Acusicola margulisae n. sp. (Copepoda: Ergasilidae) from freshwater fishes in a Nicaraguan crater lake based on morphological and molecular evidence

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Abstract The ergasilid copepod *Acusicola margulisae* n. sp. is described based on material from three species of cichlid, *Amphilophus citrinellus* (Günther), *Parachromis managuensis* (Günther), and *Oreochromis* sp., and from the poecilid *Poecilia mexicana* (Steindachner), in the crater Lake Asososca León, Nicaragua. This constitutes the 15th species described in the genus *Acusicola* Cressey, 1970. The new species differs from all its congeners by the relatively longer first endopodal segment of leg 1, and

the size and number of setae on second endopodal segment of leg 1. We provide the first gene sequence for a species of *Acusicola*. To examine the intraspecific genetic variation of the new species collected from different host species, sequences of the mitochondrial barcode region *cox*1 were generated. In addition, partial regions of the 18S and 28S ribosomal RNA genes were sequenced and used to infer the phylogenetic relationships of the genus *Acusicola* within the family Ergasilidae Burmeister, 1835. The phylogenetic trees yielded the isolates of *Acusicola margulisae* n. sp. as a reciprocally monophyletic lineage, and as the sister taxa of five genera of ergasilid copepods. The genus *Ergasilus* von Nordmann, 1832 was recovered as a paraphyletic group. These analyses indicate that phylogenetic relationships are not yet well resolved and more representative species and genera of the family are required to provide a robust classification of this highly diverse group of copepods.

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Introduction

Copepods are the most abundant group of crustaceans, containing mainly free-living organisms, and a few parasitic lineages that infect predominantly fishes (Klompmaker & Boxshall, 2015). Within the entirely parasitic family Ergasilidae only females are adapted to a parasitic life-style. All developmental stages in the life-cycle in both sexes are free-living, and only after fertilization the female infects its host (Boxshall & Defaye, 2008). Species of *Acusicola* Cressey & Collette, 1970 are widely distributed since most of them parasitise coastal euryhaline fishes (da Motta et al., 1995). Some of the species of *Acusicola* have been found in the USA and Central America (Cressey & Collette, 1970; El-Rashidy & Boxshall, 1999), but the largest species richness of the genus is found in the River Amazon basin in South America (Luque & Tavares, 2007; Luque et al., 2013). The genus *Acusicola* includes mainly parasitic species that are considered among the most pathogenic copepods (Kearn, 2005). Exceptionally, few species are free-living, inhabiting dwelling freshwater, brackish and marine environments (Araujo & Boxshall, 2001). The genus contains 14 species, differentiated morphologically by leg setation patterns (Araujo & Boxshall, 2001).

The Pacific coast of Nicaragua holds the largest freshwater lakes in Central America. The two large lakes, Managua and Nicaragua, originated due to tectonic activity less than 1 Mya (Bussing, 1976). This region is also relevant because of the existence of several crater lakes of volcanic origin, formed within the last few thousand years (Waid et al., 1999; Barluenga & Meyer 2004). The crater lakes were seeded by waves of colonisation from populations in the large lakes, followed by rapid diversification and sympatric speciation. Crater lakes are ideal model systems for studying very recent speciation events associated with isolation and local adaptation. The

Crater lake Asososca León is one of these small and isolated lakes, which attracted special interest due to its degree of isolation and relatively impoverished fauna compared to surrounding lakes. It has an estimated age of a few thousand years (Siebert & Simkin, 2002; Elmer et al., 2010), and its fish fauna is potentially derived from the close-by larger Lake Managua, although the time of colonisation of Lake Asososca Leon is still under debate (see Barluenga & Meyer, 2010). The fish fauna of this lake includes two cichlid species, the Midas cichlid *Amphilophus citrinellus* (Günther) and the jaguar guapote *Parachromis managuensis* (Günther), also present in the surrounding lakes, an introduced cichlid, the African tilapia, *Oreochromis* sp. (Günther), and one poecilid, *Poecilia mexicana* (Steindachner) (see Waid et al., 1999; McCrary et al., 2007; Barluenga & Meyer, 2010).

