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ORIGINAL PAPER

Ergasilus tipurus n. sp. (Copepoda: Ergasilidae), A Parasite of Brazilian Amazon Fish Species

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Abstract

Introduction Copepods are common components in all types of fish assemblages, being present in fishes of all ecosystems. The *Ergasilidae Burmeister*, 1835 is one of the biggest families in the order *Cyclopoida Burmeister*, 1834, with the majority of species found on freshwater fishes.

Material and methods *Ergasilus tipurus* n. sp. is described from the nasal fossae of *Calophysus macropterus* and *Sorubim lima* captured in the rivers Guaporé and Mamoré in the State of Rondônia, Brazil, and from the nasal fossae and the gills of *Rhaphiodon vulpinus* collected from floodplain lakes of the Solimões River in the State of Amazonas, Brazil.

Results The new species differs from all known species of *Ergasilus* from Brazilian waters by: (1) antennule setal formula 3:7:3:4:2:5; (2), having three-segmented first endopod of leg I; and (3) having leg V with a single well-developed segment with one middle distal setae, two distal setae placed laterally and two basal papillae, each provided with a simple seta.

Discussion From all *Ergasilus* species described in the neotropics, this is the first species that can be found parasitizing the gills and nasal fossae of the reported hosts.

Keywords Copepod parasite · Gills · Nasal fossae · Neotropics

Introduction

Copepods are common components in all types of fish assemblages, being present in fishes of all ecosystems [5]. They are considered the largest and most diverse group of crustaceans and the most abundant among groups of multicellular organisms [4].

The Ergasilidae Burmeister, 1835 is one of the biggest families in the order Cyclopoida Burmeister, 1834, with the majority of species found on freshwater fishes. Only adult females of ergasilids are found parasitizing the gills, fins, nasal fossae, embedded in host tissues or in the urinary bladder of actinopterygian fishes [5, 15].

In the Americas, North America and Brazil are the territories with the most studies and knowledge about species of Ergasilidae [16]. Species representing 16 genera of ergasilid copepods have been reported from the neotropical realm [4], but some authors assume that only a small proportion of existing ergasilids has hitherto been reported [10, 11, 15]. Twenty-three freshwater species of *Ergasilus* von Nordmann, 1832 are known parasitizing the gills of neotropical teleosts [17].

The presently reported study focuses on copepod parasites of the genus *Ergasilus* found in the nasal fossae of two species: *Calophysus macropterus* (Lichtenstein, 1819) and *Sorubim lima* (Bloch and Schneider, 1801) collected from the State of Rondônia and from the nasal fossae and gills of *Rhaphiodon vulpinus* Spix and Agassiz, 1829 collected from Amazon floodplain lakes. The specimens were sampled within an extended time period during different expeditions. A morphological study of these specimens has revealed that they represent a new species of *Ergasilus*, which is described herein.

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Materials and Methods

Calophysus macropterus and *Sorubim lima* were captured in the rivers Guaporé and Mamoré in the State of Rondônia, Brazil, in November 1984 and October 1985 (07°13′43″S, 59°50′66″W). *Rhaphiodon vulpinus* were captured in six floodplain lakes of the Brazilian Amazon between March and December 2013. The sampled lakes were: Baixio (03°17′27.2″S, 60°04′29.6″W); Preto (03°21′17.1″S, 60°37′28.6″W); Ananá (03°53′54.8″S, 61°40′18.4″W); São Tomé (03°49′39.0″S, 61°25′24.6″W); Araçá (03°45′04.3″S, 62°21′25.9″W) and Maracá (03°50′32.8″S, 62°34′32.4″W), all lakes located between the cities of Manaus and Coari in the State of Amazonas.

Copepod parasites were removed from the nostrils and gill filaments of the hosts with dissecting needles and fixed in 70% alcohol. The parasites were transported to the Fish Parasitology Laboratory of the National Institute of Amazon Research (INPA), Manaus AM, where permanent slide preparations were made using the phenol–balsam method explained in Thatcher [18].

Drawings were prepared with the help of a light optical microscope with phase contrast using a drawing tube. Measurements were made utilizing a measuring ocular and expressed in micrometres (µm). Type specimens and vouchers were deposited in the Crustacean Collection of the National Institute of Amazon Research (INPA), Manaus, AM, Brazil.

