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FISH PARASITOLOGY - ORIGINAL PAPER

Integrative taxonomy of a new species of *Therodamas* (Ergasilidae) infecting the Amazonian freshwater fish *Leporinus fasciatus* (Anostomidae)

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Abstract
Crustaceans of the subclass Copepoda are an important component of the invertebrate aquatic fauna. They occur in all aquatic environments and include some representatives that are free-living organisms and others that have a parasitic lifestyle. The genus *Therodamas* comprises marine and freshwater copepods whose females are parasites of fish in their adult phase, with only seven species described so far. During a field survey of fish parasites in the Jari River, a large tributary of the Amazon River system, in Brazil, we found a new species of the genus *Therodamas* infecting *Leporinus fasciatus*. *Therodamas longicollum* n. sp. is the second strictly freshwater species known. Phylogenetic analysis showed that the new species is grouped in the family Ergasilidae, and divergence estimates showed that *T. longicollum* n. sp. diverged from its ancestor at around 66.34 Ma, in the late Upper Cretaceous. *Therodamas longicollum* n. sp. differs from its congeneric in that it does not have lobes and/or expansion of the anterior neck region. Besides describing a new *Therodamas* species, thereby increasing the diversity of the genus to eight species, this study points out the existence of a lineage of these copepods that has adapted to the freshwater environment of the Amazon. This study also corroborates the genus *Therodamas* as part of the family Ergasilidae.

Keywords *Therodamas longicollum* · Copepoda · Fish parasite · Characiformes · Phylogeny · Divergence estimate

Introduction

It has been estimated that Copepoda diverged at around 444 Ma (Eyun 2017; Walter and Boxshall 2020). Today, species of Copepoda account for the largest biomass of all animals on Earth (Ju-Shey 1994; Boxshall and Defaye 2008). These small crustaceans can be found as free-living planktonic, meiobenthic, or deep-sea organisms, or as parasites or in other forms of association with other organisms, in marine, freshwater, and estuarine environments (Ju-Shey 1994; Boxshall and Defaye 2008; Eyun 2017).

Ergasilidae Von Nordmann, 1832, comprise some 264 copepods species divided into 30 genera (Walter and Boxshall 2020). The genus *Therodamas* Krøyer, 1863, was created to accommodate *Therodamas serrani* Krøyer, 1863, and currently comprises seven species: *Therodamas mexicanus* Suárez-Morales et al., 2008; *Therodamas sphyricephalus* Thomsen, 1949; *Therodamas serrani* Krøyer, 1863; *Therodamas frontalis* El-Rashidy and Boxshall 2001; *Therodamas fluviatilis* Paggi, 1979; *Therodamas dawsoni* Cressey, 1972; and *Therodamas elongatus* Thatcher, 1986 (*Therodamas tamarae* Motta Amado & Rocha, 1996) (Krøyer 1863;

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Thomsen 1949; Cressey 1972; Paggi 1976; Thatcher 1986; El-Rashidy and Boxshall 2001; Suárez-Morales et al. 2008).

During a field survey of fish parasites in the Jari River, a large tributary of the Amazon River system, in the eastern Amazon region of Brazil, we found a new cyclopoid species of the genus *Therodamas*, infecting *Leporinus fasciatus* Spix and Agassiz, 1829 (Anostomidae). Here, we present the species description, based on optical and scanning electron microscopy and on molecular data. Phylogenetic and divergence time estimate hypotheses are also provided in relation to the new species.

Material and methods

Host fish collection

In January 2018, 30 specimens of *L. fasciatus* were collected using fishing nets in the lower Jari River, near the Jarilândia district of the municipality of Vitória do Jari, in the Amapá State, Brazil (1° 9′ 4.24″ S 51° 59′ 24.87″ W). The host identification was carried out in accordance with Queiroz et al. (2013).

Parasitological analysis procedures

The *L. fasciatus* specimens were anesthetized in eugenol solution (2-methoxy-4-prop-2-enylphenol; phenol) and euthanized by means of medullary transection. They were then necropsied, and the gill arches were removed and fixed in formalin (5%) for morphological analysis and in ethanol for DNA sequencing. The samples were taken to the Aquaculture and Fishery Laboratory of Embrapa Amapá, Macapá, Amapá State, Brazil.

In the laboratory, the gill arches were examined, and the copepods were removed. Specimens for the morphological study were transferred to 5% potassium hydroxide and placed in an oven at 50 °C for 7–10 min, to facilitate detachment of parasites adhering to the gills and prevent their structural rupture. Recovered parasite specimens were maintained in potassium hydroxide 5% solution and again heated to 50 °C until they had completely clarified. The specimens were then assembled in glass slide using pure glycerin, sealed with paraffin, and examined. For DNA analysis, the copepod specimens removed from the gills fixed in ethanol were used. They were removed through mechanical action, using appropriate forceps.

The terminology used for the body and appendages of the copepods was in accordance with previous studies (El-Rashidy and Boxshall 2001). All measurements were performed under a Carl Zeiss Axio Imager A2 light microscope with differential interference contrast and phase contrast optics equipped with Axio Cam and AxioVision AxioVs 40V4.8.2 software. The average measurements, expressed in micrometers (μm), were followed by the range and the number of samples (*n*) measured. Illustrations were made with the aid of a drawing tube coupled to a microscope. Subsequently, the illustrations were prepared in the CorelDraw 2019 software and processed using the Adobe Photoshop CS6 software.