During a survey of the local freshwater fish parasite fauna of the Lake Asososca León in Nicaragua, we collected ectoparasitic copepods from the gills of both native and introduced fish species. Some of these copepod individuals were found to represent an undescribed species of *Acusicola*. Here, we describe the new species based on morphological and molecular data. In addition, molecular data are used to explore the phylogenetic position of the genus *Acusicola* within the family Ergasilidae Burmeister, 1835. We report the first sequence data for a species of this ergasilid genus.

Materials and methods

Specimen collection

During two fieldwork expeditions at the end of the wet season (November-December 2017, 2018) 75 fish were captured in the crater lake Asososca León, Nicaragua: 48 Midas cichlids (*A. citrinellus*); 17 jaguar guapotes (*P. managuensis*); 6 tilapias (*Oreochromis* sp.); and 4 guppies (*Poecilia mexicana*). Fishes were euthanised with an overdose of tricaine methane sulfonate. The gills were then removed and examined under a stereomicroscope to isolate the parasites. Ectoparasites were preserved in individual vials with 100% ethanol for further morphological and molecular analysis.

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Morphological analysis

For morphological characterisation, some specimens were mounted on separate slides, cleared in lactic acid and then examined under an Olympus SZ61 stereomicroscope, and under a Leica DMLB compound microscope. A subset of the specimens was dissected. Drawings were made with the aid of a drawing tube attached to the compound microscope at magnifications of 400× and 1000×. Drawings were then scanned, redrawn using Inkscape 0.91 software, and assembled into figure plates using Gimp 2.8 software. Measurements were taken using an ocular micrometer and are given in micrometres, as the range, followed by the mean in parentheses. For scanning electron microscopy (SEM), some specimens were dehydrated in a series of ethanol and then subjected to critical-point drying with carbon dioxide, sputter-coated with gold, and then examined with a SEM Hitachi Stereoscan Model SU1510 (Hitachi Ltd, Tokyo, Japan). Copepod body and appendage terminology follows El-Rashidy & Boxhall (1999) and Araujo & Boxshall (2001).

Molecular data generation and phylogenetic analyses

DNA was isolated using DNAzol Reagent (Molecular Research Center, Cincinnati, OH, USA) or Speedtools tissue DNA extraction kit (Biotools, Madrid, Spain) according to the manufacturer's instructions. The barcode region of the cytochrome *c* oxidase subunit 1 (*cox*1) gene was amplified using the forward primer 507F (5′-AGT TCT AAT CAT AAR GAT ATY GG-3′; Nadler et al., 2006) and the reverse primer HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′; Folmer et al., 1994). The ribosomal genes 28S and 18S were amplified with the primers designed by Song et al. (2008): 28S rDNA (28SF, 5′-ACA ACT GTG ATG CCC TTA G-3′ and 28SR, 5′-TGG TCC GTG TTT CAA GAC G-3′); 18S rDNA (18SF, 5′-AAG GTG TGM CCT ATC AAC T-3′ and 18SR, 5′-TTA CTT CCT CTA AAC GCT C-3′). The amplification was performed with the following conditions: 94°C for 2 min; 30 cycles of 94°C for 1 min, annealing temperature of 48°C (for *cox*1) or 54°C (for 18S and 28S rDNA), and 72°C for 2 min, with a final extension step at 72 °C for 7 min. The PCR products were purified using ExoSAP-IT (Thermo Scientific, CA, USA) and sequenced in both directions with the BigDye Terminator Cycle Sequencing Ready

Reaction kit (Applied Biosystems, TX, USA). Sequencing was carried out at the Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud (Biology Institute, UNAM, Mexico) or at Macrogen sequencing service (Macrogen Inc., Madrid, Spain). Forward and reverse sequences were assembled using Geneious v7 (Kearse et al., 2012). An alignment was constructed for each molecular marker by adding all sequences available on GenBank (Supplementary Table S1). Each dataset was aligned using Clustal Omega web service (Sievers et al., 2011) and verified in Mesquite v3.10 (Maddison & Maddison, 2016). The model of sequence evolution for each matrix was implemented in the ATGC bioinformatics platform using the Smart Model Selection (SMS) (Lefort et al., 2017), and the AIC criterion of selection. The optimal model of molecular evolution was TN93+G+I for 18S rDNA and GTR+G for 28S rDNA. The mitochondrial data was not used in a phylogenetic reconstruction given the low representation of related homologous sequences in the databases for this molecular marker.

