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(Copepoda: Ergasilidae): A Gill Parasite of
the Freshwater Fish *Prochilodus lineatus*
(Valenciennes, 1837) (Actinopterygii:
Prochilodontidae) from the Neotropical
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Acta Parasitologica

ISSN 1230-2821

Acta Parasit.

DOI 10.1007/s11686-020-00270-7



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Rhinergasilus unguilongus n. sp. (Copepoda: Ergasilidae): A Gill Parasite of the Freshwater Fish *Prochilodus lineatus* (Valenciennes, 1837) (Actinopterygii: Prochilodontidae) from the Neotropical Region, Brazil

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Received: 24 April 2020 / Accepted: 11 August 2020

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Abstract

Purpose To describe, based on morphological features, a new copepod species of *Rhinergasilus*, *Rhinergasilus unguilongus* n. sp., collected from gills of the Streaked prochilod, *Prochilodus lineatus* (Valenciennes, 1837), sampled in the Veados and Paranapanema Rivers, two tributaries of the Jurumirim Reservoir (Upper Paranapanema River, Paraná River Basin), São Paulo State, Brazil.

Methods Fish were collected using multi-panel gill nets. The gills of each fish were washed and examined for copepods using a stereo microscope. 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 microscope equipped with a drawing tube.

Results The new copepod species can be distinguished from its two congeners, *Rhinergasilus piranhus* (type-species) Boeger et Thatcher, 1988 and *Rhinergasilus digitus* Narciso, Brandão, Perbiche-Neves et Silva, 2020, due to its extremely long antennary claw (etymology of the species), being longer than the other antennary segments; third leg lacking any interpodal plate; and third endopodal segment of third leg lacking a distal spine.

Conclusions Based on the morphological differences, we erected a new species of *Rhinergasilus*. The new copepod represents the second report of a *Rhinergasilus* species parasitizing a fish from the Jurumirim Reservoir. It also represents the first report of this genus in *P. lineatus*.

Keywords Cyclopoida · Crustacea · Ectoparasite · Paranapanema · Ribeirão dos veados · Streaked prochilod

Introduction

The Ergasilidae Burmeister, 1835 is a unique family among cyclopoid copepods in which only post-mated adult females are parasites while the developmental stages from nauplii to copepodites (♂♀) and adult males are free-living organisms [1]. Ergasilids are recorded in a wide variety of aquatic habitats, from marine to freshwater environments, and in almost every continent and zoogeographic region, excepted Antarctica [2, 3]. This family currently comprises 263 species from 30 valid genera, parasitic mainly on fishes—Actinopterygii

(majority) and Elasmobranchii—with few species living in bivalve molluscs [4–7]. In fishes, fertilized females can be found attached to gills, nostrils, fins, embedded into host tissues (i.e. mesoparasitic species) or inside the urinary bladder [5, 8, 9]. Despite their small size, the damage inflicted by these copepods to their host tissues, especially in massive infections, can lead to significantly reducing the weight and the growth rate of their hosts or even to serious mortalities [10].

In Brazil, Ergasilidae is considered one of the most important and speciose families of parasitic copepods with over 60 described species from 17 genera [11]. Most of the Brazilian ergasilids parasitize gills, but species from nine genera also occur inside the nostrils of their fish hosts, namely *Brasergasilus* Thatcher et Boeger, 1983, *Ergasilus* von Nordmann, 1832, *Gamispatulus* Thatcher et Boeger, 1984, *Gamidactylus* Thatcher et Boeger, 1984, *Gamispinus* Thatcher et Boeger, 1984, *Pseudovaigamus* Amado, Ho et Rocha, 1995, *Rhinergasilus* Boeger et Thatcher,

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1988, *Therodamas* Krøyer, 1863, and *Vaigamus* Thatcher et Robertson, 1984 [12, 13]. The genus *Rhinergasilus* was originally proposed based on a species typically found in nostrils—*Rhinergasilus piranhus* Boeger et Thatcher, 1988—type-species, but the other described species, *Rhinergasilus digitus* Narciso, Brandão, Perbiche-Neves et Silva, 2020 was found attached to the gills of the red-tailed lambari, *Astyanax fasciatus* (Cuvier, 1819), expanding the variety of sites in hosts infected with species of this genus [14].

