



A New Species of *Rhinergasilus* Boeger et Thatcher, 1988 (Copepoda: Ergasilidae) from Gills of *Astyanax fasciatus* (Cuvier, 1819) (Actinopterygii: Characidae)

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Abstract

Purpose To describe a new species of a parasitic copepod, *Rhinergasilus digitus* n. sp. (Cyclopoida, Ergasilidae), collected from the gills of the red-tailed lambari *Astyanax fasciatus* (Characiformes, Characidae) in two tributaries of the Jurumirim Reservoir (Upper Paranapanema River), São Paulo State, Brazil: Ribeirão dos Veados and Paranapanema River.

Methods Fish were collected using multi-panel gills nets. The gill of each fish was washed and examined in a stereo microscope for copepods. The copepods found were stored in 70% ethanol, cleared in lactic acid, and mounted in Hoyer's medium. Drawings were made with the aid of a Leica microscope DMLS equipped with a drawing tube.

Results The new species differs from its congeneric species, *Rhinergasilus piranhus* (type-species), in having comparatively biggest body size (body length: 535–598 μm in the new species vs. 237–282 μm in *R. piranhus*); second antennary segment armed with a minute sensillum near middle of inner margin and a row of spinules on outer margin; third exopodal segment of leg 1 with digitiform process; interpodal plates two and three both ornamented with spinules along posterior margin; leg 5 reduced and represented by two unequal setae.

Conclusions Based on the morphological differences described above, we erected a new species of *Rhinergasilus*. *Rhinergasilus digitus* n. sp. is the second ergasilid described from *A. fasciatus*, as well as it represents the first report of this genus in a characid fish.

Keywords Cyclopoida · Crustacea · Ectoparasite · Jurumirim · Paranapanema · Ribeirão dos veados

Introduction

Ergasilidae Burmeister, 1835 represents one of the most important and biggest families of parasitic copepods of the order Cyclopoida Burmeister, 1834. It current comprises 28 valid genera and over than 260 species [1–3]. Ergasilids are recorded in all continents and zoogeographic regions excluding Antarctica, and occur in a wide variety of aquatic habitats: marine, brackish, and freshwater (the majority) [4]. Due to their unique

life cycle, which is characterized by the presence of a parasitic phase (post-mated adult females) and a free-living phase (larvae ♀♂ and adult males) [1, 4], most ergasilids are only known by their parasitic adult females. Females are usually found attached to gills, fins, nasal fossae, embedded into the host tissues or in the urinary bladder of actinopterygian fishes, with few species on elasmobranchs and bivalve mollusks [3].

The Brazilian territory is inhabited by the richest biodiversity of Ergasilidae worldwide comprising over than 60 species from 17 genera [4, 5]. Most of the Brazilian ergasilids are found parasitizing the gills of freshwater fishes, with few records in other sites (e. g.: nasal fossae or urinary bladder) [3, 6]. Ergasilids of six genera are typically found associated with nasal fossae of Brazilian fishes, especially those species from the Amazon region, as follow: *Brasergasilus* Thatcher et Boeger, 1983; *Gamidactylus* Thatcher et Boeger, 1984; *Gamispatulus* Thatcher et Boeger, 1984; *Gamispinus* Thatcher et Boeger, 1984; *Rhinergasilus* Boeger et Thatcher, 1988; and *Vaigamus* Thatcher et Robertson, 1984

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[3]. Recently, *Ergasilus tipurus* Varella, Morey et Malta, 2019 and *Therodamas elongatus* (Thatcher, 1986) were recorded from nasal fossae of Amazonian fishes, increasing the number of genera in this family with species that parasitize nasal fossae of fishes in Brazil [3, 7].

Rhinergasilus is a monotypic genus described by Boeger and Thatcher [8] as a parasite of the nasal fossae of *Pygocentrus nattereri* Kner, 1858 from the Amazon region. The type-species, *Rhinergasilus piranhus* Boeger et Thatcher, 1988 can be easily distinguished from other ergasilids by the unique combination of diagnostic features, as follow: four-segmented antenna, three pairs of legs biramous, and legs 4 and 5, both reduced and represented by setae [8]. Since its description, specimens of *Rhinergasilus* has been reported in nasal fossae of fishes from three families (Acestorhynchidae, Prochilodontidae, and Serrasalminidae) sampled in Amazonas, Minas Gerais and Paraná States, Brazil (see Table 4 for a list of fishes parasitized with *Rhinergasilus* species).