To reconstruct the phylogenetic history of the group, two phylogenetic approaches were used, Maximum Likelihood (ML) and Bayesian inference (BI). The ML analysis were carried out in PhyML 3.0 (Guindon et al., 2010) and nodal support for the tree was assessed thorough bootstrap analysis with 1,000 replicates. The BI analysis was run in MrBayes (Huelsenbeck & Ronquist, 2001) using the CIPRES platform (Miller et al., 2010); the analysis included two simultaneous runs of Markov chain Monte Carlo for 10 million generations, sampling every 500 generations, with a heating parameter value of 0.2 and a "burn-in" of 25%. A 50% majority-rule consensus tree representing the posterior probability distribution of clades was generated. The trees were visualised in FigTree v1.4.4 (Rambaut, 2012). Outgroup species were selected following Song et al. (2008). Based on the rDNA data, the uncorrected p-distance was calculated for comparison among members of the family Ergasilidae, while the mitochondrial dataset was used to assess the levels of intraspecific genetic variation among isolates from different host species. The estimations were performed using the software MEGA7 (Kumar et al., 2016), with a bootstrap procedure based on 10,000 replicates.

Order Cyclopoida Burmeister, 1834
Family Ergasilidae Burmeister, 1835
Genus *Acusicola* Cressey, 1970

***Acusicola margulisae* n. sp.**

Type-host: *Amphilophus citrinellus* (Günther) (Perciformes: Cichlidae), Midas cichlid.

Other hosts: *Parachromis managuensis* (Günther), *Oreochromis* sp. (both Cichlidae) and *Poecilia mexicana* (Steindachner) (Poeciliidae).

Type-locality: Asososca León crater lake (12°25′57.191″N, 86°39′41.687″W), Nicaragua.

Type-material: Colección de Parásitos de Peces del Noroeste del Pacífico at CIAD-Mazatlán, Sinaloa, Mexico (CPPNP): holotype female ex *A. citrinellus* (CPPNP 1375); 6 paratype females ex *P. managuensis* (CPPNP 1376); and 17 paratype females from *Oreochromis* sp. (CPPNP 1377 and 1378). Colección Nacional de Crustáceos, Universidad Nacional Autónoma de México (CNCR): CNCR 35552 (ex *Poecilia* sp.); CNCR 35553 (ex *Oreochromis* sp.); and CNCR 35554 (ex *Amphilophus citrinellus*).

Site on host: Gills.

Representative DNA sequences: MN852694-MN852696 (18S); MN852849-MN852851 (28S); MN854838-MN854870 (*cox*1).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Acusicola margulisae* n. sp. is urn:lsid:zoobank.org:act:F7CC2485-5336-484E-8550-B3746C606C88

Etymology: The name of the species is in honour of the late Lynn Margulis for her contributions in the field of evolutionary biology.

Description (Figs. 1–5)

Adult female. [Based on 10 specimens.] Body slender, cyclopiform (Figs. 1A, B, 4A, B, D). Body length 1,000–1,297 (1,172) from anterior margin of prosome to posterior margin of caudal rami. Prosoma consisting of oblong cephalosome and 4 pedigerous somites gradually tapering posteriorly. Dorsal surface of cephalosome with nauplius eye located near frontal margin, inverted T-shape marking, and sensillae. Area between cephalosome and first pedigerous somite depressed, with posterior margin of cephalosome distinct on lateral view (Figs. 1A, 4A), but indistinct in dorsal view (Fig. 1B). Urosome comprising short fifth pedigerous somite, ventrally and laterally expanded genital double-somite and 3 free abdominal somites. Genital double-somite with patch of tiny spinules on medio-ventral surface and row of spinules along postero-ventral margin (Fig. 1C). Abdominal somites decreasing gradually in size from anterior to posterior, each bearing row of spinules on postero-ventral margin. Caudal ramus about 1.25 times as long as wide (Figs. 1C, 4C), furnished with small patch of tiny spinules on anteroventral surface and 4 caudal setae; innermost seta VI (Huys & Boxshall, 1991) longest.

