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RESEARCH ARTICLE

Proposal of *Tiddergasilus* gen. nov. (Ergasilidae: Cyclopoida) for *T. iheringi* comb. nov. from the gills of *Hoplias malabaricus* (Erythrinidae: Characiformes) from Brazil

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ABSTRACT. *Ergasilus* von Nordmann, 1832 is the type and the most speciose genus of Ergasilidae, with more than 150 valid species. The first species of this genus, *Ergasilus iheringi* Tidd, 1942, was described from the Neotropical Region, from the gills of *Hoplias malabaricus* (Bloch, 1794). Since then, 35 new species of Ergasilidae have been described from the Neotropics. However, a reanalysis of the available type specimens of *E. iheringi* revealed features that suggest that it represents a lineage independent from *Ergasilus*. Consequently, we propose *Tiddergasilus* **gen. no**v. (Ergasilidae: Cyclopoida) to accommodate *T. iheringi* (Tidd, 1942) **comb. no**v. The species was re-examined and redescribed based on available type specimens. *Tiddergasilus* **gen. no**v. is characterized by species with exopod of leg 4 2-segmented; antenna small and robust, with a short second segment (approximately three times shorter than the first segment), and claw with a unique morphology (short, recurved with a sub-proximal indentation in the inner margin). A phylogenetic analysis, based on a matrix of 15 taxa and 16 morphological characters in PAUP* software 4.0a152, resulted in 15 equally parsimonious trees. Based on this analysis, *Tiddergasilus* is closely related to a group composed of Neotropical species of *Ergasilus* – sharing with them antennule with 6-segments. When the highly homoplastic character "number of segments in the antennule" is removed from the analysis, *Tiddergasilus* forms a polytomy with the clade of genera of the former Vaigamidae, *Ergasilus*, and the clade *Rhinergasilus+Brasergasilus+Pindapixara*.

KEY WORDS. Copepoda, Ergasilidae, taxonomy.

INTRODUCTION

Ergasilus von Nordmann, 1832 is the oldest genus of Ergasilidae, being the type and the most species-rich genus of the family, including more than 150 valid species (Walter and Boxshall 2017) distributed on all continents except Antarctica (Lacerda et al. 2007; Boxshall and Defaye 2008). The Neotropical Region has the richest freshwater fauna of parasitic ergasilids (Boxshall and Defaye 2008). The first representative of Ergasilidae described from the Neotropics was *E. iheringi* Tidd, 1942, a gill parasite of *Hoplias malabaricus* (Bloch, 1794). Since then, 35 new species of Ergasilidae have been described from the Neotropical region.

Reanalysis of the available type specimens of *E. iheringi* revealed that the species belongs to an undescribed genus of Ergasilidae. In this study, we redescribe the species, re-evaluate its phylogenetic relationships and propose a new genus, *Tiddergasilus* gen. nov., to accommodate the species originally described by Tidd (1942).

MATERIAL AND METHODS

The specimens were obtained on loan from the United States National Museum, Washington, D.C. (USNM). Nine adult females preserved in ethanol were cleared with lactic acid, mounted on temporary slides, and examined under a BX51 DIC microscope. One specimen was dissected with glass microprobes and mounted in Hoyer's mounting medium (Humason 1979) on a semi-permanent slide. Illustrations were prepared with a drawing tube attached to the microscope. All measurements are