During a parasitological survey of fishes from two tributaries (Ribeirão dos Veados and Paranapanema River) of the Jurumirim Reservoir, Upper Paranapanema River, São Paulo State, Brazil, we detected several ectoparasitic ergasilids parasitizing the gills of the red-tailed lambari, *Astyanax fasciatus* (Cuvier, 1819). A morphological analysis of these specimens indicated that they represent a new species of *Rhinergasilus*, which is described herein.

Materials and Methods

Specimens of *A. fasciatus* were collected from April 2011 to October 2012 from two tributaries of the Jurumirim Reservoir: (1) Paranapanema River, Jurumirim Reservoir (23° 29'16.54" S, 48° 37'12.88" W), municipality of Angatuba, São Paulo State, Brazil; and (2) Ribeirão dos Veados River, Jurumirim Reservoir (23° 16'2.49" S, 48° 38'15.72" W), municipality of Itatinga, São Paulo State, Brazil. Fish were collected using multi-panel gill nets (3–14 cm mesh) soaked for 14 h. Hosts were individually stored in plastic bags and placed in a freezer before necropsy. The gill of each fish was thawed by placing it in a Petri dish with tap water and examined for copepods using a stereomicroscope. Copepods were removed from the gill using a fine needle, stored in 70% ethanol, cleared in lactic acid, and mounted in Hoyer's medium. Whenever necessary, some specimens were dissected in glycerol medium and then each part was mounted on individual slides. Coverslips were sealed with transparent nail varnish.

Morphological analyses and measurements of whole/dissected copepods were made using a microscope with differential interference contrast optics (Leica DMLB 5000, Leica Microsystems). Drawings were made with the aid of a microscope (Leica DMLS, Leica Microsystems, Wetzlar, Germany) equipped with a drawing tube. All measurements are in micrometers (μm) and presented as the mean and

standard deviation followed by the range in parenthesis. Anatomical terms followed Boxshall and Montú [9], and that key was used to identify copepod specimens to the genus level. Abbreviations used throughout the text to refer to the name of the structures and segments present in Ergasilidae are shown in Table 1. The nomenclature used for the antennary segmentation has followed the following considerations: the ergasilid antenna is four-segmented (comprising coxobasis and three endopodal segments) and the claw is an armature element derived from the third endopodal segment [10].

Ecological descriptors such as prevalence, mean abundance, and intensity were calculated in accordance with Bush et al. [11].

Type specimens (holotype and paratypes) were deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), municipality of Manaus, Amazonas State, Brazil.

Results

Family Ergasilidae Burmeister, 1835

Rhinergasilus digitus n. sp. (Figs. 1–3; Tables 2, 3)

Description of adult female (based on 11 female specimens; no male observed): Body cycloform (Fig. 1a), comprising prosome, urosome, and caudal rami; prosome consisting of cephalosome and PS-1; PS-1 fused to cephalosome; and 3 free pedigerous somites. Cephalothorax bullet-shaped (Figs. 1a, 2a), with maximum width at level of buccal apparatus (Table 2), ornamented with two pairs of sensilla laterally (Fig. 2a). Pedigerous somites decreasing gradually in width from anterior to posterior; PS-2 narrower than cephalothorax, with paired integumental windows laterally on tergite (Fig. 1a); PS-4 reduced, smaller and thinner than other prosome somites (Fig. 1a, b).