Antennule (Figs. 1D, 5A, B) 5-segmented. First segment longest. Second to fifth segments gradually tapering distally. Setal formula (s, setae; ae, aesthetascs): 12s: 6s: 4s: 2s+ae: 6s+ae. Antenna (Figs. 2A, 5C–E) 4-segmented, comprising short coxobasis, 3-segmented endopod and terminal claw; first endopodal segment longest, about 6 times as long as wide, with transverse striation in distal part and minute setules along both outer and inner margins; second endopodal segment (Fig. 2B) with basal outer process, medial constriction, and forming 2 inner lobes; third endopodal segment smallest (arrowed in Fig. 2B); terminal claw short, curved and with fossa on inner margin near tip. Mandible consisting of 3 blades each with sharp teeth (Fig. 2C). Maxillule bearing 1 short and 2 long setae (Fig. 2C). Maxilla comprising large, unarmed syncoxa with 1 pore and basis, with dense array of curved spinules distally (Fig. 2C). Maxilliped absent.

Swimming legs 1 to 4 (Figs. 2D, 3A, B) biramous. Spinulate wide intercoxal sclerites present between
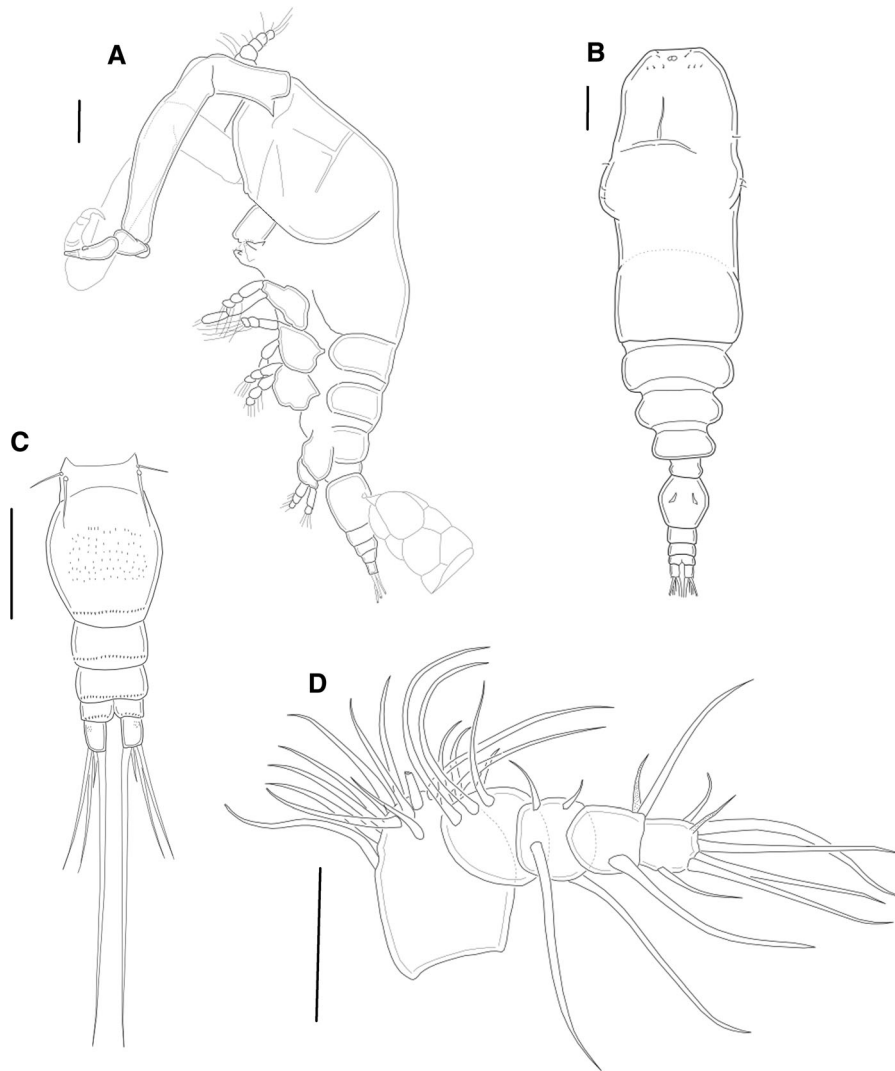
Fig. 1 *Acusicola margulisae* n. sp., holotype female. A, Habitus, lateral view; B, Habitus, dorsal view; C, Urosome, ventral view; D, Antennule. *Scale-bars*: A–C, 100 μm; D, 50 μm