expressed in micrometers. A parsimony analysis was performed with a matrix of 15 taxa and 16 morphological characters using PAUP 4.0a152 (Swofford 2002). The characters used were based on previous phylogenetic analyses (Amado et al. 1995, Tang and Kalman 2008); in addition, new characters were included in the analysis. The included taxa are those genera with species occurring in the freshwater habitats of the Neotropical Region. Species of these genera form a putative monophyletic group, as suggested by the following putative synapomorphy: endopod of leg 1 with two segments. Rooting was based on a functional outgroup determined on preliminary phylogenetic analysis of known genera of Ergasilidae. Three genera were chosen as functional outgroups: *Acusicola*, *Miracetyma,* and *Amplexibranchius*. Species of these genera are characterized by having a latching groove in the third segment for receiving the opposite claw (Thatcher 1984). Clade stability was measured using Bremer's support (Bremer 1994), calculated in PAUP according to Bremer (1994). All character-states were equally weighted, unordered, and non-applicable character states were coded as 'missing'. A heuristic search analysis consisted of 1,000 random stepwise-addition replicates. Character matrix and morphological character states are presented in Tables 1 and 2.

Table 1. Morphological characters used in the analysis of the genera of the Ergasilidae in the Neotropical Region; the number in parentheses indicates the code of each state.

#	Characters
1	Rostral spine absent (0), or present (1)
2	Retrostylets absent (0), or present (1)
3	Antennule 5-segmented (0), or 6-segmented (1)
4	Latching antenna absent (0), or present (1)
5	Second segment of antennae normal (0), reduced (1), or absent (2)
6	Antenna tipped with 2 claws (0), or 1 claw (1)
7	Claw short (0), or elongated (1)
8	Leg 1 endopod 3-segmented (0), or 2-segmented (1)
9	Leg 1 second endopodal segment normal (0), or modified (1)
10	Spines on Leg 1 endopod present (0), or absent (1)
11	Spines on Leg 2 exopod absent (0), or present (1)
12	Spines on Leg 2 endopod present (0), or absent (1)
13	Leg 4 biramous (0), represented by one seta (1), or absent (2)
14	Leg 4 exopod 2-segmented (0), or 1-segmented (1)
15	Leg 4 endopod 3-segmented (0), or 2-segmented (1)
16	Egg sac with multiple rows (0), or composed of single row (1)

TAXONOMY

Ergasilidae Burmeister, 1835

Tiddergasilus gen. nov.

http://zoobank.org/D0DA8A5E-4434-49F2-B2FD-D31E04AAAAC3

Diagnosis. Adult female body comprising prosome and urosome; cephalosome well defined; antenna prehensile presenting coxobasis, two segments, hooked claw; antennule 6-segmented; mandible presenting two blades (median and posterior), basis of maxilla partially toothed on convex margin; four pairs of legs; leg 1 exopod 3-segmented, endopod 2-segmented; legs 2 and 3 differ in number of setae on endopod; legs 2 and 3 exopod and endopod 3-segmented; leg 4 exopod 2-segmented, endopod 2-segmented; abdominal somites with spinules ventrally; caudal rami armed with 4 setae, egg sac long with 2-3 rows of eggs.

Type species. *Tiddergasilus iheringi* (Tidd, 1942) comb. nov.

Etymology. The generic epithet is in honor of Wilbur M. Tidd, author of *Ergasilus iheringi*.

Table 2. Character matrix for 16 morphological characters used in the analysis of the genera of the Ergasilidae in Neotropical Region.

Taxon	Character															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acusicola	0	0	1	1	0	1	0	1	0	0	1	0	0	0	0	0
Amplexibranchius	0	0	1	1	0	1	0	1	1	1	0	0	0	0	0	0
Brasergasilus	0	0	0	0	2	1	1	1	0	0	1	0	1	-	-	1
Ergasilus	0	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0
Gamidactylus	0	1	1	0	0	0	0	1	0	0	1	1	0	1	1	1
Gamispatulus	1	1	1	0	0	0	0	1	0	0	1	1	0	1	1	1
Gamispinus	0	1	1	0	0	1	0	1	0	0	1	0	0	1	1	1
Gauchergasilus	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0
Miracetyma	0	0	1	1	0	1	0	1	1	1	0	0	0	0	0	0
Pindapixara	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1
Prehendorastrus	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0
Pseudovaigamus	1	1	1	0	0	1	0	1	0	0	1	1	0	0	0	1
Rhinergasilus	0	0	1	0	1	1	1	1	0	1	1	0	1	-	-	1
Tiddergasilus gen. nov.	0	0	0	0	1	1	0	1	0	0	1	0	0	0	1	0
Vaigamus	1	1	0	0	0	1	0	1	0	0	1	0	0	1	1	1

Tiddergasilus iheringi (Tidd, 1942), comb. nov.