Urosome consisting of PS-5, genital double-somite, and three free abdominal somites (Fig. 1b); PS-5 (Fig. 1b)

Table 1 Abbreviations of body parts and segments used throughout the text to describe copepods

Abbreviation	Meaning
PS-1 (2–5)	To indicate the first (second to fifth) pedigerous somite
AS-1 (2, 3)	To indicate the first (second, third) abdominal somite
P1 (2–5)	To indicate the first (second to fifth) leg
enp	Endopod
exp	Exopod
enp-1 (2, 3)	To indicate the first (second, third) endopodal segment
exp-1 (2, 3)	To indicate the first (second, third) exopodal segment

reduced, unornamented; genital double-somite (Fig. 1b), 1.5 times wider than long, bearing paired slit-like genital apertures dorsally, ornamented with transverse row of spinules on ventral surface; abdominal somites decreasing in width from anterior to posterior, each somite ornamented with posterior spinule row along ventral margin (Fig. 1b); AS-3 (= anal somite) deeply incised posteriorly (= anus). Caudal rami (Fig. 1b), about 1.5 times longer than wide;

each ramus ornamented with paired spinule rows on ventral surface and armed with four setae, all naked: seta 1 and 3 shortest, both setae inserted on ventral surface; seta 2 and 4, both setae inserted on posterior margin; seta 4 longest, about three times longer than seta 2.

Antennule six-segmented (Fig. 2b), setal formula: 1, 7, 3, 2 + 2 ae, 1 + 1 ae, 5 + 2 ae (total 24). Antenna (Fig. 2c) 4-segmented comprising coxobasis, and 3-segmented enp;