Due to the small amount of specimens collected in 1984 and 1985 and the quality of the slides, the parasites were just described in the doctoral theses of Varella [23] but were not published. In 2013 in another expedition, the same parasite was found in another host (*R. vulpinus*) with sufficient specimens to elaborate a better description and publish the work.

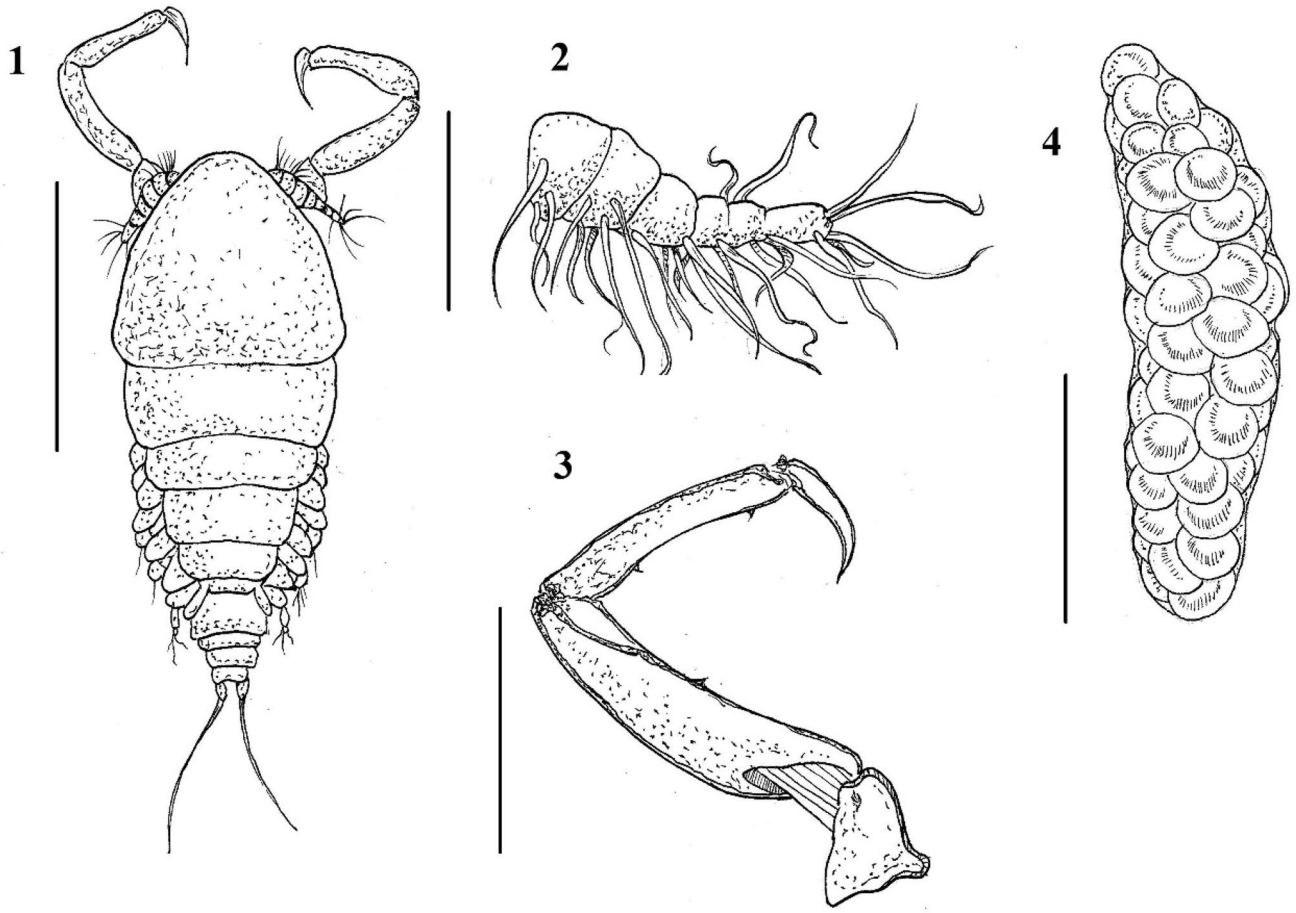

Fig. 1–4 *Ergasilus tipurus* n. sp. female, from *Calophysus macropterus*, *Sorubim lima* and *Rhaphiodon vulpinus*; (1) whole specimen; (2) antennule; (3) antenna; (4) egg sac. Scales: 1 = 300 µm; 2 = 50 µm; 3 = 100 µm; 4 = 100 µm

Table 1 Measurements in micrometres (μm) of 14 adult females of *Ergasilus tipurus* n. sp.