During the survey of the parasitic fauna of fishes from the Jurumirim Reservoir in Brazil (Upper Paranapanema River Basin, a tributary of Upper Paraná River Basin), we found several ergasilids parasitizing the gills of the Streaked prochilod, *Prochilodus lineatus* (Valenciennes, 1837). A morphological analysis of these copepods indicates that they represent an undescribed species of *Rhinergasilus*, which is herein described.

Materials and Methods

Thirty-seven specimens of the Streaked prochilod fish, *Prochilodus lineatus* (Valenciennes, 1837), were collected from April 2011 to April 2012 in 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) Veados River, Jurumirim Reservoir (23°16'2.49" S, 48°38'15.72" W), municipality of Itatinga, São Paulo State, Brazil. This study was approved by the ethics committee of the Institute of Biosciences (CEEAA #120) from São Paulo State University (Unesp), Botucatu, São Paulo State, Brazil. All applicable international, national, and/or institutional guidelines for the use and care of animals were followed. Fish collections in both municipalities (Angatuba and Itatinga) were authorized by the Department of Fisheries Development and Inspection (license #SP/538/88). Fish were collected using multi-panel gill nets (3–14 cm mesh) soaked for 14 h. Each specimen was individually stored in plastic bags and placed in a freezer before necropsy. The gills were then removed and examined for copepods using a stereomicroscope. Copepods were removed from the gills using entomological needles and then stored in 70% ethanol for further morphological analysis.

For morphological characterization, some specimens were cleared in lactic acid and then mounted in Hoyer's medium in semi-permanent slides. 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 (LeicaDMLS, Leica Microsystems, Wetzlar, Germany) equipped with a drawing tube. All measurements are in micrometres (μm) and presented as the range followed by the mean in parenthesis. Anatomical terms followed Boxshall and Montú [15] and Boxshall and Halsey [5], and that key was used to identify copepod specimens to the family and genus level. The nomenclature used for the antennary segmentation has followed the following considerations: the ergasilid antenna is 4-segmented (comprising coxobasis and three endopodal segments) and the claw is an armature element derived from the third endopodal segment [16]. Ecological descriptors such as prevalence and mean intensity were calculated following Bush *et al.* [17].

Type specimens (holotype and paratypes) were deposited in: (1) the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), municipality of Manaus, Amazonas State, Brazil; and (2) the Zoological Collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP), municipality of São Paulo, São Paulo State, Brazil.