Figs 1–10

Type host. *Hoplias malabaricus* (Bloch, 1974)

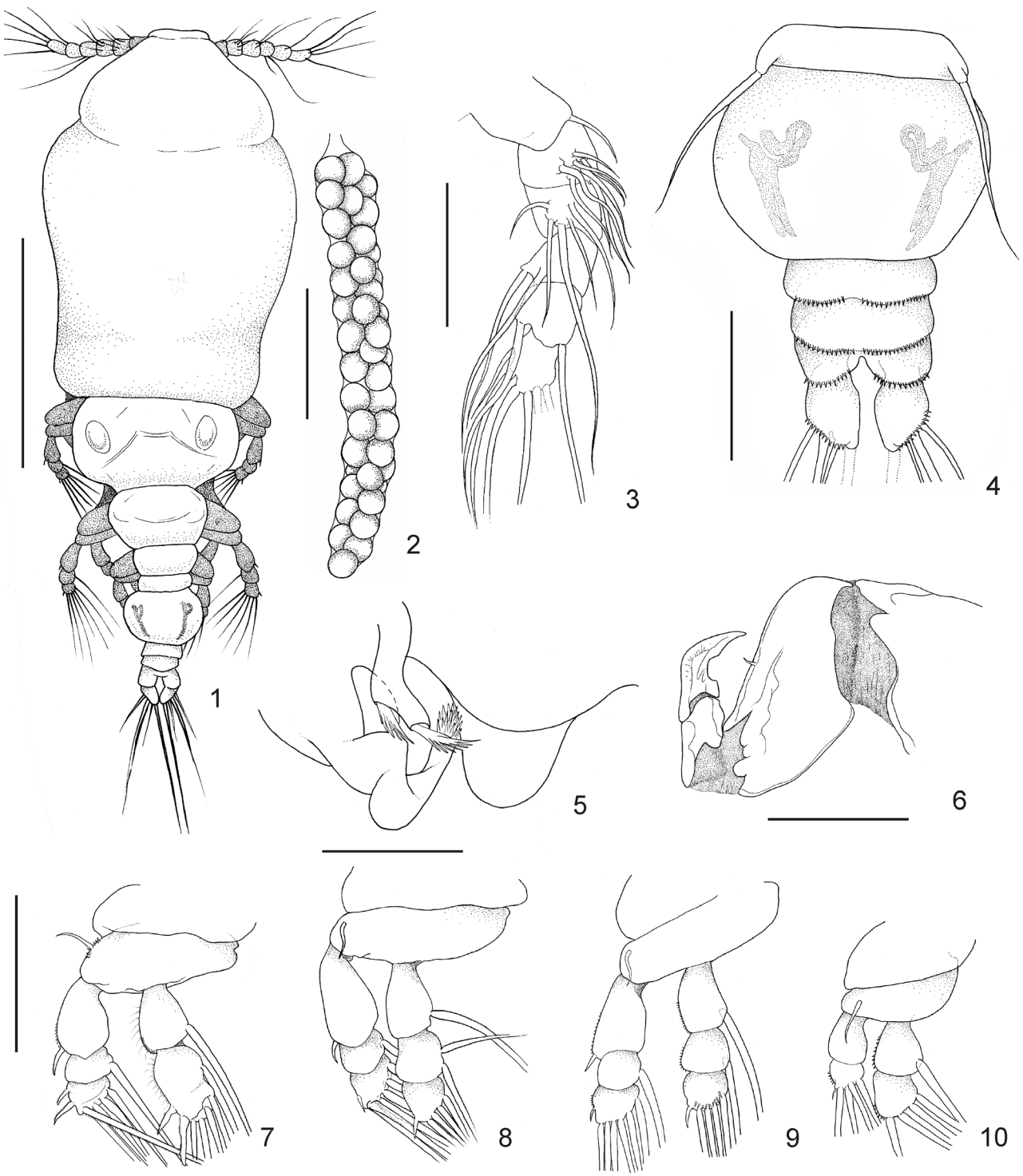
Site on host. Gill filaments.