Structure	*R. vulpinus*		*C. macropterus* and *S. lima*	
	Length	Width	Length	Width
Body	708–900 (809)	361–420 (391)	640–694 (659)	232–290 (259)
Cephalothorax	416–477 (445)	361–420 (391)	323–372 (350)	232–290 (259)
Free thoracic somites				
I	39–60 (44)	210–309 (255)	54–62 (58)	162–204 (183)
II	44–70 (53)	195–280 (232)	42–60 (53)	132–154 (144)
III	38–62 (49)	164–210 (190)	44–55 (49)	93–108 (99)
IV	45–53 (50)	119–166 (143)	22–29 (26)	63–80 (73)
Abdominal somites				
II (Genital double somites)	70–80 (73)	86–122 (109)	44–59 (54)	74–92 (84)
III	20–30 (24)	74–87 (80)	20–30 (26)	54–66 (60)
IV	20–27 (22)	60–80 (69)	24–24 (24)	46–56 (51)
V (anal)	16–24 (20)	57–66 (61)	12–21 (18)	34–47 (42)
VI (Caudal rami)	19–24 (20)	20–20 (20)	16–20 (18)	18–20 (18)
Caudal setae	156–228 (186)		204–212 (208)	
Egg sac	414–584 (527)	140–210 (176)	367–327 (350)	73–77 (75)

Collected from *Rhaphiodon vulpinus* (9 individuals), *Calophysus macropterus* (3 individuals) and *Sorubim lima* (2 individuals)

Table 2 Measurements in micrometres (μm) of the antennule and antenna of 14 adult females of *Ergasilus tipurus* n. sp.

Structure	*R. vulpinus*		*C. macropterus* and *S. lima*	
	Length	Width	Length	Width
Antennule	127–140 (133)	35–54 (45)	88–97 (93)	26–34 (30)
Antenna segment				
1	80–96 (87)	66–89 (76)	56–66 (62)	53–60 (57)
2	210–238 (224)	75–91 (84)	170–186 (180)	36–42 (39)
3	140–168 (156)	40–51 (45)	123–138 (132)	28–32 (31)
Claw	112–132 (124)	23–40 (30)	68–76 (73)	17–20 (19)

Collected from *Rhaphiodon vulpinus* (9 individuals), *Calophysus macropterus* (3 individuals) and *Sorubim lima* (2 individuals)

Table 3 Spine and setal formula of swimming legs of *Ergasilus tipurus* n. sp.

	Exopod	Endopod
Leg I	I—0, I—1, II—5	0—1, 0—1, II—4
Leg II	I—0, 0—1, 0—6	0—1, 0—2, I—4
Leg III	I—0, 0—1, 0—6	0—1, 0—2, I—4
Leg IV	I—0, 0—5	0—1, 0—2, I—3
Leg V	0—5	

Roman numbers = spines; Arabic numbers = setae

Results

Order Cyclopoida Burmeister, 1834.

Family Ergasilidae Burmeister, 1835.

Ergasilus tipurus n. sp. (Figs. 1, 12).

Diagnosis (based on 14 females studied and measured; measurements in Tables 1 and 2). Cephalosome subtriangular with antennary area slightly projecting anteriorly. Head with first thoracic somite incorporated into cephalosome; fusion with second somite marked by suture. Thorax (Fig. 1) with five free somites, first being as long as cephalosome, and others, progressively decreasing in size. Each sternite provided with a series of small spines. Genital somite subrectangular (Fig. 6).

Abdomen with three somites. Caudal rami with long and terminal seta, two short lateral setae, and small ventral spines.