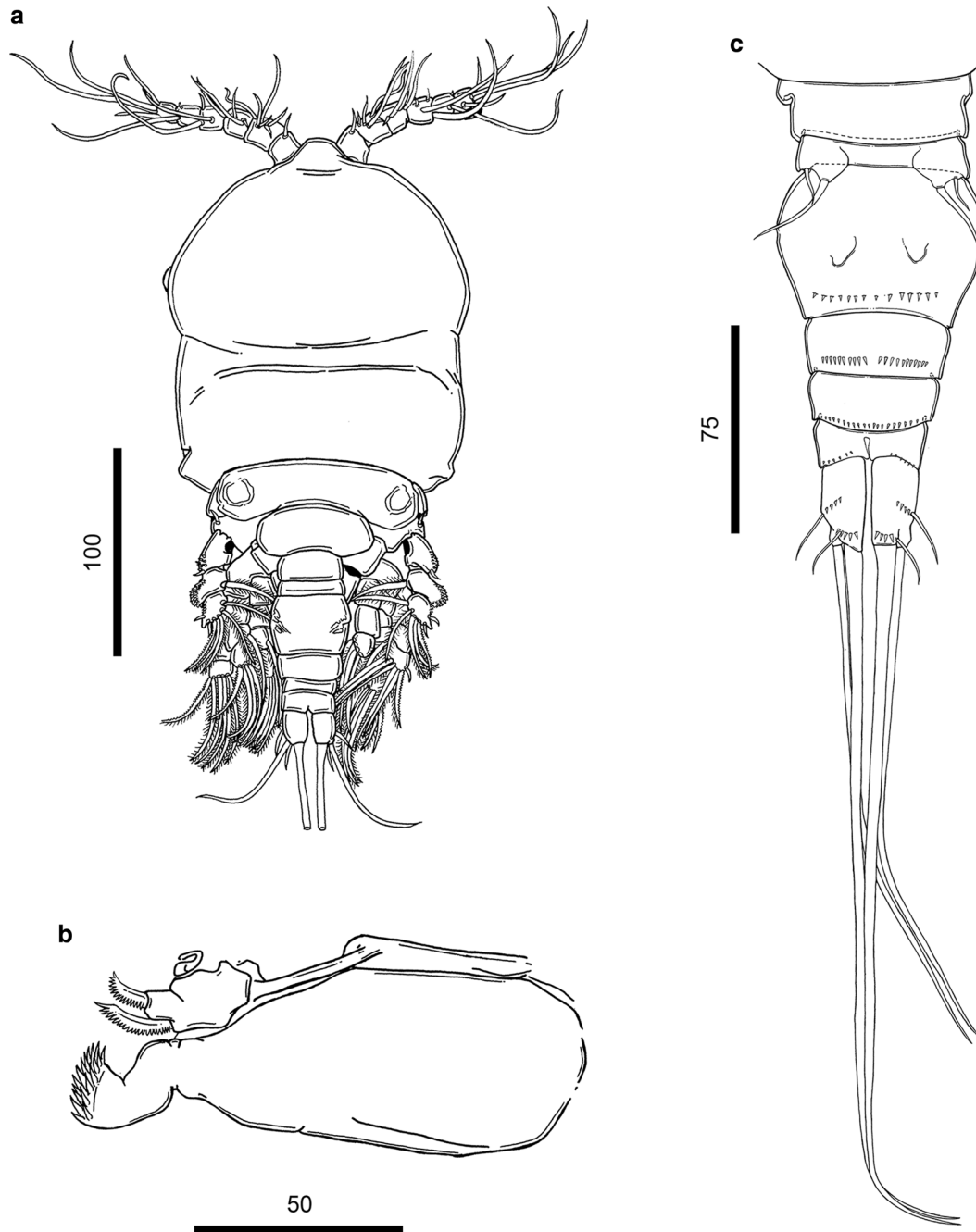


Fig. 1 *Rhinergasilus digitus* n. sp. adult female. **a** body, dorsal view. **b** buccal apparatus. **c** urosome, ventral view. Scale bars in micrometers (μm)

Table 2 Measurements in micrometers (μm) of adult females of *Rhinergasilus digitus* n. sp.

Character	Mean \pm SE	Range
Total length ^a	592 \pm 55	535–698
Cephalothorax length	328 \pm 41	287–414
Cephalothorax width	272 \pm 22	242–314
Antennule length	144,5 \pm 12	126–161
Antenna segment 1 length	101 \pm 6	89–114
Antenna segment 2 length	93 \pm 3	89–101
Antenna segment 3 length	35 \pm 2,5	30–39
Antenna segment 4 length	6 \pm 1	5–8
Claw length	71 \pm 3	66–76
Pedigerous somite 2 length	45 \pm 3	41–47
Pedigerous somite 2 width	181 \pm 3	178–183
Pedigerous somite 3 length	42 \pm 1,5	42–44
Pedigerous somite 3 width	110 \pm 6	103–114
Pedigerous somite 4 length	18 \pm 4	14–28
Pedigerous somite 4 width	66 \pm 7	54–74
Pedigerous somite 5 length	14 \pm 2	10–16
Pedigerous somite 5 width	63 \pm 5	54–70
Genital double-somite length	54 \pm 3	49–58
Genital double-somite width	74 \pm 4	68–82
Abdominal somite 1 length	23 \pm 3	17–25
Abdominal somite 1 width	58 \pm 5	52–67
Abdominal somite 2 length	24 \pm 3	19–28
Abdominal somite 2 width	52 \pm 5	45–61
Abdominal somite 3 length	22 \pm 2	18–25
Abdominal somite 3 width	48 \pm 5	41–56
Caudal ramus length	34 \pm 3	28–40
Caudal ramus width	21 \pm 3	17–26
Caudal ramus seta 1 length	24 \pm 3	18–29
Caudal ramus seta 2 length	117 \pm 12	96–133
Caudal ramus seta 3 length	27 \pm 3	23–31
Caudal ramus seta 4 length	293 \pm 19	267–332

SE standard error

^aLess caudal rami setae**Table 3** Armature of swimming legs of *Rhinergasilus digitus* n. sp. adult female

Swimming leg	Coxa	Basis	Endopod	Exopod
P1	0–0	1–0	0–1; 0–2	I-0; 0–1; II-5
P2	0–0	1–0	0–1; 0–2; I-4	I-0; 0–1; I-6
P3	0–0	1–0	0–1; 0–2; I-4	I-0; 0–1; 0–6

(Roman numeral=spines; Arabic numerals=setae). P1–P4 first to fifth swimming leg

coxobasis (= first segment) broad, with short naked seta; enp-1 (= second segment) ornamented with spinule row along outer margin and minute sensillum near middle of

inner margin; enp-2 (= third segment) three times shorter than enp-1, unornamented; enp-3 (= fourth segment) reduced, unornamented; and terminal claw; claw curved, with fossa on concave margin.