Type locality. Campina Grande, Paraíba, Brazil.

Male. Unknown

Type specimens. Holotype and Paratype in USNM Catalog #79255.

Redescription (based on 9 females). Body comprising prosome and urosome; prosome consisting of non-inflated cephalosome, rostrum broad and well defined. Cephalosome well defined. Cephalosome and first pedigerous somite partially fused, followed by three free pedigerous somites. Cephalothorax length less than twice width, more than half



Figures 1–10. *Tiddergasilus iheringi* comb. nov. adult female. (1) dorsal view; (2) egg sac; (3) antennule; (4) urosome, caudal ramus; (5) mouth parts; (6) antenna; (7) leg 1; (8) leg 2; (9) leg 3; (10) leg 4. Scale bars: 1–2 = vertical 250 µm, 3–10 = 50 µm.

body length. Antennule 6-segmented, tapering distally, with 23 setae, aesthetascs not observed. Antenna small and robust, with coxobasis, 2-segmented endopod, and terminal claw. Coxobasis short, with small seta at inner distal corner, membrane between coxobasis and first endopodal segment not inflated; first endopodal segment lacking spinules on internal margin, submedian sensillum present, lacking cuticular elevation; second segment (approximately trhee times shorter than first), lacking sensillum; claw with unique morphology (short, recurved with sub-proximal indentation in inner margin). Maxillule not observed; mandible with middle and posterior blades, anterior blade absent, middle blade toothed along posterior edge. Maxilla lacking spinulate seta; basis partially toothed on convex margin. Swimming legs 1–4 biramous each with 2-segmented protopod comprising coxa and basis. Interpodal plates of all legs with two rows of spinules ventrally. The armature of legs (spines, Roman numerals; setae, Arabic numerals) as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	I-0; 0-1; II-4	0-1; II-5
Leg 2	0-0	1-0	I-0; 0-1; I-6	0-1; 0-2; I-4
Leg 3	0-0	1-0	I-0; 0-1; I-6	0-1; 0-1; I-4
Leg 4	0-0	1-0	0-0; I-4	0-1; 0-4

Leg 1. Coxa with smooth margins, lacking spinules; basis with spinules and outer seta. Exopod 2-segmented; first segment non-pilose, with spinules distally on outer margin, and a spinulate distal spine; second segment lacking spinules and distal spine, with single seta; third segment lacking spinules, lacking pectinate seta, with four pilose setae and one spinulate seta, plus two distal spinulate spines. Endopod 2-segmented; first segment with spinules distally on outer margin, pilose, with single seta; second segment with spinules on outer margin, with five setae, plus 2 spinulated spines, one long, one short. **Leg 2.** Coxa lacking spinules; basis lacking spinules, with outer seta. Exopod 3-segmented; first segment non-pilose, with spinules on outer margin and non-spinulate distal spine, lacking seta; second segment non-pilose, lacking spinules, with single seta; third segment with spinules distally on outer margin, with six setae, plus non-spinulated distal spine. Endopod 3-segmented; first segment non-pilose, with spinules on outer margin and with single seta; second segment non-pilose, with spinules on outer margin, with single seta, distal spine absent; third segment with 4 setae and spinules immediately anterior to setae, plus non-spinulated distal spine. **Leg 3.** Coxa lacking spinules; basis lacking spinules, with outer seta. Exopod 3-segmented; first segment non-pilose, with spinules on outer margin and non-spinulate distal spine, lacking seta; second segment non-pilose, lacking spinules, with single seta, distal spine absent; third segment with spinules distally on outer margin, with six setae, plus non-spinulate distal spine. Endopod 3-segmented; first segment non-pilose, with spinules on outer margin and with single seta; second segment non-pilose, with spinules on outer margin, with single seta, distal spine absent; third segment with 4 setae and spinules immediately anterior to setae, plus non-spinulate distal spine. **Leg 4.** Coxa lacking spinules; basis with outer seta, lacking spinules. Exopod 2-segmented; first segment non-pilose, lacking spinules and seta, distal spine absent; second segment with spinules on outer margin, with 4 setae, plus non-spinulate distal spine. Endopod 2-segmented; first segment non-pilose, with spinules on outer margin, with single seta; second segment with spinules distally on outer margin, with 4 setae, distal spine absent. **Leg V** reduced to single seta.