For scanning electron microscope analysis, copepod specimens fixed in 70% ethanol were transferred to a glutaraldehyde solution (2.5%) in 0.15-M phosphate buffer (pH 7.3) for 24 h, post-fixed in 1% osmium tetroxide solution for 2 h, washed with same buffer for 10 min, and dehydrated in an increasing ethanol sequence. A final drying were then performed using hexamethyldisilazane (HMDS) for 5 min (Bray et al. 1993). The samples were mounted on metal stubs, sputter-coated with gold–palladium, and examined under a Leo 440 Stereoscan microscope at the Federal University of São Paulo (UNIFESP).

The ecological terms of prevalence and average intensity and average abundance were used in accordance with the definitions of Bush et al. (1997). The type specimens were deposited in the Museum of Zoology "Adão José Cardoso" University of Campinas (UNICAMP), São Paulo, Brazil.

DNA extraction and amplification

DNA was extracted from a single ergasilid specimen using the DNeasy® Blood & Tissue Kit, in accordance with the animal tissue protocol (QIAGEN, CA, USA). It was quantified in a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA) at 260 nm. Fragments of the small subunit ribosomal DNA (SSU-rDNA) were amplified using the primers 18SF (5'— AAG GTG TGM CCT ATC AAC T—3') and 18SR (5'—TTA CTT CCT CTA AAC GCT C—3') (Song et al. 2008). PCR runs were conducted in 25-μL reaction volumes comprising 100 ng of DNA, 5 × Go Taq Flexi buffer (Promega, Madison, WI, USA), 10 mmol of dNTP, 25 mmol of $MgCl_2$, 10 pmol of each primer and 1 × Go Taq G2 Flexi DNA polymerase (Promega, Madison, WI, USA), and ultrapure water.

The PCR cycling was performed in a Nexus Mastercycler® (Eppendorf, Hamburg, Germany). The cycling comprised an initial denaturation step at 94 °C for 5 min followed by 30 denaturation cycles at 94 °C for 30 s, with annealing at 54 °C for 30 s and extension at 72 °C for 1 min, followed by a terminal extension at 72 °C for 5 min (Song et al. 2008). The PCR products were analyzed by means of electrophoresis on 1.0% agarose gel (0.045 M Tris–borate, 0.001 M EDTA, pH 8.0). This was stained with SYBR™ Safe (Thermo Fisher Scientific, MA, USA) and analyzed using a Syngene transilluminator.

The purification was carried out using the QIAquick PCR purification kit (QIAGEN, CA, USA), in accordance