Buccal apparatus (Fig. 1c) comprising mandible and maxilla; mandible armed with two blades (anterior and posterior blade); each blade with spinules along posterior margin; maxilla two-segmented, comprising syncoxa and basis; syncoxa broad, unornamented; basis covered with multiples spinules; maxillule vestigial, unornamented.

P1–P3 biramous (Fig. 3a–c), each comprising coxa, basis, endopod (inner ramus) and exopod (outer ramus). P1 (Fig. 3a); coxa unornamented; basis with bare outer seta and posterior margin produced posteriorly (arrowed in Fig. 3a); enp two-segmented, all segments with spinules along outer margin; enp-2 (= distal segment) with rounded end, armed with 2 setae; exp 3-segmented, all segments with spinules along outer margin; exp-1 (= proximal segment) with bristles on inner margin; exp-3 (= distal segment) with digitiform process (arrowed in Fig. 3a), armed with two serrated spines, one semi-plumose seta (serrated outer margin) and four plumose setae.

P2 (Fig. 3b); coxa unornamented; basis with bare outer seta; enp 3-segmented; enp-1 (= proximal segment) with bristles on outer margin; enp-2 and -3, both with spinules on outer margin; enp-3 (= distal segment) armed with one serrated spine and four plumose setae; exp 3-segmented, all segments with spinules along outer margin; exp-1 (= proximal segment) with bristles on inner margin; exp-3 (= distal segment) armed with one serrated spine, one semi-plumose seta (serrated outer margin) and five plumose setae. P3 (Fig. 3c) similar to P2, except for lacking spine on exp-3.

P4 absent (Fig. 1b). P5 reduced and represented by two unequal setae (Fig. 1b). Spine and setal formula of biramous swimming legs as presented in Table 3.

Intercoxal sclerites slender, unornamented, with both ends directed posteriorly (Fig. 3a–c). Interpodal plates of P2–P3 with a transverse spinule row on ventral surface (Fig. 3b, c); interpodal plate of P1, unornamented (Fig. 3a). Egg sac paired (Fig. 3d), multiseriate.

Taxonomic Summary

Type host: *Astyanax fasciatus* (Cuvier, 1819) (Characiformes: Characidae), red-tailed lambari.

Site of infection: Gill filaments.

Prevalence: 32.5%.

Intensity of infection: 4.3 ± 1.2 (1–39).

Type locality: Ribeirão dos Veados River, Jurumirim Reservoir, Upper Paranapanema River (23° 16'2.49" S, 48° 38'15.72" W), municipality of Itatinga, São Paulo State, Brazil.