Results

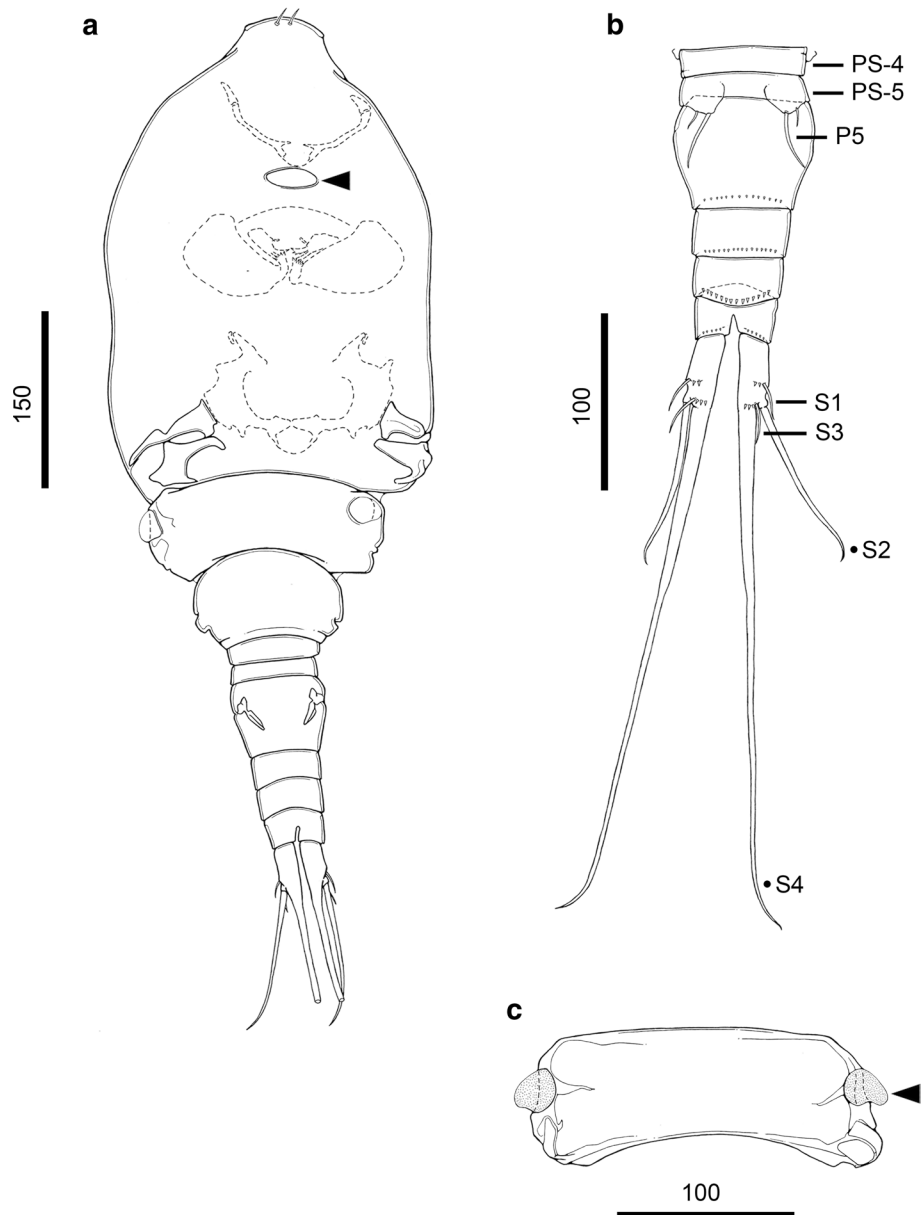
Family Ergasilidae Burmeister, 1835

Rhinergasilus unguilongus (Figs. 1, 2, 3; Tables 1, 2; Measurements Based on Six Female Specimens)

Description of adult female (based on six specimens; males not observed): Body cyclopiform (Fig. 1a), comprising prosome, urosome, and caudal rami; prosome consisting of cephalosome and first pedigerous somite; first pedigerous somite fused to cephalosome; and three free pedigerous somites (second to fourth). Cephalothorax bullet-shaped (Fig. 1a), with maximum width at level of buccal apparatus (Table 1); dorsal surface with elliptical integumental window (arrowed in Fig. 1a). Rostrum quadrangular, ornamented with one pair of setules anteriorly. Free pedigerous somites decreasing gradually in width (on transverse axis) from anterior to posterior (Fig. 1a); second pedigerous somite narrower than cephalothorax, with paired integumental windows laterally on tergite (Fig. 1c); third pedigerous somite longer (on longitudinal axis) than previous somite, with rounded lateral margins; fourth pedigerous somite reduced, shorter and narrower than other prosome somites (Fig. 1a, b).

