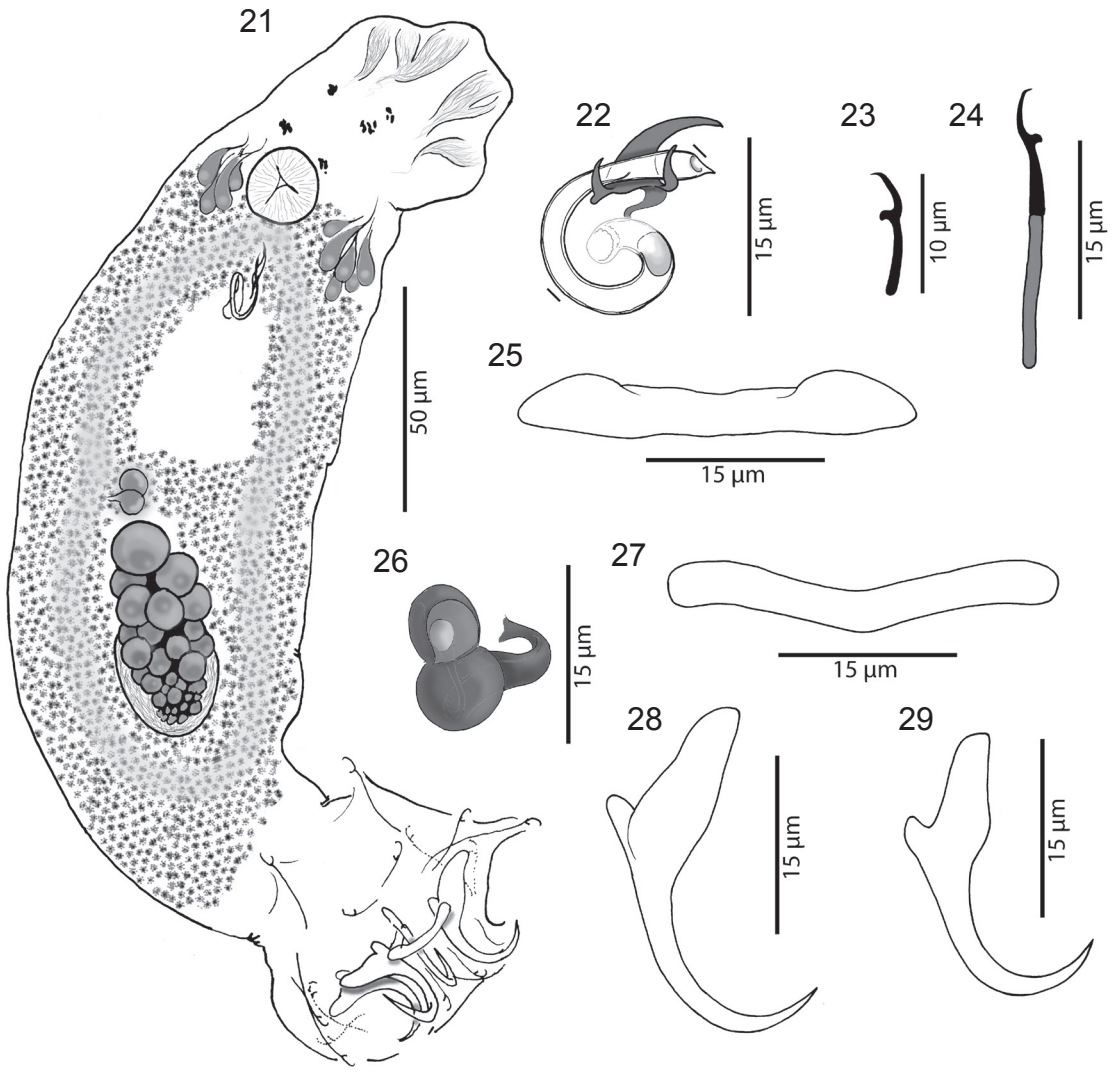
Figs. 12–20. *Marumbius dorsivaginatus* sp. n. from *Characidium pterostictum* and *C. lanei*. **Fig. 12.** Holotype (ventral view). **Fig. 13.** Male copulatory complex. **Fig. 14.** Ventral bar. **Fig. 15–16.** Ventral anchor. **Fig. 17.** Dorsal bar. **Fig. 18.** Hook pair 2. **Fig. 19.** Hook pair 1. **Fig. 20.** Dorsal anchor.