swimming legs (Fig. 3C). Armature on rami as follows (Roman and Arabic numerals indicating spines and setae, respectively).

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

Leg 1 (Figs. 2D, E, 5F, G) coxa unarmed. Basis with single outer plumose seta. Exopod 3-segmented, with rows of spinules on outer margin of all segments; first segment with small outer spine; second segment with inner plumose seta and a small process (arrowed in Fig. 2D) near base of seta; third segment with small spine on outer corner, long apical spine and 5 plumose setae. Endopod (Fig. 2E) 2-segmented, both segments with rows of spinules on outer margin; first segment about 1.3 times as long as exopodal ramus, with plumose inner seta; second segment with 2 apical spines and 5 setae (one of them tiny located on inner
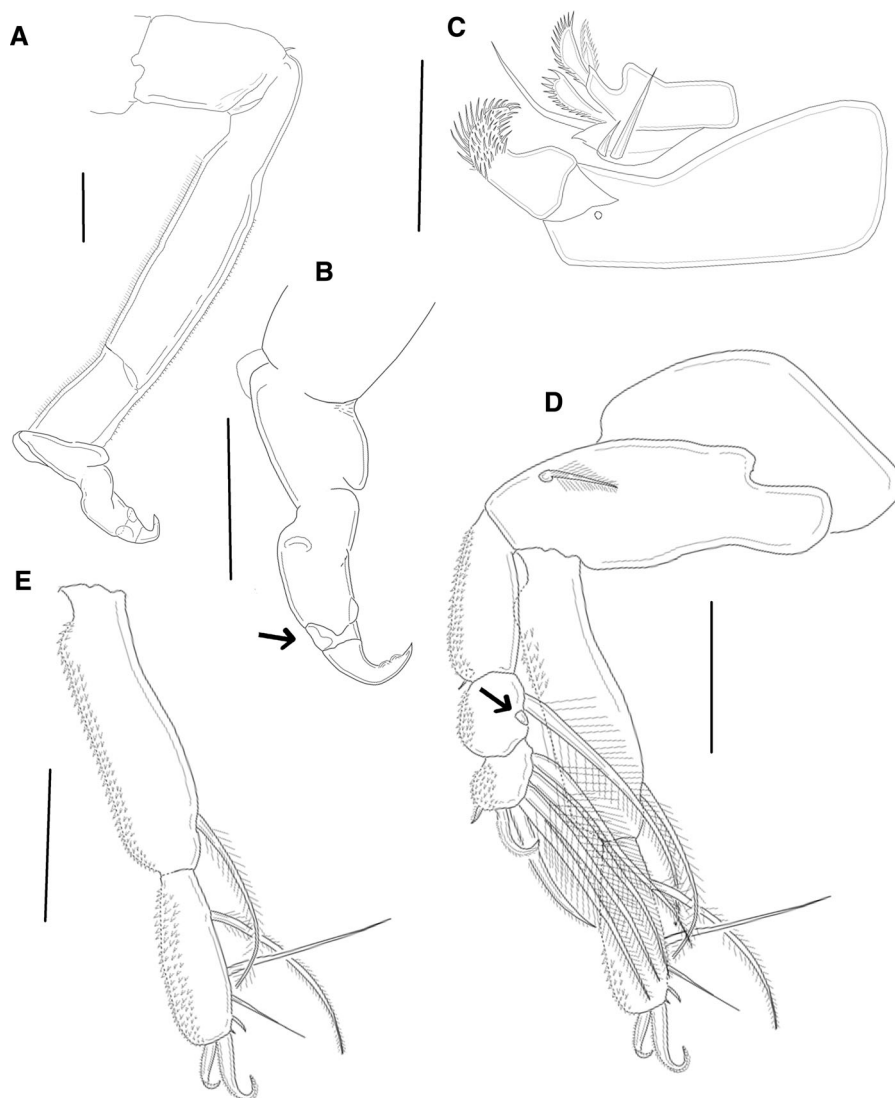
Fig. 2 *Acusicola margulisae* n. sp., holotype female. A, Antenna; B, Distal subchela of antenna, with vestigial third endopodal segment arrowed; C, Mandible, maxillule and maxilla, ventral view; D, Leg 1, anterior view (arrow showing small process at second exopodal segment); E, Leg 1 endopod, anterior view. *Scale-bars*: A, B, 100 μm; C–E, 50 μm