Antennule (Fig. 2) with six similar segments, with 24 simple setae; setal formula: 3:7:3:4:2:5.

Antenna (Fig. 3) with four segments: first being subtriangular, without spines; second most robust, slightly curved and with sensillum in median internal region; third presenting parallel margins with two median sensilla (one proximal and other distal), and fourth (claw) curved and having single pore at median margin, near distal end.

Mouth parts (Fig. 5): mandible two segmented; basal segment subrectangular, armed with subterminal blade with bristles and finely pectinated palp, terminal segment having teeth at posterior distal margin. Maxillule, subrectangular with two short setae, basal segment presenting connection between maxilla and mandible; terminal segment curved with spines on anterior margin.

Legs (Figs. 9–12). Spine and setal formula of swimming legs are presented in Table 3. Legs I–IV biramous, with rami

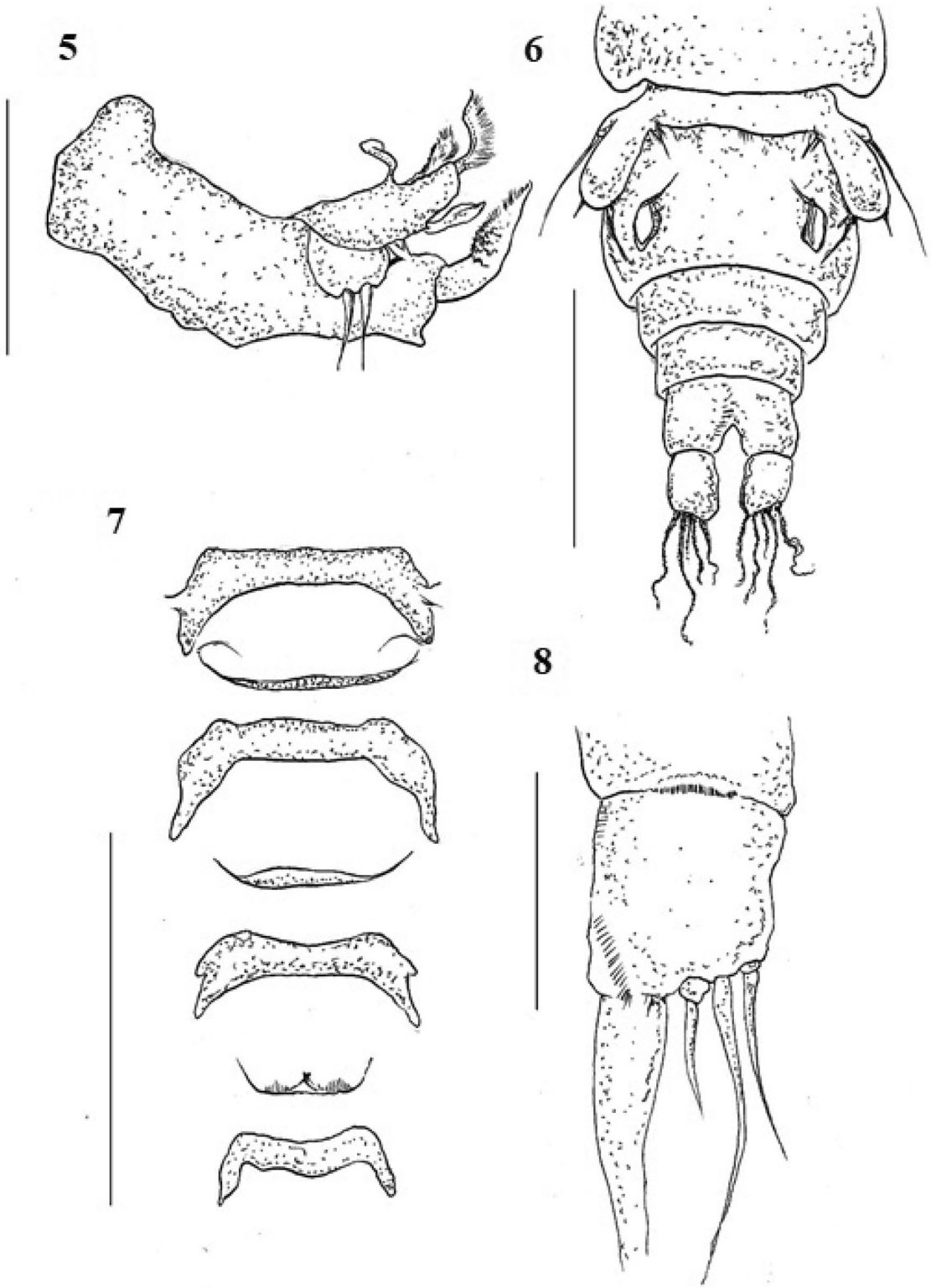

Fig. 5–8 *Ergasilus tipurus* n. sp. female, from *Calophysus macropterus*, *Sorubim lima* and *Rhaphiodon vulpinus*; (5) mouth parts; (6) abdomen and caudal rami, ventral view; (7) sternite, ventral view; (8) Uropod. Scales: 5 = 50 µm; 6 = 100 µm; 7 = 100 µm; 8 = 50 µm