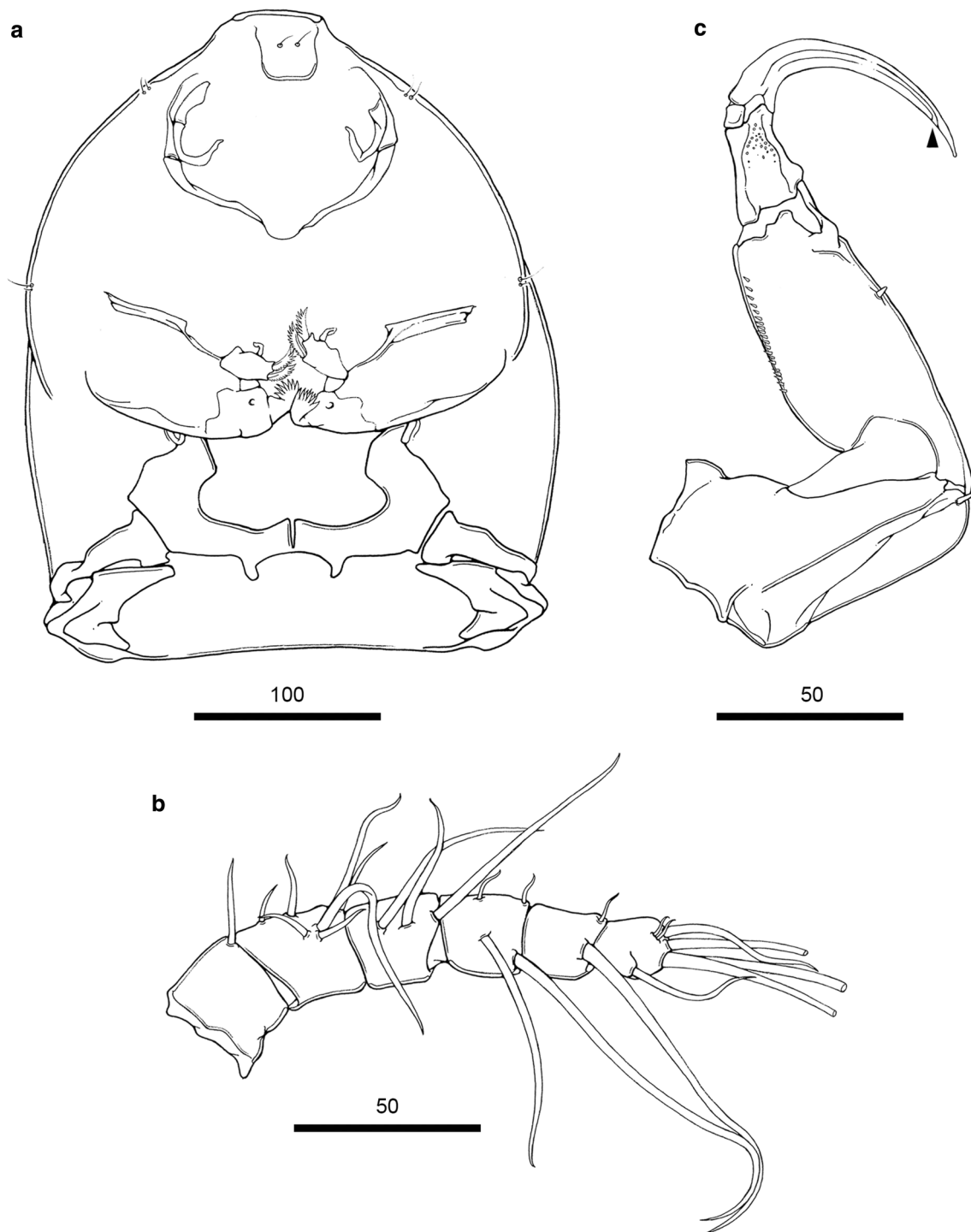


Fig. 2 *Rhinergasilus digitus* n. sp. adult female. **a** cephalothorax, ventral view. **b** antennule. **c** antenna, with fossa on concave margin (arrow-head). Scale bars in micrometers (μm)

Other locality: Paranapanema River, Jurumirim Reservoir, Upper Paranapanema River ($23^{\circ} 29'16.54''$ S, $48^{\circ} 37'12.88''$ W), municipality of Angatuba, São Paulo State, Brazil.

Specimens deposited: The numbers of holotype and paratypes are: Holotype INPA 2515, Paratypes INPA 2516, INPA 2517, INPA 2517, INPA 2519 and INPA 2520.