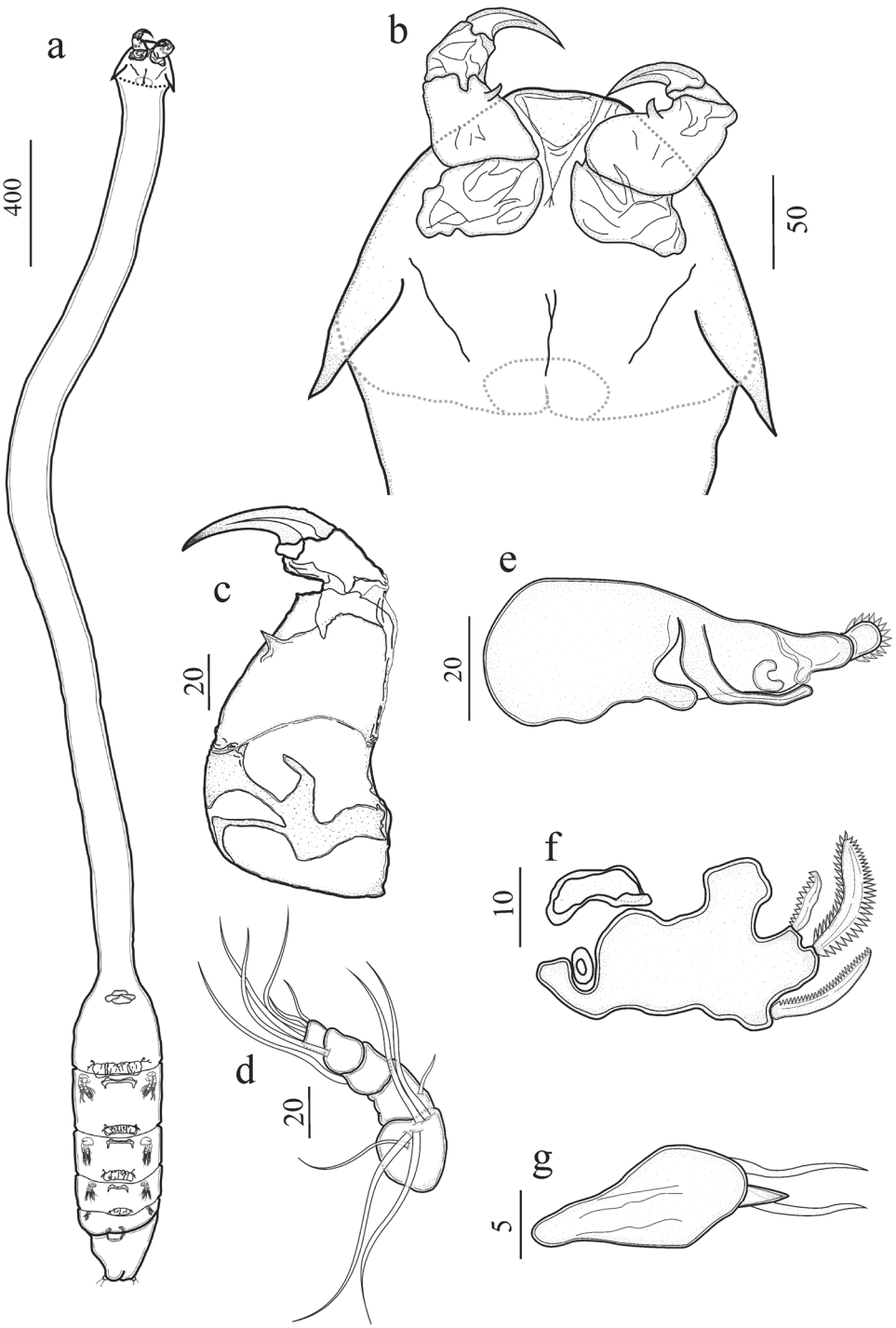


Fig. 1 **a**–**g** *Therodamas longicollum* n. sp. **a** Holotype, whole mount (ventral view). **b** Antennal region of the head (ventral view). **c** Antenna. **d** Antennule. **e** Maxilla. **f** Mandible. **g** Maxillule. Scale bar in micrometer

with the manufacturer's instructions. Direct sequencing was done using PCR primers in both directions using a BigDye 102 Terminator v. 3.1 cycle sequencing kit in an Applied Biosystems ABI 3.500 analyzer (Applied Biosystems, CA, USA). The sequences were assembled and edited using the Geneious 7.1.3 software (Bioinformatics software for sequence data analysis). BLASTn searches (Altschul et al. 1997) were performed against the NCBI nucleotide database, with the aim of determining sequence similarity.

Sequence alignment, phylogenetic analysis, and divergence time estimation

Alignment was performed using the partial SSU-rDNA sequences that were available in GenBank from 42 copepod

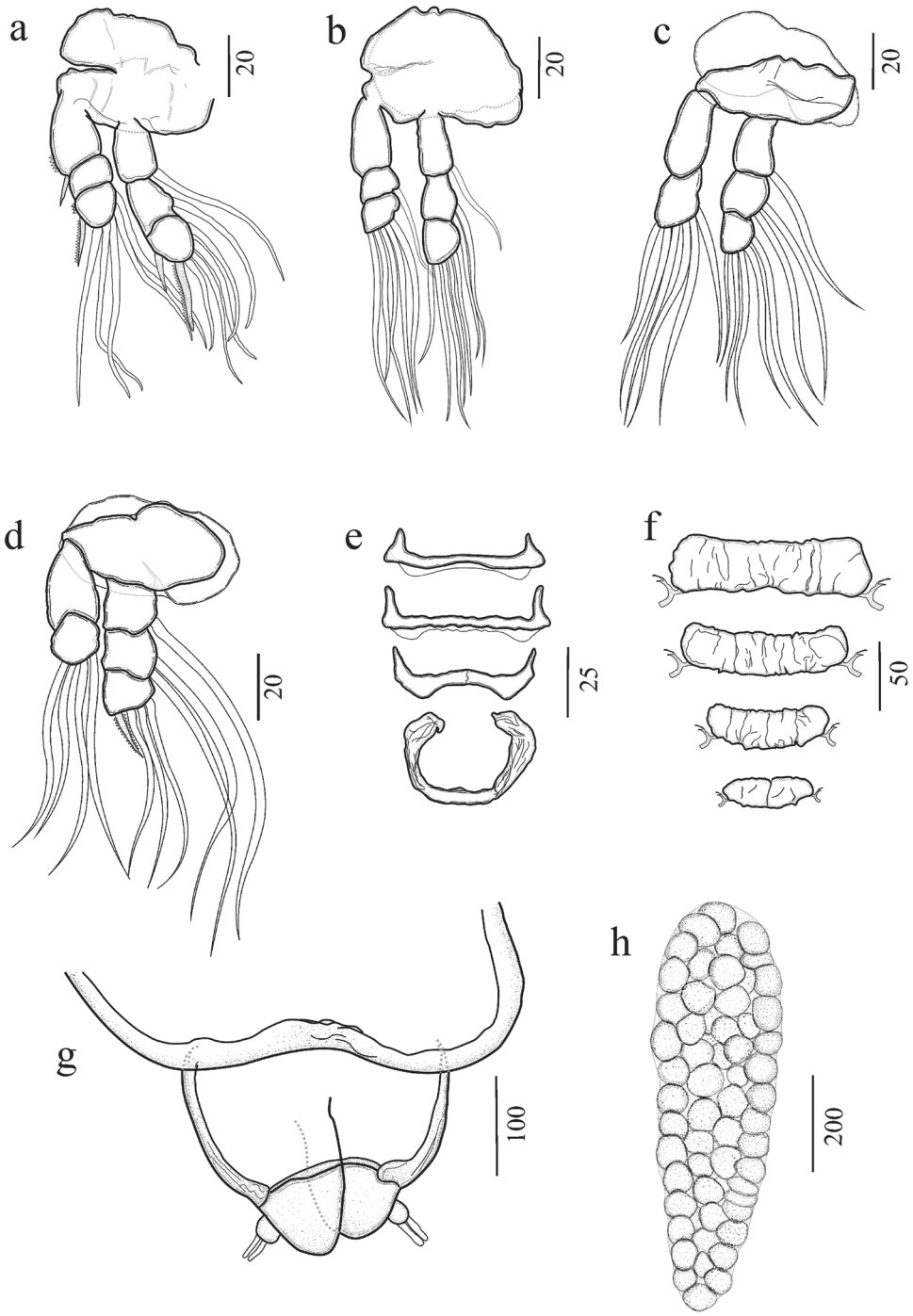


Fig. 2 **a**–**h** *Therodamas longicollum* n. sp. **a** Leg I. **b** Leg II. **c** Leg III. **d** Leg IV. **e** Intercoxal sclerites. **f** Tergites of pedigerous somites. **g** Urosome. **h** Egg sac. Scale bar in micrometer