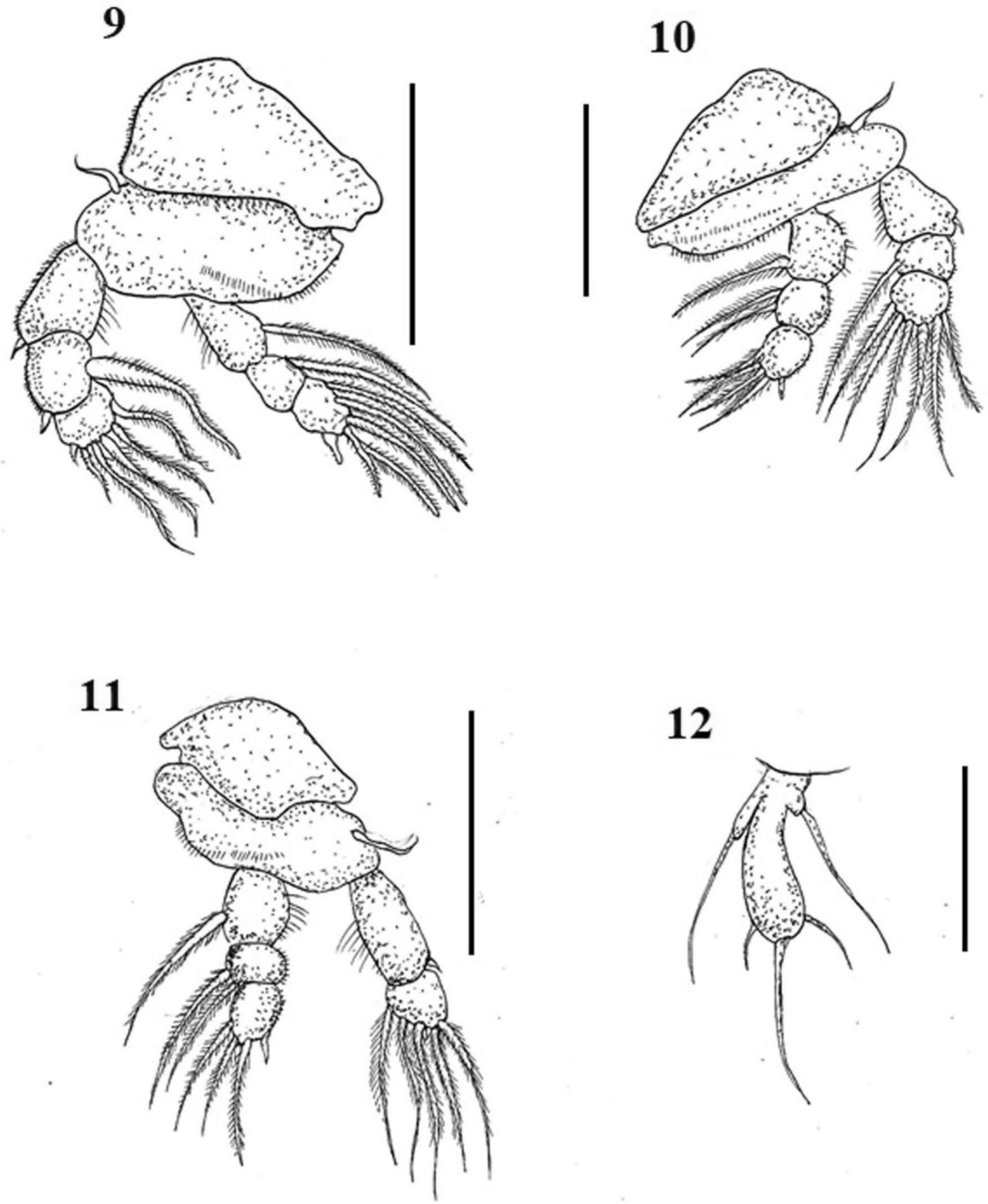

Fig. 9–12 *Ergasilus tipurus* n. sp. female, from; *Calophysus macropterus*, *Sorubim lima* and *Rhaphiodon vulpinus* (9) Leg I; (10) Leg II and III; (11) Leg IV; (12) Leg V. Scales: 9–11 = 50 µm; 12 = 20 µm

three-segmented, except for fourth, two-segmented, exopod. Leg I (Fig. 9) with coxa provided with series of small spines on outer margin and others on inner distal region; bases with simple external seta and series of small spines on inner distal margin, reaching base of endopod. First endopodal segment with series of small spines and bristles on outer margin and plumose inner seta; second with series of spines on outer margin and with plumose inner seta; third with small external spines, two strong distal spines and four plumose setae. First exopodal segment with two series of small spines on outer margin, terminal spine, and bristles on inner margin; second with two series of spines on outer margin, distal

spine and plumose seta on inner margin; third with two series of small spines on outer margin, two terminal spines and five plumose setae, having most distal an external pectinated face.

Legs II and III similar to Leg I (Fig. 10). Coxa with small spines on outer margin; base with simple external seta and two series of small spines on inner distal margin reaching base of endopod. First endopodal segment with small spines and bristles on outer margin and plumose seta; second segment with series of small spines on outer margin and two plumose inner setae; third segment with series of small spines on outer margin, distal spine, and four plumose