Urosome consisting of fifth somite, genital double-somite, and three free abdominal somites. Genital double-somite barrel-shaped narrowing posteriorly, ventral surface lacking spinules. Abdominal somites with a row of spinules on posteroventral margins; third abdominal somite bipartite. Caudal rami as long as wide, each armed with one long, one medium and two smaller (lateral and ventral) setae, rows of spinules present ventrally, immediately anterior to smaller setae. Egg sac many times longer than wide, composed of 2–3 rows of eggs.

Phylogeny

An initial hypothesis of the evolutionary relationships of Ergasilidae genera was constructed manually using Hennigian Argumentation (Hennig 1966, Wiley 1981). The topology of the resulting cladogram was tested with PAUP* to confirm that it was one of the most-parsimonious trees. This hypothesis is presented in Figure 11 (tree length 28; C.I. = 0.68 and R.I. = 0.76) – it is one of 15 equally parsimonious trees obtained with PAUP*. Bremer Support is presented in gray numbers on each respective node.

Three main clades were observed within the Neotropical Ergasilidae. One is comprised of the three outgroup genera: *Acusicola* Cressey, 1970, *Miracetyma* Malta, 1993 and *Amplexibranchius* Thatcher & Paredes, 1985. Another clade is composed of *Gamidactylus* Thatcher & Boeger, 1984, *Gamispatulus* Thatcher & Boeger, 1984, *Pseudovaigamus* Amado, Ho & Rocha, 1995, *Vaigamus* Thatcher & Robertson, 1984 and *Gamispinus* Thatcher & Boeger, 1984, which represents members of the clade formerly referred to as the Vaigamidae. Although this clade corroborates the results of past morphological analyses (Amado et al. 1995, Tang and Kalman 2008), the relationships between the included genera differ.

The same is true for *Acusicola*, *Amplexibranchius* and *Miracetyma*. As in the previous phylogenies (Amado et al. 1995, Tang and Kalman 2008), those genera are sister taxa, but their relative phylogenetic relationships differ in the present analysis, with the sister-group *Amplexibranchius* and *Miracetyma* supported the following synapomorphies: second endopod segment of leg 1 modified (Character 9), endopod of leg 1 lacking spines, and endopod of leg 2 lacking spines (Characters 10 and 12). Accordingly, this is the most robust clade in the phylogeny, presenting the highest Bremer support.