species of the families Caligidae, Chondracanthidae, Eudactylinidae, Taeniacanthidae, Lernaeidae, and Ergasilidae (which are parasites of fish) and one species of the family Mytilicolidae (which are parasites of bivalves), plus the sequence of *Therodamas* species obtained in the present study. A sequence from *Sebekia purdieae* Riley, Spratt & Winch, 1990 (Sebekidae) was used as an outgroup. The sequences were aligned using the standard parameters of the MUSCLE algorithm (Edgar 2004), implemented in Geneious 7.1.3 (Kearse et al. 2012), and the ends of the alignments were trimmed. To assess occurrences of substitution saturation, the ISS index was estimated using the DAMBE 5 software (Xia 2013). The number of base substitutions per site between the sequences was calculated and standard error estimates were obtained using an initialization procedure with 2,000 replicates.

Bayesian inference (BI) analysis was conducted using MrBayes 3.2 (Ronquist and Huelsenbeck 2003) on the

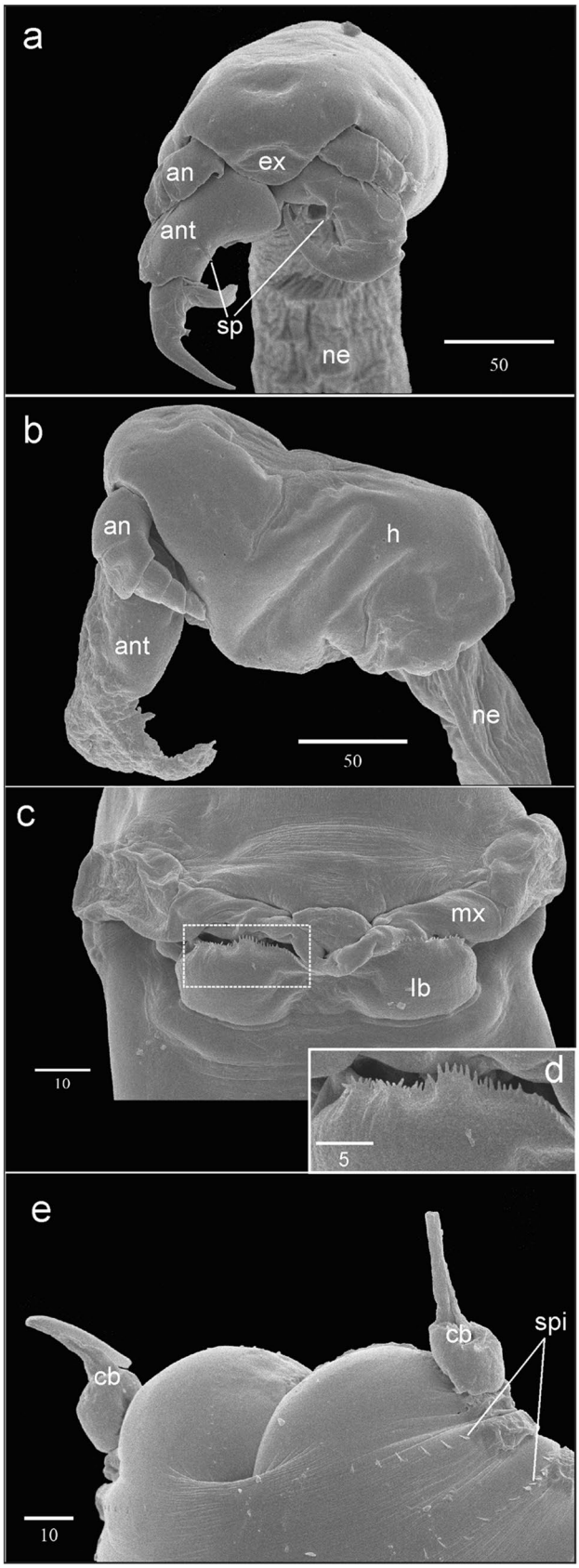


Fig. 3 **a**–**e** Scanning electron microscopy of *Therodamas longicollum* n. sp. **a** Head region (frontal view). **b** Head region (lateral view). **c** Oral region. **d** Labrum. **e** Urosome. Abbreviations: an, antennule; ant, antenna; ex, carapace expansion; sp, spine; ne, neck; h, head; mx, maxilla; lb, labrum; cb, caudal branch; spi, spinules. Scale bar in micrometer

CIPRES platform, applying the model of evolution GTR + I + G obtained by jModelTest analysis under the Bayesian information criterion (BIC) (Posada 2008). Posterior probabilities were estimated from 10 million generations via Markov Chain Monte Carlo (MCMC) algorithms. The first 25% of the generations were defined as burn-ins and were discarded. A consensus tree (majority rules) was estimated using the remaining topologies (Miller et al. 2010).

Divergence times were estimated by means of alignment considering only the SSU-rDNA sequences of the ergasilid species used in the BI analysis. A sequence from *Anthosoma crassum* (Abildgaard, 1794) (Dichelesthiidae) was used as the representative species of the fossil family for calibrating the clock, and *Mytilicola orientalis* Mori, 1935 (Mytilicolidae), was used as an external group. The analysis was performed using the BEAST v2.4.3 software (Bouckaert et al. 2014), with species tree inference method and the relaxed lognormal clock, set to the Yule process option (Drummond et al. 2006). The calibration of the molecular clock was based on the fossil record of *Kabatarina pattersoni* Cressey and Boxshall, 1989 (Dichelesthiidae), in association with calculation of the previous probabilities modified using Bayes' theorem (Bouckaert et al. 2014). Two runs of 100 million chains were carried out and the quality of the convergences was verified using the Tracer 1.7 software (Rambaut et al. 2018). Racing qualities were considered when ESS values above 200,000 were observed. The extracts resulting from the trees were created in Treeannotator 2.3.1 (Bouckaert et al. 2014) with a burn-in of 10% of the total number of trees generated.