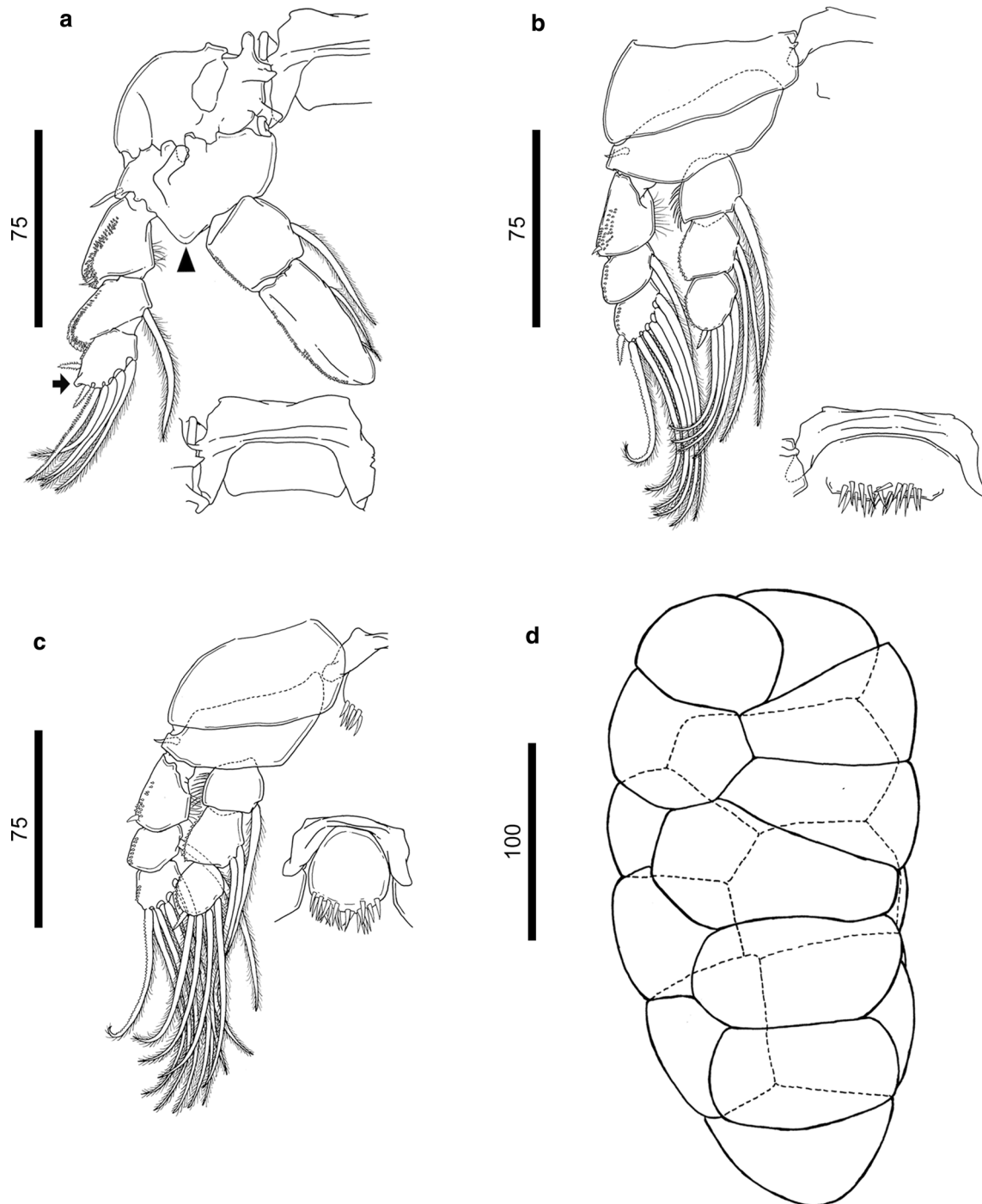


Fig. 3 *Rhinergasilus digitus* n. sp. adult female. **a** leg 1, basis produced posteriorly (arrowhead) and with third exopodal segment with a digitiform process (seta). **b** leg 2. **c** leg 3. **d** egg sac. Scale bars in micrometers (μm)

Specimens examined: *Rhinergasilus piranhus* Boeger and Thatcher, 1988 – Holotype INPA PA 309-1, and Paratypes PA 309-2 to 309-5.

The Zoobank LSID is: urn:lsid:zoobank.org:pub:FAE7C164-50E2-4969-83D3-E30A23BB1B74.

Etymology: From Latin, the specific epithet refers to the presence of a digitiform process on exp-3 (=terminal segment) of P1.