Urosome consisting of fifth pedigerous somite, genital double-somite and three free abdominal somites (first to third) (Fig. 1b); fifth pedigerous somite reduced (Figs. 1a, b), unornamented, similar in size to fourth prosome somite; genital-double somite (Fig. 1b) about 1.3 times wider than

Fig. 1 *Rhinergasilus unguilongus* n. sp.—adult female. a body, dorsal view, cephalothorax with dorsal elliptical window (arrowhead). b urosome, ventral view. c second pedigerous somite, with paired integumental windows laterally on tergite (arrowhead). Scale bars in micrometers (μm). P5 = fifth leg. PS-4 = fourth pedigerous somite. PS-5 = fifth pedigerous somite. S1 = seta 1. S2 = seta 2. S3 = seta 3. S4 = seta 4



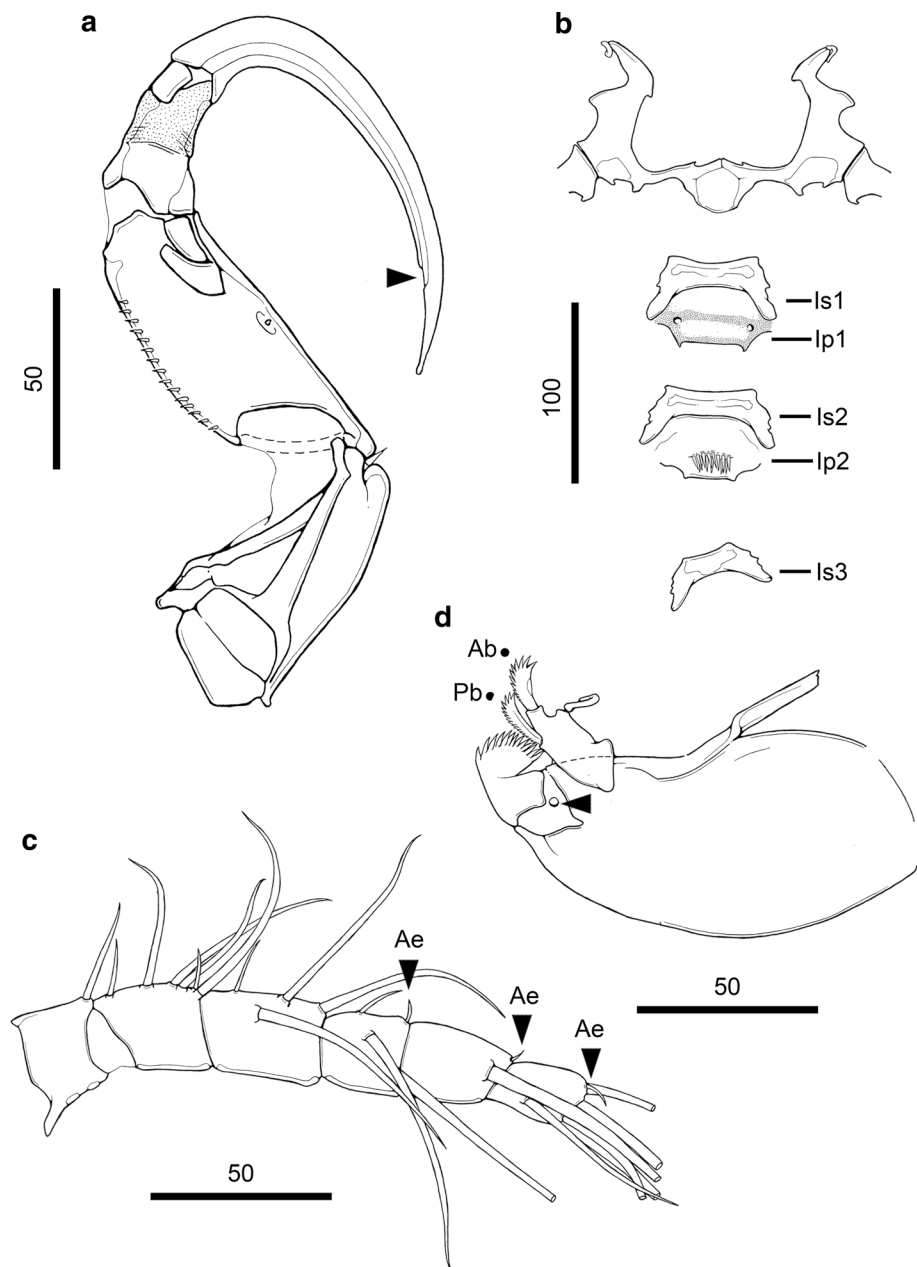
long, bearing paired slit-like genital apertures dorsally; ventral surface ornamented with transverse row of spinules; abdominal somites (Fig. 1b) decreasing gradually in width (on transverse axis) from anterior to posterior, each somite ornamented with transverse row of spinules ventrally; third abdominal somite (or anal somite) deeply incised posteriorly (anus).