All the phylogenetic trees generated in this study were viewed in FigTree version 1.3.1 (Rambaut 2020) and edited in CorelDRAW 2019.

Results

Taxonomic summary

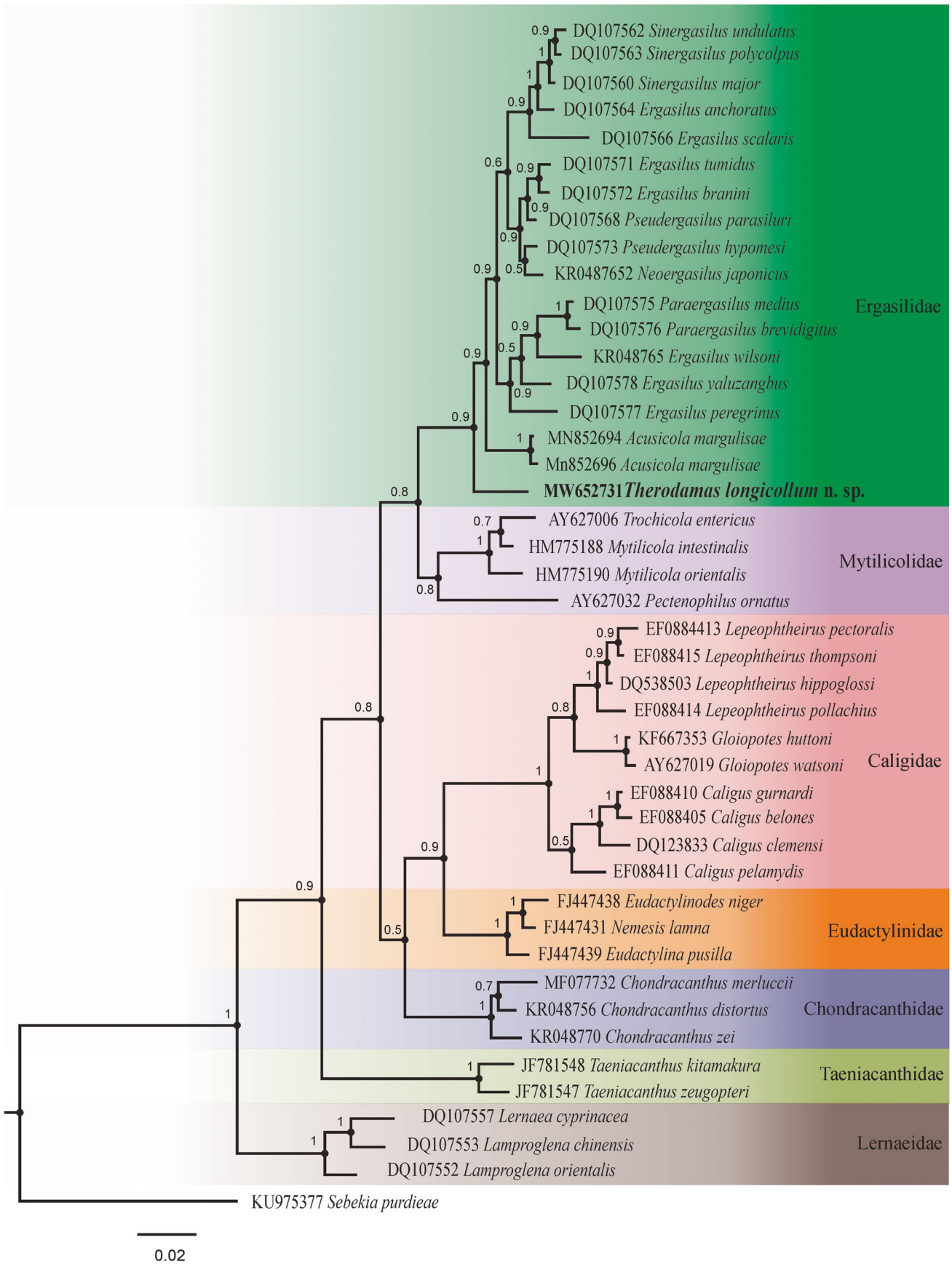
Class Hexanauplia Oakley, Wolfe, Lindgren & Zaharof, 2013

Subclass Copepoda Milne-Edwards, 1840

Order Cyclopoida Burmeister, 1834

Family Ergasilidae Von Nordmann, 1832

Genus Therodamas Krøyer, 1863



◂**Fig. 4** Bayesian tree using SSU-rDNA data for six families of Copepoda fish parasites and one bivalve parasite, applying the model of evolution GTR + I + G determined by jModelTest 0.1. Different colors represent families of parasites. Nodal supports are indicated for BI with posterior probabilities. Values for weakly supported nodes (MI < 70) are not shown

Therodamas longicollum n. sp. (Figs. 1–2).