hook pairs 2–4, 6 and 7 composed by 1–2 subunits, hook pairs 1 and 5 lacking proximal subunit, length of proximal subunits (when present) varying according to hook pair, completely or partially overlapping gonads, and MCO represented by an incomplete coil of a sclerotized tube articulated to the accessory piece by a copulatory ligament. Only the Neotropical species of *Nothothecium* Boeger et Kritsky, 1988, *Nothozothecium* Boeger et Kritsky, 1988, *Nothothecioides* Kritsky, Boeger et Jégu, 1997, *Enallothecium* Kritsky, Boeger et Jégu, 1997 and *Odothecium* Kritsky, Boeger et Jégu, 1997 present a single dorsal vagina, either lateral or at the body midline. Similar to both species of *Marumbius*, the species of the above genera also share hooks composed by two subunits, completely or partially overlapping gonads and MCO articulated to the accessory piece by a copulatory ligament. However, the dorsal vaginal opening in species of *Nothothecium*, *Nothozothecium*, *Nothothecioides*, *Enallothecium* and *Odothecium* is usually located lateral to the body midline and the vaginal duct always loops the left intestinal caecum, whereas the vaginal opening in *Marumbius* spp. is dorsal, on the body midline, and the vaginal duct is short and does not loop the caeca. *Marumbius* spp. differ from the species of these genera also by lacking the proximal subunit in hooks pair 1 and 5.

Marumbius dorsivaginatus sp. n.

Figs. 12–20, 32, 35, 37, 38

Description (based on 25 specimens): Body 242 (206–275; n = 6) long; greatest width 72 (27–99; n = 10) at level of gonads. Cephalic lobes moderately developed; each lobe with 1–2 head organs. Eyespots 4, accessory granules scarce in cephalic region. Pharynx subspherical, 27 (14–36; n = 21) in diameter.



Figs. 21–29. *Marumbius amplexus* sp. n. from *Characidium pterostictum* and *C. lanei* **Fig. 21.** Composite (ventral view). **Fig. 22.** Male copulatory complex. **Fig. 23.** Hook pair 1. **Fig. 24.** Hook pair 2. **Fig. 25.** Ventral bar. **Fig. 26.** Vagina. **Fig. 27.** Dorsal bar. **Fig. 28.** Ventral anchor. **Fig. 29.** Dorsal anchor.

Testis ovate, 40 (34–52; n = 3) long, 15 (14–19; n = 3) wide; vas deferens looping left intestinal caecum; seminal vesicle elongate; prostatic reservoir not observed. MCO 24 (21–28; n = 18) long, an incomplete loop; base of MCO wide, funnel shaped; accessory piece a rod-shape piece (distally blunt, proximally spoon-like) with an elongate flap on its proximal two thirds. Germarium elongate, 39 (35–44; n = 3) long, 16 (12–18; n = 3) wide. Uterus lateral to seminal vesicle. Vaginal pore single, dorsal; vagina non-sclerotized, inconspicuous. Vitellaria dense.

Haptor 39 (17–55; n = 19) long, 55 (24–72; n = 19) wide; bilaterally expanded. Hooks similar in shape, with depressed thumb, straight shaft, short point (hook measurements in Table 3). Ventral anchor 13 (11–15; n = 10) long, base 6 (4–7; n = 10) wide, delicate, with poorly differentiated roots, straight shaft, point delicate, wavy. Dorsal anchor 23 (20–26; n = 10) long, base 13 (12–15; n = 11) wide, with short deep root, elongate superficial root, shaft and point evenly curved. Ventral bar 17 (15–18; n = 15) long, with slightly expanded ends. Dorsal bar 28 (23–31; n = 15) long, with slightly expanded ends. Eggs ovate, with smooth shell, short polar filament.

Type host: *Characidium pterostictum* Gomez.

Other host: *Characidium lanei* Travassos.

Site of infection: Gills.

Type locality: Rio Marumbi, municipality of Morretes, Paraná, Brazil (25°29'27"S; 45°49'67"W), in March and August 2010 and May 2011 (holotype collected on 18 March 2010).