Caudal rami (Fig. 1b) about 1.8 times longer than wide; each ramus ornamented with paired spinule rows ventrally, armed with four naked seta; seta 1 and 3 shortest, both setae inserted on ventral surface; seta 2 and 4, both inserted on posterior margin; seta 4 longest, about 2.5 times longer than seta 2.

Antennule 6-segmented (Fig. 2c), fourth to sixth segments carrying distal asthetascs (ae) on posterior margin

(arrowed in Fig. 2c); setal formula: 1, 6, 4, 3 + 1 ae, 1 + 1 ae, 5 + 1 ae (total 23). Antenna 4-segmented (Fig. 2a), comprising coxobasis and 3-segmented endopod (=enp); coxobasis (first segment) about 1.5 times longer than wide, armed with short naked seta; first endopodal segment (=enp-1) (second segment) as long as coxobasis, ornamented with spinule row on outer margin and minute sensillum near middle of inner margin; second endopodal segment (=enp-2) (third segment) decreasing in width distally, maximum width near to proximal joint (enp-1-enp-2 joint), unornamented, distal half with rough surface; third endopodal segment (=enp-3) (fourth segment) reduced, about two times longer than wide, unornamented; and terminal claw, claw curved, longer than other antennary segments, with rounded tip and fossa; fossa located on concave margin near to tip (arrowed in Fig. 2a).

Fig. 2 *Rhinergasilus unguilongus* n. sp.—adult female. a antenna, ventral view, antennary claw with fossa on concave margin (arrowhead). b intercoxal sclerites and interpodal plates. c antennule, fourth to sixth segment of antennule with distal aesthetascs on posterior margin (arrowhead). d buccal apparatus, syncoxa of maxilla with pore near to basis insertion (arrowhead). Scale bars in micrometers (μm). Ab = anterior blade. Ae = aesthetascs. Is1–Is3 = first to third intercoxal sclerites. Ip1–Ip2 = first to second interpodal plate. Pb = posterior blade