Description of adult females: Based on 25 adult specimens mounted on pure glycerin. Body divided into four regions: antenna, neck, post-antenna, and trunk (Fig. 1a). Total female length–anterior margin of the prosome to the posterior of the urosome: 5284 (3783–6571; $n = 12$); and 372 (150–550; $n = 22$) in width, in the first somite portion. Head measuring 188 (159–208; $n = 15$) in length and 210 (171–269; $n = 12$) in width, containing inverted U-shaped cephalic shield, with the presence of two spines developed at each end of the posterior margin (Fig. 1b). An expansion of the head carapace toward the frontal region totally covers the bases of the antennules and partially the bases of the antennae (Fig. 3a). A pair of anteroventral antennas, containing four segments, presence of a spine on the inner margin in the second segment, and a strong and curved terminal claw (Figs. 1c, 3a). A pair of antennules with five segments and setal formula: 5, 1, 1, 1, 4 (Figs. 1d, 3b). Long preoral neck measuring 3919 (2450–5175; $n = 13$) in length and 152 (90–260; $n = 23$) in width, of totally cephalic origin separating the antennal and oral regions (Figs. 1a, 3b). Oral complex located at the junction of the neck with trunk and mouth parts typical of Ergasilidae. Maxilla with two segments. The proximal one (synccoxa) is the largest and has three expansions: the first located in the lower position is short and toward the distal region; the second is elongated, originates in the up region, goes down, and turns toward the distal region; and the third is the smallest, has C-shaped, and is positioned distally. The distal segment of the maxilla (basis) contains a small expansion facing the basal position and the distal end is armed with sharp teeth (Fig. 1e). Mandible armed with three blades positioned in the distal region. The one positioned in the median portion is the widest and armed with sharp teeth on both sides; those located in the upper and lower positions are narrower and have teeth only in the upper region (Fig. 1f). Maxillule has a pentagonal shape and distal region armed with a thorn and two arrows (Fig. 1g). Large labrum ornamented with small denticles (Fig. 3c, d). Trunk measuring 1342 (1025–1775; $n = 21$) in length, formed from the post-antennary cephalothorax, with the presence of four evident somites, and defined by the presence of a tergite. Four pairs of legs on the trunk (legs I, II, III, and IV). Leg I contains an endopodite and three-segmented exopod; segments 1 and 3 of the exopod are ornamented with a row of spinules in the external posterolateral region (Fig. 2a). Leg II contains a three-segmented endopod and an exopod; the last endopodal and exopodal segments are ornamented with a row of spinules in the external posterolateral region (Fig. 2b). Legs III and IV contain a three-segmented endopod and a two-segmented exopod (Fig. 2c–d). The setal formula and spines of the legs are shown in Table 1. Four sclerites are distributed along the trunk: the first three are similar in shape and size and the fourth is modified into a U shape (Fig. 2e). Four pedigerous somites and tergites are evident and distributed along the trunk in the dorsal region, all equal in shape and with front ends ornamented with "branch" projections. There are small decreases in size between the 1st, 2nd, 3rd, and 4th tergites (Fig. 2f). The urosome comprising the fifth somite and urosomites is barely visible and is indicated by crossline ornamentation of spinules on the posterior margins. This was only observed using SEM (Fig. 3e). The caudal branch defined at the base and composed of two setae (Figs. 2g, 3e). The egg sac is cylindrical and multiseriate (Fig. 2h).

Type host: Leporinus fasciatus Bloch, 1794.

Prevalence: 36.7%.

Mean intensity: 1.6.

Mean abundance: 0.6.

Site of infection: Gills.

Type locality: Jari River, near the Jarilândia District, municipality of Vitória do Jari, Amapá State, Brazil (1° 9′ 4.24″ S 51° 59′ 24.87″ W).

Specimens deposited: Ten glass slides, each one with a syntype, were deposited in the Museum of Zoology "Adão José Cardoso," University of Campinas (UNICAMP), São

Table 1 Setal formula and spines of the legs of *Therodamas longicollum* n. sp., *Therodamas dawsoni*, and *Therodamas elongatus*

	Therodamas longicollum n. sp.				*Therodamas dawsoni*				*Therodamas elongatus*			
	Coxa	Basis	Endopod	Exopod	Coxa	Basis	Endopod	Exopod	Coxa	Basis	Endopod	Exopod
Leg I	0–0	0–0	0–1, 0–1, II-4	I-0, 0–0, I-5	0–0	0–0	0–1, 0–1, II-4	I-0, 0–0, I-6	0–0	0–0	0–1, II-5	I-0,0–1,0–5
Leg II	0–0	0–0	0–1, 0–2, 0–5	0–0, 0–1, 0–5	0–0	0–0	0–1, 0–1, 0–5	I-0, 0–1, 0–6	0–0	0–0	0–1, 0–2, I-4	I-0,0–1,0–6
Leg III	0–0	0–0	0–1, 0–2, 0–5	0–0, 0–6	0–0	0–0	0–1, 0–2, 0–5	I-0, 0–1, 0–6	0–0	0–0	0–1, 0–2, I-4	I-0,0–1,0–6
Leg IV	0–0	0–0	0–1, 0–2, I-3	0–0, 0–4	0–0	0–0	0–1, 0–2, 0–4	0–0, 0–5	0–0	0–0	0–1, 0–1, I-4	0–0, 0–5