Tiddergasilus gen. nov. is a putative sister taxon of Neotropical species of *Ergasilus*. The two genera share antennule with 6-segments (Character 3). The hypothetical ancestor of this group of *Ergasilus* and *Tiddergasilus* gen. nov. forms a polytomy

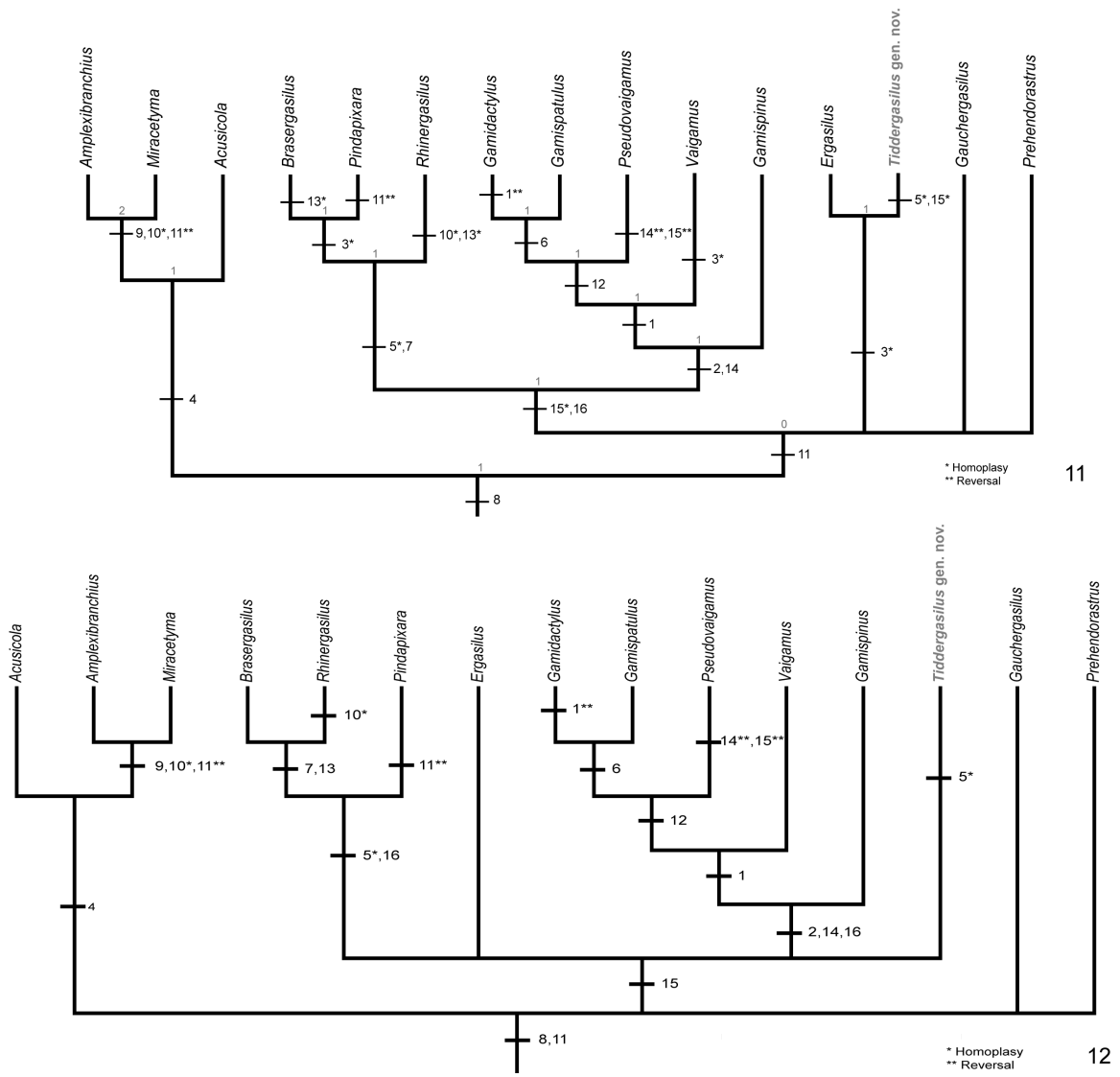


Figure 11–12. (11) Morphological phylogeny of 15 genera of Ergasilidae showing Bremer support values and character changes supporting each node. Bremer support values are indicated in gray numerals; characters that indicate a homoplasy or reversal events are indicated. (12) Morphological phylogeny of 15 genera of Ergasilidae without the Character 3, showing Bremer support values and character changes supporting each node. Bremer support values are indicated in gray numerals; characters that indicate a homoplasy or reversal events are indicated.