Prevalence: *C. pterostictum* 87% (99/114), *C. lanei* 21% (16/77).

Specimen deposited: Holotype (CHIOC 37893), 24 paratypes (CHIOC 37884–37892; 37894–37895; USNPC 107191–107192; HWML 49864 (n = 5); IPACS M – 543).

Table 3. Measurements (in micrometers) of hooks of *Marumbius dorsivaginatus* sp. n.

	Total				Subunit 1 of shank			
	n	mean	min	max	n	mean	min	max
Hook 1	-	-	-	-	13	12	10	13
Hook 2	11	20	19	22	11	14	13	15
Hook 3	11	20	17	23	11	13	12	15
Hook 4	10	20	16	22	10	14	12	15
Hook 5	-	-	-	-	13	13	11	15
Hook 6	7	17	16	18	13	13	11	14
Hook 7	4	17	16	18	13	13	12	13

Table 4. Measurements (in micrometers) of hooks of *Marumbius amplexus* sp. n.

	Total				Subunit 1 of shank			
	n	mean	min	max	n	mean	min	max
Hook 1	-	-	-	-	3	13	11	14
Hook 2	3	28	22	33	3	13	13	14
Hook 3	3	23	18	26	3	13	12	14
Hook 4	3	36	21	45	3	14	14	14
Hook 5	-	-	-	-	2	14	13	14
Hook 6	2	18	18	19	2	12	11	13
Hook 7	3	26	17	36	3	14	13	14

Etymology: The specific name is derived from the position of the vagina in this species (*dorsum* in Latin = dorsal; *vagina* in Latin = vagina).

Remarks. The species presents unequal anchor pairs with a reduced ventral pair that presents a delicate point, often distorted in mounted specimens.

Marumbius amplexus sp. n. Figs. 21–29, 36, 39, 40

Description (based on four specimens): Body fusiform. Cephalic lobes moderately developed; each lobe with 2–3 head organs. Eyespots 4, accessory granules present. Pharynx subspherical, 21 (19–24; n = 2) wide.

Testis ovate; vas deferens, seminal vesicle, prostatic reservoir not observed. MCO 23 (21–24; n = 2) long, an incomplete loop, counterclockwise; base of MCO with slightly sclerotized, elongate, cylindrical base; accessory piece hook-shaped, with proximal support for MCO. Vitellaria dense; vagina mid-dorsal, composed by two sclerotized, spherical subunits.

Haptor 70 (64–76; n = 3) long, 115 (95–140; n = 3) wide; laterally expanded. Hooks similar in shape, with depressed thumb, straight shaft, short point; hook pair 4 significantly longer than remaining hooks; hook measurements in Table 4. Ventral anchor 27 (22–29; n = 3) long, base 13 (12–15; n = 3) wide, with well differentiated roots; superficial root elongate; deep root short; shaft, point evenly curved. Dorsal anchor 32 (29–33; n = 3) long, base 17 (17–18; n = 3) wide, with short deep root, elongate superficial root, shaft and point evenly curved. Ventral bar 37 (35–39; n = 3) long, with slightly expanded ends. Dorsal bar 28 (24–31; n = 2) long, with slightly expanded ends. Eggs not observed.

Type and only host: *Characidium lanei* Travassos.

Site of infection: Gills.

Type locality: Rio Marumbi, municipality of Morretes, Paraná, Brazil (25°29'27"S; 45°49'67"W), in March and August 2010 and May 2011 (holotype collected on 11 August 2010).

Prevalence: 10% (8/77).

Specimen deposited: Holotype (CHIOC 37897) and 3 paratypes (CHIOC 37896, IPCAS M – 544; USNPC 107197).

Etymology: The specific name is derived from the morphology of the hook pair 4, which projects laterally conferring a haptor morphology that appears to be ready to embrace the gill filament (*amplexus* in Latin = to embrace).

Remarks. *Marumbius amplexus* can be easily distinguished from the type and the only other species in the genus, *M. dorsivaginatus*, by the presence of hook pair 4 conspicuously longer than the remaining hook pairs, by the presence of anchors of similar shape and size, and by the presence of a sclerotized distal vaginal complex composed by two subspherical subunits. Contrary to *M. dorsivaginatus*, *M. amplexus* is relatively rare and is known solely from *C. lanei*.