Roman numerals indicate spines; Arabic numerals indicate seta

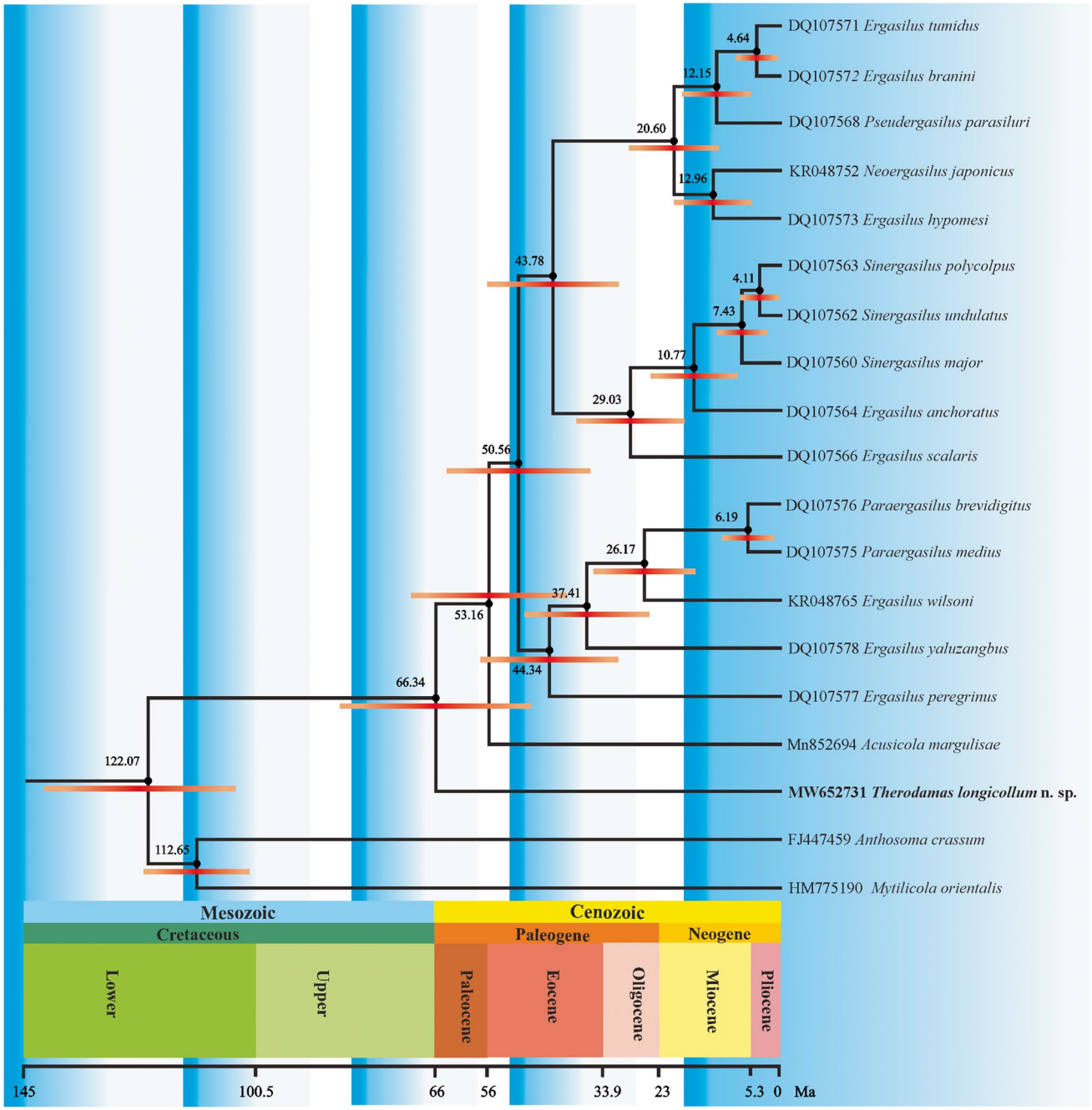


Fig. 5 Estimated divergence time for copepod species. Orange bars in the nodes indicate 95% higher posterior density (HPD) of the posterior Bayesian distribution of molecular time estimates. The geological time scale is in accordance with the International Chronostratigraphic Chart (http://www.stratigraphy.org, v2020/01)

Paulo, Brazil (ZUEC CRU 4371, 4372, 4373, 4374, 4375, 4376, 4377, 4378, 4379 and 4380).

GenBank accession number: partial SSU-rDNA sequences (1,316 bp) were deposited in GenBank under the numbers MW652731.

Etymology: The species name refers to the neck long of the species (Latin, *longi* = long + *collum* = neck).

Remarks: Therodamas longicollum n. sp. differs from *T. mexicanus*, *T. sphyricephalus*, *T. serrani*, *T. frontalis*, *T. fluviatilis*, and *T. dawsoni* through the absence of lobes in the neck and neck proportionally lengthiest than the trunk, and from *T. elongatus* through the lack of expansion of the anterior neck region. The new species, nevertheless, resembles *T. dawsoni* through the presence of an exopodite and a three-segmented endopodite in legs I and II, a three-segmented endopodite in leg III, and

a two-segmented exopodite and a three-segmented endopodite in leg IV. However, they differ through the presence of bristles and thorns in *T. dawsoni* and through the presence of a two-segmented exopodite in leg III of *T. longicollum* n. sp., whereas this is three-segmented in *T. dawsoni* (Table 1). Regarding *T. elongatus*, the resemblances relate to the neck long, three-segmented endopodites of legs II, III, and IV and the two-segmented exopodites of legs I, II, and IV. However, in *T. longicollum* n. sp., the endopodite of leg I is three-segmented and the exopodite of leg III is two-segmented, while in *T. elongatus*, they are respectively two- and three-segmented. The new species also differs from *T. elongatus* through the presence of bristles and thorns on the legs, along with the antenna bristle formula 5, 1, 1, 1, 4 in the new species and 0, 1, 3, 0, 4 in *T. elongatus* (Table 1). It was not possible to compare the morphology of *T. longicollum* n. sp. with that of *T. dawsoni*, since in the latter species, the number of bristles present in each segment of the antennae has not been reported and the drawings alone are insufficient (Cressey 1972).