setae. First exopodal segment with terminal spine, small external spines and bristles on inner margin; second with series of small spines on outer margin and with plumose seta; third with series of small spines on outer margin, pectinated seta on outer margin with five terminal plumose setae.

Leg IV (Fig. 11) with smooth coxa and base provided with simple seta on outer margin with series of small spines on distal half of inner margin reaching base of endopod. Endopod three-segmented, first segment with small spines and bristles on outer margin and inner plumose seta; second segment with small spines on outer margin with two lateral plumose setae; third segment with small spines on outer margin, terminal spine, and three plumose setae. Exopod two-segmented, first with distal spine, small spines on outer margin, and bristles on inner margin; second segment with series of spines on outer margin and five terminal plumose setae.

Leg V (Fig. 12) constituting of single segment, well developed, with long seta in middle of distal part and two setae on distal end located laterally and two basal papillae, each provided with simple seta.

Egg sac (Fig. 4) with multiple series of elliptical eggs, in number between 28 and 36 eggs.

Taxonomic Summary

Type host: *Calophysus macropterus* (Lichtenstein, 1819).

Another host: *Sorubim lima* (Bloch and Schneider, 1801); *Rhaphiodon vulpinus* Spix and Agassiz, 1829.

Site on host: nostrils and gill filaments.

Type locality: Brazil, State of Rondônia, Guajará-Mirim, near Surpresa, Guaporé River (11°52′S, 64°56′W).

Date of collection: 21 September 1985.