with *Gauchergasilus* Montu & Boxshall, 2002 and *Prehendorastrus* Boeger & Thatcher, 1990. Removal of the highly homoplastic character 3 from the analysis results in a polytomy with the clade of genera of the former Vaigamidae, *Ergasilus*, and the clade *Rhinergasilus+Brasergasilus+Pindapixara* (Fig. 12, treelength 24; C.I. = 0.70 and R.I. = 0.76).

Five homoplasious character states were observed: (1) antennule 6-segmented in *Ergasilus* + *Tiddergasilus*, *Vaigamus*, and *Brasergasilus* + *Pindapixara* (Character 3); (2) second segment of antenna reduced in *Brasergasilus* + *Pindapixara* + *Rhinergasilus* and *Tiddergasilus* (Character 5); (3) spines on second endopod absent in *Amplexibranchius* + *Miracetyma* and *Rhinergasilus* (Character 10); (4) leg 4 absent in *Rhinergasilus* and *Brasergasilus* (Character 13). (5) leg 4 endopod 2-segmented in *Brasergasilus* + *Pindapixara* + *Rhinergasilus* + *Gamidactylus* + *Gamispatulus* + *Vaigamus* + *Gamispinus* and *Tiddergasilus* (Character 15).

The analysis also shows four reversals: loss of rostral spine in *Gamidactylus* (Character 1); spines on exopod 2 absent in *Pindapixara* (Character 11); leg 4 with exopod 2-segmented and endopod 3-segmented in *Pseudovaigamus* (Characters 14 and 15).

DISCUSSION

The present redescription, based on the study of type specimens, revealed some features not reported in the original description made by Tidd (1942). These are: (1) number of setae on antennule (16 reported by Tidd, 23 reported here); (2) the presence of a submedian sensillum on the first endopodal segment of the antenna; (2) abdominal somites ornamented with spinules on their posteroventral margins; (4) caudal rami with four setae; (5) basis of all legs armed with outer seta; (6) leg 1 with pectinate seta on distal exopod segment; (7) leg 4 with exopod 2-segmented; distal spine on two exopod; and (8) interpodal plates between legs ornamented with two rows of spinules ventrally.

Based on the phylogenetic analysis, a combination of five features can be considered diagnostic of *Tiddergasilus*: (1) leg 1 endopod 2-segmented; (2) spines on exopod of leg 2 present; (3) antennule 6-segmented; (4) antenna small and robust, with a short second segment (approximately 3 times shorter than the first segment), claw with a unique morphology (short, recurved with a sub-proximal indentation in the inner margin); and (5) endopod of leg 4 2-segmented. Although a putative sister group of the Neotropical species of *Ergasilus*, the single species of *Tiddergasilus* gen. nov., *T. iheringi*, possesses morphological features that can easily distinguish it as a new genus. *Tiddergasilus iheringi* can be distinguished from *Ergasilus* spp. by the morphology of antennae and the well-defined rostrum.

Tiddergasilus gen. nov., like species of many other genera of Ergasilidae, presents exopod of leg 4 2-segmented; and antenna small and robust, with the short second segment, similar to species of *Majalincola* Tang & Kalman, 2008, *Paeonodes* Wilson, 1944, *Teredophilus* Rancurel, 1954, *Therodamas* Kroyer, 1862 and *Urogasilus* Rosim, Boxshall & Ceccarelli, 2012.

A more detailed redescription of certain features (e.g. number of setae on antennule) was hampered by the conditions of the preserved specimens. Some structures such as setae can be lost with time. For instance, the antennulemay presents more setae than those reported herein, and the maxillule could not be observed.

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