distal corner). Spines of both rami fringed with spinules on outer margin.

Leg 2 short (Fig. 3A) with short outer basipodial seta unarmed. Basis with outer plumose seta. Exopod 3-segmented, with rows of spinules on outer margin of all segments; first segment longest, with outer spine and row of setules on inner margin; second segment with inner plumose seta and small process (arrowed in Fig. 3A) near base of seta; third segment shortest, with minute outer spine and 6 apical plumose setae. Endopod 3-segmented; first segment longest, with

row of setules on outer margin and plumose inner seta; second segment with rows of spinules on outer margin and plumose inner setae; third segment with rows of spinules on outer margin, apical spine fringed with spinules on outer margin and setules on inner margin, and 4 plumose setae.

Leg 3 similar to Leg 2, except for the absence of the minute spine on third exopodal segment.

Leg 4 (Fig. 3B) coxa unarmed. Basis with 1 outer plumose seta. Exopod 2-segmented; first segment longest, unarmed, with row of setules on both outer
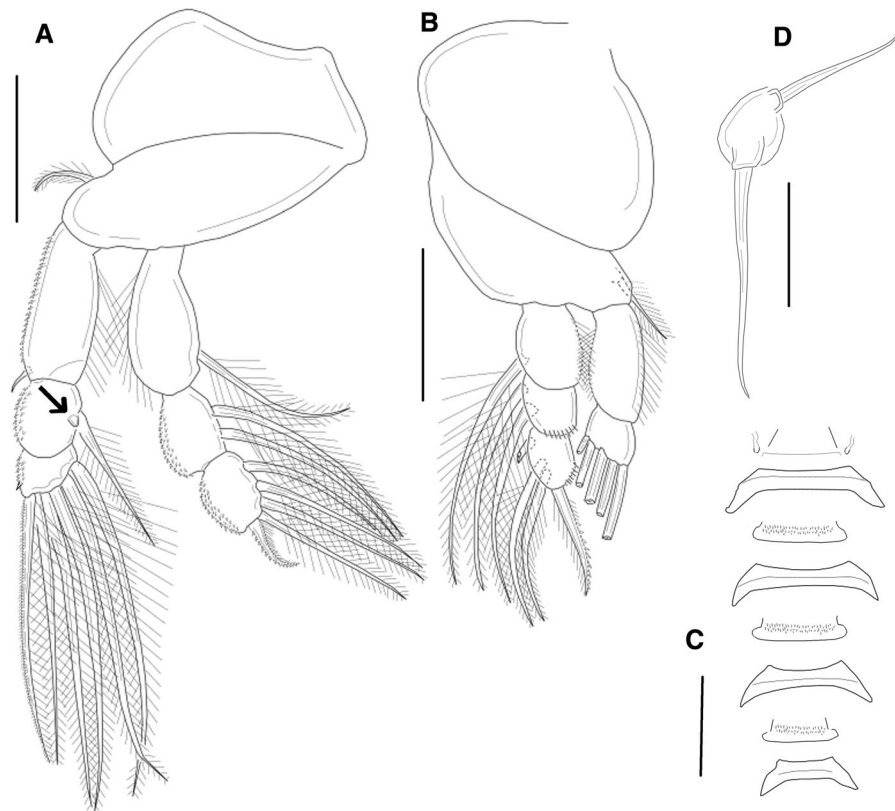
Fig. 3 *Acusicola margulisae* n. sp., holotype female. A, Leg 2, anterior view (arrow showing small process at second exopodal segment); D, Leg 5. *Scale-bars*: A, B, 50 μm; C, 100 μm; D, 25 μm