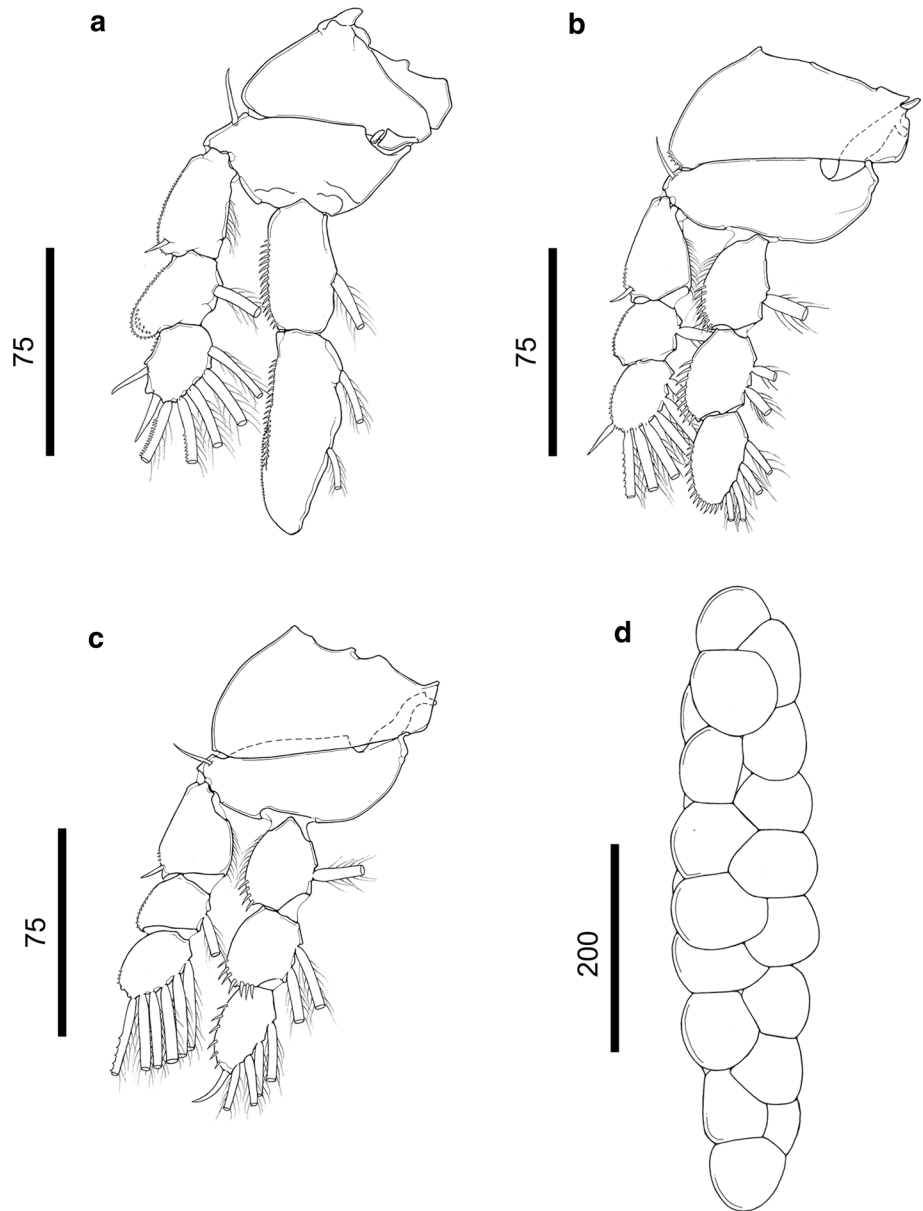


Buccal apparatus comprising mandible, maxillule and maxilla (Fig. 2d); mandible armed with two blades (anterior and posterior blade), each blade with spinules along posterior margin; posterior blade slightly longer and thinner than anterior blade; maxillule vestigial, unornamented; maxilla 2-segmented, comprising syncoxa (first segment) and basis (second segment); syncoxa broad, with a pore near to basis insertion (arrowed in Fig. 2d); basis covered with multiple spinules.

First to third leg biramous (=P1–P3) (Fig. 3a–c), each leg comprising coxa, basis, enp (inner ramus) and exopod (=exp) (outer ramus). P1 (Fig. 3a) with coxa unornamented; basis with bare outer seta; enp 2-segmented, both segments

with spinules along outer margin; enp-2 (= distal segment) with rounded end, armed with two plumose setae (i.e. seta with setules along both margins) on inner margin; exp 3-segmented, all segments with minute spinules along outer margin; spinules smaller than those present in enp; exp-1 (= proximal segment) about 1.5 times longer than following segments, ornamented with setules along inner margin, and armed with distolateral spine; exp-2 (= middle segment) with protruding outer margin, armed with plumose setae on inner margin; exp-3 (= distal segment) with small digitiform process (located between spines) and armed with two spines, two semi-plumose setae (seta with serrated outer margin) and three plumose setae.

Fig. 3 *Rhinergasilus unguilon-*
gus n. sp. – adult female. **a** leg
1. **b** leg 2. **c** leg 3. **d** egg sac.
Scale bars in micrometers (μm)



P2 (Fig. 3b) with coxa ornamented with lateral minute spinules (4 spinules); basis with bare outer seta; enp 3-segmented, all segments ornamented with spinules along outer margin; enp-1 (= proximal segment) with setules on outer margin; enp-2 (= middle segment) with two plumose setae on inner margin; enp-3 (= distal segment) with a rounded end, armed with four plumose setae, lacking spines; exp 3-segmented, all segments ornamented with minute spinules on outer margin; spinules smaller than those present in enp; exp-1 (= proximal segment) about 1.5 times longer than following segments, ornamented with setules on inner margin, armed with distolateral spine; exp-2 (= middle segment) armed with one seta on inner

margin; exp-3 (= distal segment) armed with one straight spine, one semi-plumose seta and five plumose setae.