Table 4 Fishes parasitized by *Rhinergasilus* species in Brazil

	Host	Host family	Infection site	Locality (River)	State	Country	References
<i>Rhinergasilus digitus</i> n. sp.	<i>Astyanax fasciatus</i> (Cuvier, 1819)	Characidae	Gills	Paranapanema River Ribeirão dos Veados River	São Paulo State	Brazil	Present study
<i>Rhinergasilus piranhus</i> Boeger et Thatcher, 1988	<i>Acestrorhynchus lacustris</i> (Lütken, 1875)	Acestrorhynchi- dae	Nasal fossae	Upper Paraná River floodplain	Paraná State	Brazil	[5, 13–16]
	<i>Acestrorhynchus falcistrostris</i> (Cuvier, 1819)	Acestrorhynchi- dae	Nasal fossae	-	Rondônia State	Brazil	[17]
	<i>Colossoma macropomum</i> (Cuvier, 1816)	Serrasalmidae	Nasal fossae	Purus River Solimões River	Amazonas State	Brazil	[7, 18]
	<i>Pygocentrus nattereri</i> Kner, 1858	Serrasalmidae	Nasal fossae	Furo do Catalão Marchantaria Island Purus River Solimões River	Amazonas State	Brazil	[5, 7, 8, 15, 16, 19, 20]
	<i>Serrasalmus altispinis</i> Mercky, Jégu et Santos, 2000	Serrasalmidae	Nasal fossae	Purus River Solimões River	Amazonas State	Brazil	[7, 21]
	<i>Prochilodus nigricans</i> Spix et Agassiz, 1829	Prochilodontidae	Nasal fossae	Purus River Solimões River	Amazonas State	Brazil	[22]
<i>Rhinergasilus</i> sp.	<i>Pygocentrus piraya</i> (Cuvier, 1819)	Serrasalmidae	Gills	Três Marias Reservoir	Minas Gerais State	Brazil	[15, 16, 20, 23]

Discussion

The present specimens were identified as members of the ergasilid genus *Rhinergasilus* by their possession of the diagnostic features proposed by Boeger and Thatcher [8] including the presence of three pairs of nonreduced legs, PS-4 and PS-5 greatly reduced, antennule 6-segmented and antenna 4-segmented.

The new species, *R. digitus* n. sp., resembles the type-species, *Rhinergasilus piranhus* in possessing third antennary segment (= enp-2) short, being shorter than previous antennary segments, antenna armed with a relatively long, curved claw, and P1 enp with modified distal segment (= second endopodal segment); in both species this segment lacks spines, has a rounded end and is armed with few setae, e. g., two setae in *R. digitus* and three setae in *R. piranhus*. Despite those similarities, the new species differs from the type-species in a number of features. The armature of antennary segments in *R. digitus* n. sp. differs from that of *R. piranhus*: in *R. digitus* n. sp. the second antennary segment (= enp-1) carries a minute sensillum near the middle of inner margin and a row of spinules on outer margin, whereas in *R. piranhus* this segment lacks any armature or ornament. The

ornamentation of P1 is also different, *R. piranhus* has coxa and basis both ornamented with a row of spinules near inner posterior margin, whereas in *R. digitus* n. sp. those segments do not have such ornaments. Besides, the third exopodal segment of P1 in *R. digitus* n. sp. bears a digitiform process (located between the two distal spines) which is absent in the type-species. Another distinct difference between these two species is that in *R. piranhus* the first and second interpodal plates are both ornamented with spinules along posterior margin, while in *R. digitus* n. sp., the second and third interpodal plates have spinules (first interpodal plate without spinules in the new species). Moreover, the P4 and P5 are both represented by a seta in *R. piranhus*, while in the new species, the P4 is absent and P5 is represented by two unequal setae. Finally, the body size also differs, *R. digitus* n. sp. is about twice as long as *R. piranhus*: body length (less caudal setae) 535–598 in the new species vs. 237–282 in *R. piranhus* [8].

Based on the morphological differences described above, the present specimens were considered as new species of *Rhinergasilus*. *Rhinergasilus digitus* n. sp. is the second ergasilid described from *A. fasciatus* sampled in the Jurumirim reservoir (see Narciso et al. [12] and it is the first

report of a *Rhinergasilus* species parasitizing a characid fish (Characidae). Characidae is the fourth family of Characiformes that has species parasitized by *Rhinergasilus* spp. A checklist of fishes parasitized by members of *Rhinergasilus* is provided (Table 4).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards All applicable international, national, and/or institutional guidelines for the use and care of animals were followed.

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