and inner margins; second segment with 5 long, plumose apical setae (partially drawn in Fig. 3B). Endopod 3-segmented; first segment with row of setules on outer margin and inner plumose seta; second segment with 2 inner plumose setae and row of spinules on distal margin; third segment with row of spinules on distal outer corner, apical spine fringed with spinules on outer margin and setules on inner margin, and 3 plumose setae.

Leg 5 (Fig. 3D) represented by 2 setae; each carried on separate papilla.

Remarks

The new species is distinguished from all known congeners by the relatively longer first endopodal segment of the first leg, being approximately 1.5 longer than second segment, and about 1.3 times as long as exopodal ramus. In the other species of *Acusicola*, the endopodal segments are equally long (e.g. *A. joturicola*, *A. mazatlanesis*, *A. minuta* and *A.*

spinuloderma El-Rashidy & Boxshall, 1999), or the first segment is shorter than the second one (e.g. *A. paracunula* Motta Amado & Rocha, 1996 and *A. spinulosa* Motta Amado & Rocha, 1996). The size of setae on the second endopodal segment of the first leg in *A. margulisae* n. sp. also differs from its congeners, particularly the seta located on the inner distal corner, which is much shorter in the new species than in the other species of *Acusicola*. Another characteristic observed only in *A. margulisae* n. sp. is the small inner process on the second exopodal segment of the legs 1-3.