We decided to describe and propose *M. amplexus* despite the fact that only few specimens were available. *Marumbius amplexus* sp. n. represents the second species of the newly proposed *Marumbius* and provides additional evidence for the validity and monophyly of the genus. We unsuccessfully attempted to increase the number of collected specimens of *M. amplexus* through extensive collection of the host fish species in the type locality, which included two years and several year seasons.

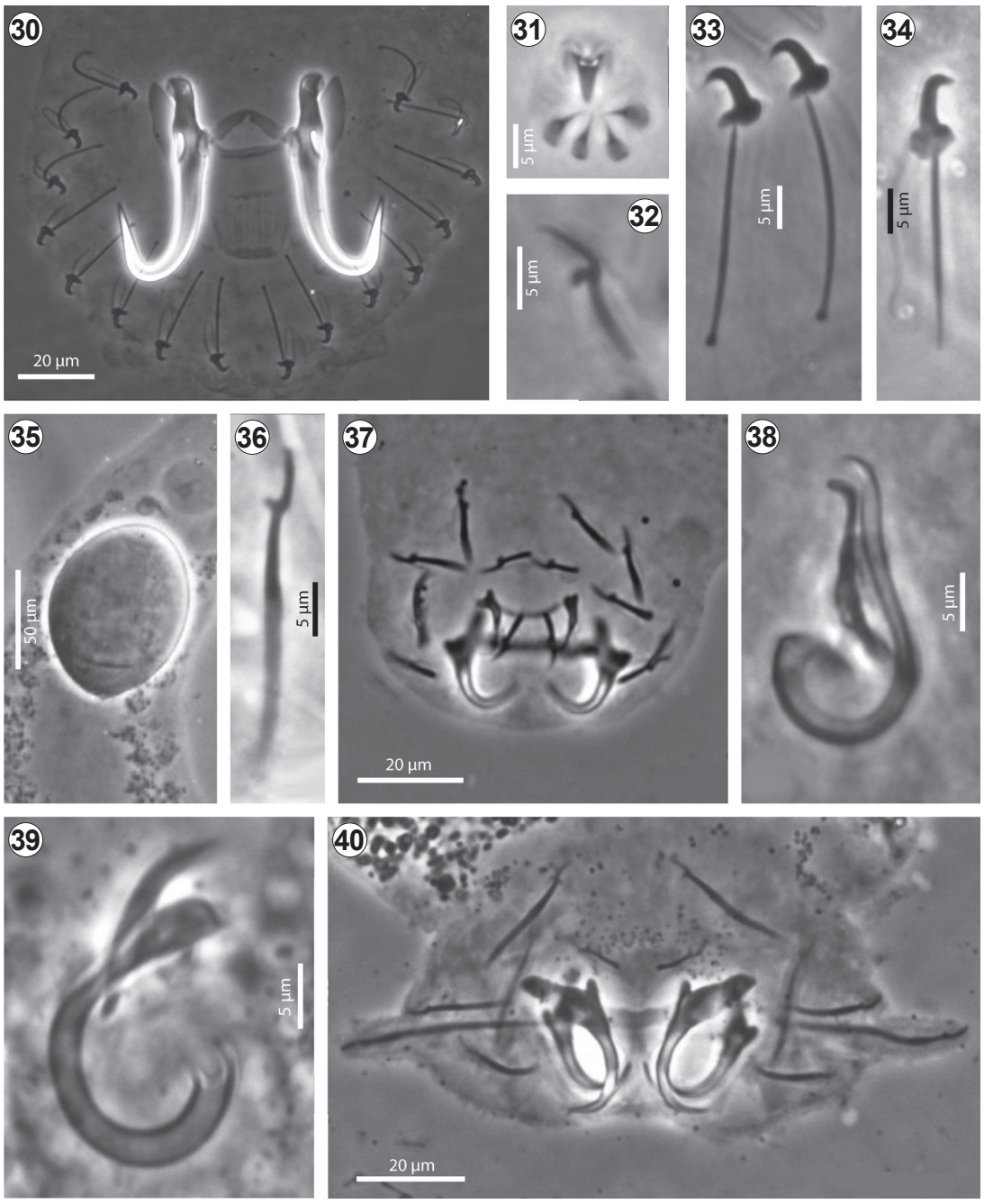
The description, although based on few specimens, which did not enable us to observe well the internal organs and systems, provides enough details and precision to allow subsequent identification of specimens and the congeneric status of the new species and *M. dorsivaginatus*. As a consequence, the proposal of *Marumbius* is, in our opinion, more robust and provides more stability to the classification of Neotropical Dactylogyridae.