P3 (Fig. 3c) with coxa unornamented; basis with bare outer seta; enp 3-segmented, all segments ornamented with spinules along outer margin; enp-1 (= proximal segment) with setules on outer margin; enp-2 (= middle segment) with two plumose setae on inner margin; enp-3 (= distal segment) armed with curved spine and four plumose setae; exp 3-segmented, all segments ornamented with minute spinules on outer margin; spinules smaller than those present in enp; exp-1 (= proximal segment) about 1.5 times longer than following segments, ornamented with setules on inner margin, armed with distolateral spine; exp-2

Table 1 Measurements in micrometers (μm) of adult females of *Rhinergasilus unguilongus* n. sp.

Character	Range (mean)
Total length ^a	655–938 (764)
Cephalothorax length	326–524 (399)
Cephalothorax width	260–347 (292)
Antennule length	138–173 (161)
Antenna segment 1 length	81–107 (93)
Antenna segment 2 length	93–101 (97)
Antenna segment 3 length	43–49 (46)
Antenna segment 4 length	12–15 (13)
Claw length	136–150 (145)
Pedigerous somite 2 length	63–109 (77)
Pedigerous somite 2 width	190–207 (200)
Pedigerous somite 3 length	55–83 (71)
Pedigerous somite 3 width	117–135 (128)
Pedigerous somite 4 length	13–24 (18)
Pedigerous somite 4 width	64–83 (77)
Pedigerous somite 5 length	16–20 (18)
Pedigerous somite 5 width	71–80 (76)
Genital double-somite length	59–71 (64)
Genital double-somite width	79–89 (84)
Abdominal somite 1 length	27–32 (29)
Abdominal somite 1 width	59–65 (61)
Abdominal somite 2 length	26–31 (28)
Abdominal somite 2 width	51–60 (55)
Abdominal somite 3 length	24–33 (28)
Abdominal somite 3 width	47–54 (50)
Caudal ramus length	39–50 (44)
Caudal ramus width	18–25 (21)
Caudal ramus seta 1 length	22–32 (29)
Caudal ramus seta 2 length	80–127 (108)
Caudal ramus seta 3 length	27–35 (31)
Caudal ramus seta 4 length	259–290 (277)

^aLess caudal rami setae

Table 2 Armature of swimming legs of *Rhinergasilus unguilongus* 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; 0–4	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-P3 = first to third swimming leg

(= middle segment) armed with one seta on inner margin; exp-3 (= distal segment) armed with one semi-plumose seta and five plumose setae, lacking spines.

Fourth leg absent (Fig. 1b). Fifth leg (Fig. 1b) reduced and represented by two unequal setae. Spine and setal formula of biramous swimming legs as presented in Table 2.

Intercoxal sclerites (Fig. 2b) slender, unornamented, with both ends directed posteriorly. Interpodal plate of P1 with lateral pores, ornamented with a pair of spine-like process on posterior margin; interpodal plate of P2 ornamented with multiple spinules, lacking pores; interpodal plate of P3, absent (Fig. 2b). Egg sac paired (Fig. 3d), multiseriate.

Taxonomic Summary

Type host: *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae), Streaked prochilod.

Site of infection: Gill filaments.

Prevalence: Four of 37 host examineds (10.8%).

Mean intensity of infection: 1.2 ± 0.2 (1–2).

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

Other locality: Paranapanema River, Jurumirim Reservoir, Upper Paranapanema River (23°29'16.54" S, 48°37'12.88" W), municipality of Angatuba, São Paulo State, Brazil.

Specimens deposited: Holotype MZUSP 41256 and two Paratypes MZUSP 41254 and MZUSP 41255 deposited in the Zoological Collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP), municipality of São Paulo, São Paulo State, Brazil; and one Paratype INPA 2530 deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), municipality of Manaus, Amazonas State, Brazil.