Material examined: holotype female (INPA-CR 2447) from nasal fossae of *Calophysus macropterus*, Brazil, Rondônia State, Guajará-Mirim, near Surpresa, Guaporé River (11°52′S, 64°56′W); two paratype females (INPA-CR 2449a, b) from nasal fossae of *Calophysus macropterus*, Brazil, Rondônia State, Guajará-Mirim, near Surpresa, Mamoré River (11°52′S, 64°56′W); two paratype females (INPA-CR 2450a, b) from nasal fossae of *Sorubim lima* (Bloch and Schneider, 1801), Brazil, Rondônia State, Guajará-Mirim, near Surpresa, Mamoré River (11°52′S, 64°56′W); two paratype females (INPA-CR 2451a, b) from nasal fossae of *Rhaphiodon vulpinus* Spix and Agassiz, 1829, Brazil, Amazonas State, Iranduba, Baixio Lake (03°20′31″S, 60°37′14″W); seven paratype females (INPA-CR 2295a, g) from gill filaments of *Rhaphiodon vulpinus* Spix and Agassiz, 1829, Brazil, Amazonas State, Iranduba, Baixio Lake (03°20′31″S, 60°37′14″W); all on permanent slides. Specimens were deposited in the Crustacea Collection of the Instituto Nacional de Pesquisas da Amazonia, Manaus, Amazonas, Brazil.

Etymology: the specific name is based on the locality of infestation from the first material collected: nasal fossae, in Nheengatu language (Língua Geral): *tipurus*.

Number of analysed fish: five *Calophysus macropterus*; two *Sorubim lima* and 13 *Rhaphiodon vulpinus*.

Prevalence: 40% in *Calophysus macropterus*; 100% in *Sorubim lima*, 8% in the nostrils of *Rhaphiodon vulpinus* and 69% in the gills.

Remarks

Ergasilus tipurus n. sp. is the first species of *Ergasilus* reported from the nasal fossae of a neotropical fish. *Ergasilus myctarothes* [24] was found in the nasal fossae of a shark, *Sphyrna zygaena* (Linnaeus, 1758) from Jamaica [24]; *E. megaceros* [25] was found in the nasal fossae of "*Ictalurus anguilla* Evermann and Kendall, 1898" (see [25], from North America; and *E. rhinos* [6] in the nasal fossae of *Centrarchus macropterus* (Lacepède, 1801), *Lepomis gibbosus* (Linnaeus, 1758) and *L. auritus* (Linnaeus, 1758) from North America [6].

Ergasilus tipurus n. sp. differs from other known neotropical species by presenting a well-developed leg V, which was previously mentioned in *E. xenomelanirisi* [7], collected from the alisphenoid cavity (cavity located near the eye), of *Atherinella brasiliensis* (Quoy and Gaimard, 1825), from São Paulo; *E. bahiensis* Amado and Rocha, 1997, collected from the gills of *Mugil curema* Valenciennes, 1836 from the Paraguasu River, in the State of Bahia; *E. atafonensis* Amado and Rocha, 1997, collected from the gills of *M. curema*, *M. trichodon* Poey, 1876, *M. liza* Valenciennes, 1836, and *M. gaimardianus* Desmarest, 1831, all collected from coastal waters and estuaries of Rio de Janeiro, Rio Grande do Sul, São Paulo, Bahia, Alagoas. Sergipe, Maranhão and Pará; *E. sergipensis* [2], collected from the plankton of some Brazilian rivers; and *E. xinguensis* Taborda, Paschoal and Luque, 2016, collected from the gills of *Geophagus argyrostictus*

Kullander, 1991 and *G. altifrons* Heckel, 1840 from the Brazilian Amazon [1, 2, 7, 17].

E. tipurus n. sp. resembles *Ergasilus arthrosis* mainly by having a well-developed leg V, the number of segments of the first endopod, conformation of the setae and spines in the legs and the presence of sensillum in the second and third segment of the antenna. *E. tipurus* n. sp. differs from *E. arthrosis* in the following aspects: (1) ornamentation of the antennule: *E. arthrosis* presents 34 whereas *E. tipurus* n. sp. 24; (2) structure of the mouth parts: in *E. arthrosis* the mandibular palp is bifurcated, whereas in *E. tipurus* n. sp. is simple; the maxillule has two short setae in *E. arthrosis* and, in the new species, the setae are longer; *E. arthrosis* does not present a binding structure between the maxillule and the mandible, as in *E. tipurus*; (3) the long caudal setae in *E. arthrosis* presents small spines, in *E. tipurus* are smooth.

Ergasilus tipurus n. sp. can be easily separated from *E. xenomelanirisi* in the shape of the body, antennule setal formula, presence of one sensillum in the second segment and two sensilla in the third antenna segments, the presence of spines in the first segment of all exopods and the presence of two basal papillae, each provided with a simple seta in the leg V.