Cacatuocotyle paranaensis Boeger, Domingues et Kritsky, 1997

Remarks. A single specimen of *C. paranaensis* (CHIOC 37898) was recovered from one *C. lanei* of more than 181 specimens of *Characidium* spp. (77 *C. lanei*, 114 *C. pterostictum* and 2 *Characidium* sp.). All morphological characters of this specimen are in accordance with the original description.

DISCUSSION

Despite the fact that no analysis on the community history of the fauna of Monogenoidea that parasitizes species



Figs. 30–40. Phase contrast micrographs of the haptoral structures of Monogenoidea from *Characidium* spp. **Fig. 30.** Haptoral structures of *Gyrodactylus carolinae* sp. n. **Fig. 31.** Male copulatory organ of *G. carolinae* sp. n. **Fig. 32.** Hook 5 of *Marumbius dorsivaginatus* sp. n. **Fig. 33.** Hooks of *G. carolinae* sp. n. **Fig. 34.** Hook of *Gyrodactylus inesperatus* sp. n. **Fig. 35.** Egg of *M. dorsivaginatus* sp. n. **Fig. 36.** Hook pair 2 of *Marumbius amplexus* sp. n. **Fig. 37.** Haptoral structures of *M. dorsivaginatus* sp. n. **Fig. 38.** Copulatory complex of *M. dorsivaginatus* sp. n. **Fig. 39.** Copulatory complex of *M. amplexus* sp. n. **Fig. 40.** Haptoral structures of *M. amplexus* sp. n.

of *Characidium* exists, it is clearly composed by multiple lineages, some of distant relationships, suggesting complex origins. Species of *Marumbius* and *Cacatuocotyle* are known solely from species of Characiformes, which suggests a longer history of association with fishes of this order. *Marumbius* sp. are known solely from species of *Characidium* (Crenuchidae), whereas *Cacatuocotyle* was originally proposed based on parasites from *Characidium* by Boeger et al. (1997) from South Brazil and subsequent species were described from *Astyanax* sp. from Mexico (Mendoza-Franco et al. 2012).

Species of *Urocleidoides* sensu stricto (see Kritsky et al. 1986), not collected in this study, are known solely from the gills of *Characidium caucanum* Eigenmann as

Urocleidoides anops Kritsky et Thatcher, 1974 from Colombia. This genus is known to contain species parasitising fishes from different taxonomic groups from three orders (Characiformes, Cyprinodontiformes and Gymnotiformes) within the Neotropics (see Mizelle and Price 1964, Kritsky et al. 1986, Jogunoori et al. 2004, Mendoza-Franco and Reina 2008, Rosim et al. 2011).

Gyrodactylids from *Characidum* spp. also appear to compose distinct lineages. As suggested in this study, *G. carolinae* is likely to be a member of a lineage of *Gyrodactylus* in which most members parasitize poeciliid fishes (Cyprinodontiformes) in the Neotropics. This phenotypic and genetic proximity suggests that the association of this parasite lineage with either cyprinidontiform or characiform fishes in the Neotropics is likely associated with at least one host switch event between these distantly related fish clades. In contrast, *G. inesperatus* is apparently not a member of this lineage or any other known lineage of Neotropical *Gyrodactylus*.

The fauna of Monogenoidea of *Characidium* spp. provides additional support for the recent proposal that ecological fitting (Janzen 1985) through host-switching (see Agosta et al. 2010) is prevalent in parasite community structuring and diversification. Hoberg and Brooks (2008) suggested that the majority of host-parasites associations result from a complex macroevolutionary mosaic involving both co-evolution and colonization. Evidences of host-switching are extensive in the structuring of the parasite community of *Characidium* spp. not only within Gyrodactylidae, as suggested by Boeger et al. (2003), but also among species of Ancyrocephalinae.

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