Specimens examined: *Rhinergasilus piranhus* Boeger and Thatcher, 1988 (type-species)—Holotype INPA PA 309–1 and Paratypes PA 309–2 to 309–5; *Rhinergasilus digitus* Narciso, Brandão, Perbiche-Neves and Silva, 2020—Holotype INPA 2515 and Paratypes INPA 2517 to 2520.

Zoobank LSID: urn:lsid:zoobank.org:pub:C10F7FB8-9748-4D18-B20D-43634E4BCB91.

Etymology: The generic name results from the combination of two Latin words: *unguis* (= claw) and *longus* (= long). This refers to the size of the antennary claw.

Discussion

The new species was identified as a member of the ergasilid genus *Rhinergasilus* in having the combination of diagnostic features listed by Boeger and Thatcher [18] for this genus, as follows: (1) first three pairs of swimming

legs not reduced; (2) fourth and fifth legs reduced; and (3) antenna 4-segmented. Furthermore, the new species also displays the same reduction pattern of the fourth and fifth legs as described for *R. digitus*, i.e., in *R. digitus* the P4 is absent and P5 is represented by two unequal setae (see Fig. 1c in Narciso *et al.* [14]). In addition to the generic features, the new species also resembles its two congeners, *R. piranhus* (type-species) and *R. digitus*, in having: (1) third antennary segment (= enp-2) unornamented and relatively short, being shorter than previous antennary segments; (2) antenna armed with relatively long and curved claw, i.e., claw with equal or larger size than other antennary segments; and (3) P1 enp with modified distal segment (= second segment), i.e., in all species this segment lacks spines, has a rounded end and is armed with few plumose setae (two or three setae).

Thus, the new species can be distinguished from its two congeners in possessing: (1) antennary claw extremely long, being longer than the first endopodal segment (rather than equal in size as in *R. piranhus*, or smaller than enp-1 as in *R. digitus*); (2) third interpodal plate absent (rather than present as in *R. piranhus* and *R. digitus*); and (3) third segment of P2 enp lacking any spine (rather than armed with distal spine as in *R. piranhus* and *R. digitus*). Moreover, the new copepod also differs from the type-species in having second antennary segment armed with a minute sensillum on inner margin and a spinule row on outer margin (rather than unornamented as in *R. piranhus*), interpodal plate of P1 unornamented (rather than ornamented with multiple spinules as in *R. piranhus*), and egg sac multiseriate (rather than uniseriate as in *R. piranhus*). Regarding *R. digitus*, the new species also differs from its congener in having endopodal segments of swimming legs (P1 to P3) ornamented with long spinules; leg endopodal spinules longer than those of exopodal segments (minute spinules), while in *R. digitus*, segments in both rami are ornamented with minute spinules.

Based on the morphological differences listed above, the present specimens were considered as a new species, *Rhinergasilus unguilongus* n. sp., for the ergasilid genus *Rhinergasilus*. It also represents the second report of a *Rhinergasilus* species parasitizing a fish in the Jurumirim Reservoir and the first report of this genus in *P. lineatus* (see Table 4 in Narciso *et al.* [14]).

Acknowledgements R.B.N thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support given to the study (132844/2018-4). R.J.S. is supported by FAPESP #2016/50377-1; CNPq #309125/2017-0; CNPq-PROTAX #440496/2015-2.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by RBN. The first version of the manuscript was written by

RBN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Proc. No.: 11/24159–3) from São Paulo State, Brazil.

Compliance with Ethical Standards

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

Ethical Approval This study was approved by the ethics committee of the Institute of Biosciences (CEEA #120). All applicable international, national, and/or institutional guidelines for the use and care of animals were followed.

Consent to Participate All authors have consented to participate in this study.

Consent for Publication All authors have consented to the publication of the manuscript.

Availability of Data and Material All data will be available for consultation. Whenever necessary, they should be requested from the corresponding author. The specimens used for description will be available in specialized scientific collections.

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