Ergasilus tipurus differs from *E. bahiensis*, by the body shape, number of setae in the antennule which in *E tipurus* is 24 while in *E. bahiensis* is 32. Ornamentation of the maxilla, which in *E. bahiensis* presents three setae of different sizes and the new species presents only two short setae. The ornamentation of the leg IV, which in *E. tipurus* presents five setae in the terminal segment of the exopod, whereas in *E. bahiensis* there are only four. The shape and ornamentation of leg V, which in *E. bahiensis* has four bristles and in the new species has five. The two species can also be differentiated based on the arrangement of spines on the thoracic sternites. In addition, *E. tipurus* is the only species of *Ergasilus* occurring in the nasal fossae and in the gills.

Ergasilus tipurus differs from *E. atafonensis* in the absence of an inflation of the articulation membrane between the segments 1 and 2 of the antennae that occur in *E. atafonensis*. The abdominal somites are long and the caudal rami short with internal setae oriented in a very particular way that is not found in the new species. Another difference can be noticed in the setae of the endopod of leg 1 of *E. atafonensis* that is plumose in the anterior part, and then become spinulous until the extremity, and in the new species the setae of all legs are only plumose.

Ergasilus tipurus differs from *E. sergipensis* in the setal formula, size of the antennae that is robust and short in *E. sergipensis* and in *E. tipurus* n. sp. longer and not so robust, and the presence of a spine in the first segment of the exopod of leg IV in *E. tipurus* n. sp. that is not present in *E. sergipensis*. The main characteristic that can differentiate these two species is the presence of two cuticular expansions ending in two spines that cover leg V and the first segment of the urosome in *E. sergipensis* that is not observed in the new species.

The new species resembles *E. xinguensis* in many aspects and can be only differentiated by some characters: (1) shape of the body; (2) number of setae in the antennule, with 32, whereas the new species presents 24; (3) the second antenna segment in *E. xinguensis* presents a long spine, whereas the new species presents a small sensillum, (4) the leg V in *E. xinguensis* bears four setae, one outer, two distally and one laterally, whereas the new species bears five setae, one distal and two in each basal papillae and two distal setae, located laterally.

Discussion

For *Callophysus macropterus* captured in the Amazon River basin, only *Ergasilus callophysus* Thatcher and Boeger 1984 is reported parasitizing the gills of this fish species (Thatcher and Boeger 1984). For *Sorubim lima* no copepod has been recorded, and for *Rhaphiodon vulpinus,* only *Miracetyma kawa* Malta, 1993 from Rondonia State in Brazil was reported. In the present study, the new species described is reported for the first time in these fish species.

In Brazilian freshwater fishes, ergasilid species from *Brasergasilus* [19]; *Gamidactylu*s Thatcher and Boeger, 1984; *Gamispatulus* Thatcher and Boeger, 1984; *Gamispinus* Thatcher and Boeger, 1984; and *Rhinergasilus* [3] have been reported parasitizing the nasal fossae of their hosts [3, 19–22].

In Amazon floodplain lakes, the ergasilids *Gamidactylus jaraquensis* Thatcher and Boeger, 1984, *Rhinergasilus piranhus* [3]; were recorded infecting the nasal fossae of *Colossoma macropomum* (Cuvier, 1816) and *Serrasalmus altispinis* Merckx, Jégu and Santos, 2000 (see [12, 13], and *Therodamas elongatus* (Thatcher, 1986) was reported in the nasal fossae of *Astronotus crassipinnis* (Heckel, 1840) (see [14].

For *Ergasilus*, all valid and unidentified species collected from the neotropical realm have been only reported infecting the gills of fishes [8, 9] with no reports of occurrence in the nasal fossae. This is the first report of an *Ergasilus* species parasitizing the gills and the nasal fossae of the same host in the neotropical realm.

The presence of *Ergasilus tipurus* n. sp. in three different fish species and in two different sites of infections indicates that both, gills and nasal fossae offer adequate conditions for the parasitism of this species. Furthermore, as this copepod can parasitize species from Siluriformes and Characiformes, new hosts, from other taxonomic orders and families may be also suitable hosts for this parasite.

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