Molecular and phylogenetic analysis and divergence time estimation

The sequencing of the SSU-rDNA of one specimen *Therodamas longicollum* n. sp. resulted in a partial sequence with 1,316 bp. The BLASTn search did not reveal any identical match between these sequences and any other SSU-rDNA sequence available in GenBank. The phylogenetic analysis showed seven distinct copepod lineages: six infecting fish (Caligidae, Chondracanthidae, Eudactylinidae, Taeniacanthidae, Lernaeidae, and Ergasilidae) and one parasitizing bivalves (Mytilicolidae). *Therodamas longicollum* n. sp. appeared as an early divergent branch within Ergasilidae. Our results also showed that species of the genus *Ergasilus* Von Nordmann, 1832, did not form a monophyletic lineage (Fig. 4). The divergence time estimate suggested that *T. longicollum* n. sp. diverged from its ancestral form at around 66.34 Ma, with a confidence interval of 50.3–87.0 Ma (Fig. 5).

Discussion

Among the seven species of the genus *Therodamas* known until now, *T. dawsoni* was described infecting marine hosts and *T. frontalis*, *T. sphyricephalus*, *T. serrani*, and *T. mexicanus* were found parasitizing host fish that circulated between marine and estuarine waters (Thomsen 1949; Cressey 1972; Araujo and Boxshall 2001; El-Rashidy and Boxshall 2001; Suárez-Morales et al. 2008). *Therodamas fluviatilis* was firstly described infecting freshwater characid fish in Argentina (Paggi 1976), but was subsequently also reported in the brackish water fish *Paralichthys orbignyanus* Valenciennes, 1839, in Southern Brazil (Paggi 1976; Veloso et al. 2005). The Amazonian freshwater copepod *T. elongatus* was reported infecting gills of the sciaenid *Plagioscion squamosissimus* Heckel, 1840 (Thatcher 1986) and respectively the nostrils and gills of the cichlids *Astronotus ocellatus* Agassiz, 1831, and *Astronotus crassipinnis* Heckel, 1840 (Morey et al. 2016). Therefore, *T. longicollum* n. sp. is only the second species of the genus reported strictly in a freshwater environment and, like *T. elongatus*, it occurs in Amazonian fish. The occurrence of other species of *Therodamas* was not observed in *L. fasciatus* or in any other host species in the studied region.

The remarkable feature of the genus *Therodamas* is the presence of a lobe in the head region (Krøyer 1863; Thomsen 1949; Cressey 1972; Paggi 1976; El-Rashidy and Boxshall 2001; Suárez-Morales et al. 2008). However, in *T. elongatus*, the lobe was absent, with only an expansion in the anterior region of the neck (Thatcher 1986; Motta Amado and Rocha 1996). As in *T. elongatus*, the specimens of *T. longicollum* n. sp. also lack the lobe in the cephalic region, and this may be suggestive that this structure was lost in freshwater species.

The BI phylogenetic tree showed the copepod species grouping according to lineages that corresponded to families (Fig. 4). *Therodamas longicollum* n. sp. appeared as an early divergent lineage of the ergasilids, thus corroborating the taxonomic status proposed by Motta Amado et al. (1995), who, using morphological data, placed the genus *Therodamas* in the family Ergasilidae. However, the future availability of sequences from other *Therodamas* spp. will be important for defining the true phylogenetic affinities of the genus.

In agreement with the studies of Song et al. (2008) and Santacruz et al. (2020), our analyses show that the genus *Ergasilus* is not monophyletic. Although Mytilicolidae is known to exclusively infect mollusks, species of its three distinct genera appeared as a sister clade of Ergasilidae, thus corroborating the results of Khodami et al. (2017). Hence, it is plausible to think that an ancestor of Ergasilidae/Mytilicolidae switched host group, thereby originating a lineage that specialized in parasitizing bivalves.

Our estimate of divergence times showed that *T. longicollum* n. sp. diverged from its ancestor at around 66.34 Ma, in the late Upper Cretaceous, a period subsequent to the important marine transgression in northwestern South America (Sempere et al. 1997; Lundberg et al. 1998). Since the majority of the known *Therodamas* species are estuarine (the others comprise a single marine species and two strictly freshwater species), this geological event may have been the basis for the lineage transition to freshwater, in a similar way as seen in relation to manatees, dolphins, stingrays, sciaenid fish, shrimps, crabs, and mollusks (Webb 1995;

Lundberg et al. 1998; Wesselingh et al. 2002; Albert et al. 2006; Lovejoy et al. 2006). This would also include monogenean parasites of fish gills (Boeger and Kritsky 2003) and myxosporeans parasites of fish gallbladder (Zatti et al. 2018). However, it has not yet established evolutionary rates for these organisms, and our analysis was based just on the SSU-rDNA. Thus, future studies considering these aspects, as well as including molecular data on other *Therodamas* species, become indispensable to evaluate the evolutionary processes.

Besides describing a new *Therodamas* species, thus increasing the diversity of the genus to eight species, this study points out the existence of a lineage of these copepods that has adapted to the freshwater environment of the Amazon region. It also corroborates the genus as part of the family Ergasilidae.

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Declarations

Ethics approval The catches access to genetic heritage was authorized by the Brazilian Ministry of the Environment (SISBio no 73550–1 and SisGen no AA4B6BA). This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was conducted under authorization from the Ethics Committee for Animal Use of Embrapa (protocol no 014/2018).

Conflict of interest The authors declare no competing interests.

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