Further, the depression on dorsal surface, between cephalosome and first pedigerous somite, observed in lateral view in the new species, has not been described for any species of *Acusicola*. *Acusicola margulisae* n. sp. most closely resembles four species of *Acusicola*, i.e. *A. tenax* (Roberts, 1965), *A. brasiliensis* da Motta Amado & Rocha, 1996, *A. minuta* Araujo & Boxshall, 2001, and *A. cunula* Cressey, 1970, in having an antenna with elongate first endopodal segment and a
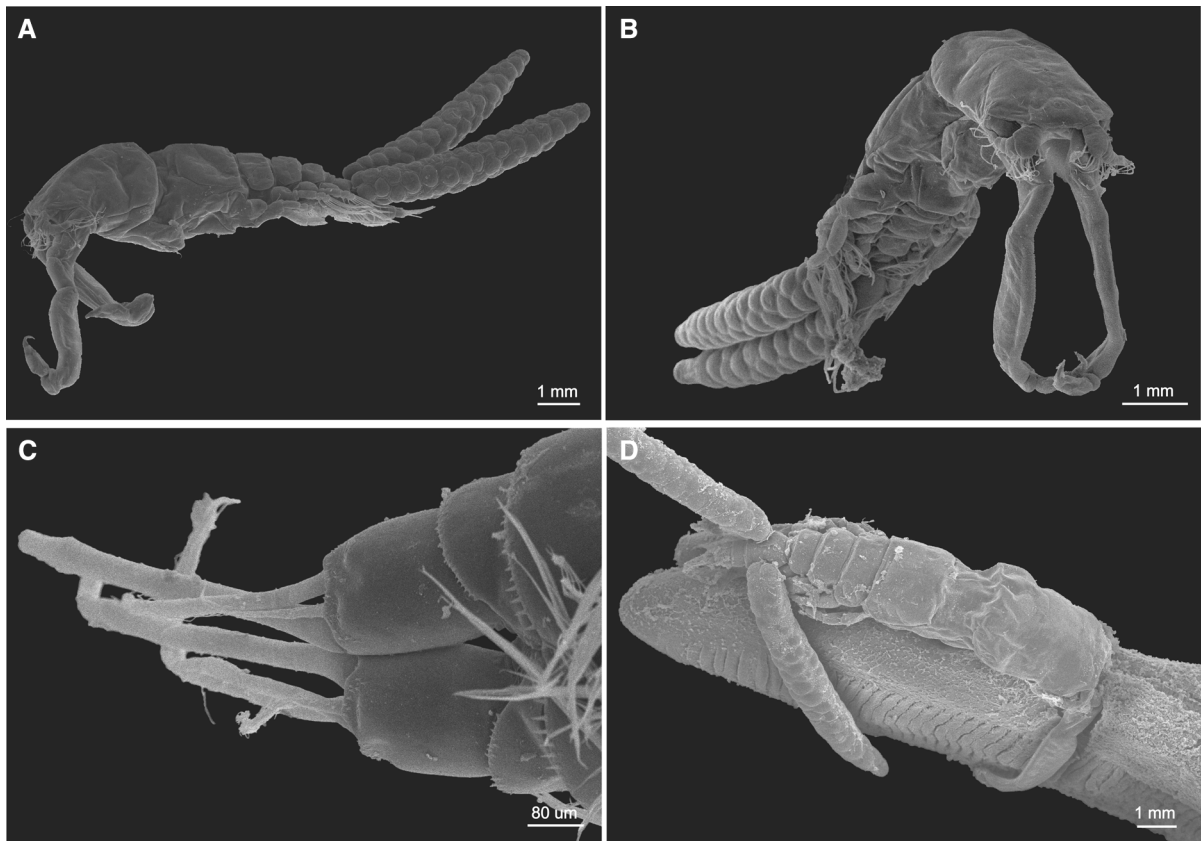
Fig. 4 SEM micrographs of adult female *Acusicola margulisae* n. sp. A, Habitus, lateral view; B, Habitus, ventrolateral view; C, Caudal rami; D, Adult female attached to host gill filament

short distal subchela; a second endopodal segment of first leg with 2 apical spines and 5 inner setae; and the apical spine on third endopodal segment of leg 4 being at least 1.5 times longer than the segment itself (Roberts, 1965; Cressey & Collete, 1970; da Motta Amado & Rocha, 1996; Araujo & Boxshall, 2001). In addition, the indistinct boundary between cephalosome and first pedigerous somite of *A. margulisae* n. sp. is also present in *A. tenax* and in *A. joturicola* El-Rashidy & Boxshall, 1999, *A. lyncengraulidis* Thatcher & Boeger, 1983, *A. mazatlanensis* El-Rashidy & Boxshall, 1999, *A. spinuloderma*, *A. spinulosa* and *A. rotunda* da Motta Amado & Rocha, 1996 (see Roberts, 1965; Thatcher & Boeger, 1983a; da Motta Amado & Rocha, 1996; El-Rashidy & Boxshall, 1999).

Acusicola margulisae n. sp. differs from *A. tenax* and *A. minuta* by having two considerably shorter inner apical setae on the second endopodal segment of leg 1. The new species differs further from *A. tenax* by

having one outer spine on the first exopodal segment of leg 1. In addition, Roberts (1965) described the antennule of *A. tenax* as being 6-segmented; however, this needs to be confirmed by examining the type-material. *Acusicola margulisae* n. sp. differs further from *A. minuta* by the absence of two inner membranous expansions on the second endopodal segment of the antenna.

Molecular analysis

The phylogenetic reconstructions using the two nuclear genes yielded *Acusicola margulisae* n. sp. as a member of the family Ergasilidae. The monophyly of the isolates of the new species was well supported based on the evidence of both nuclear markers (18S, Fig. 6; 28S, Supplementary Figure S1). Overall, ML and BI analysis recovered the same topology, with *Acusicola* as the sister taxon of a group of five genera
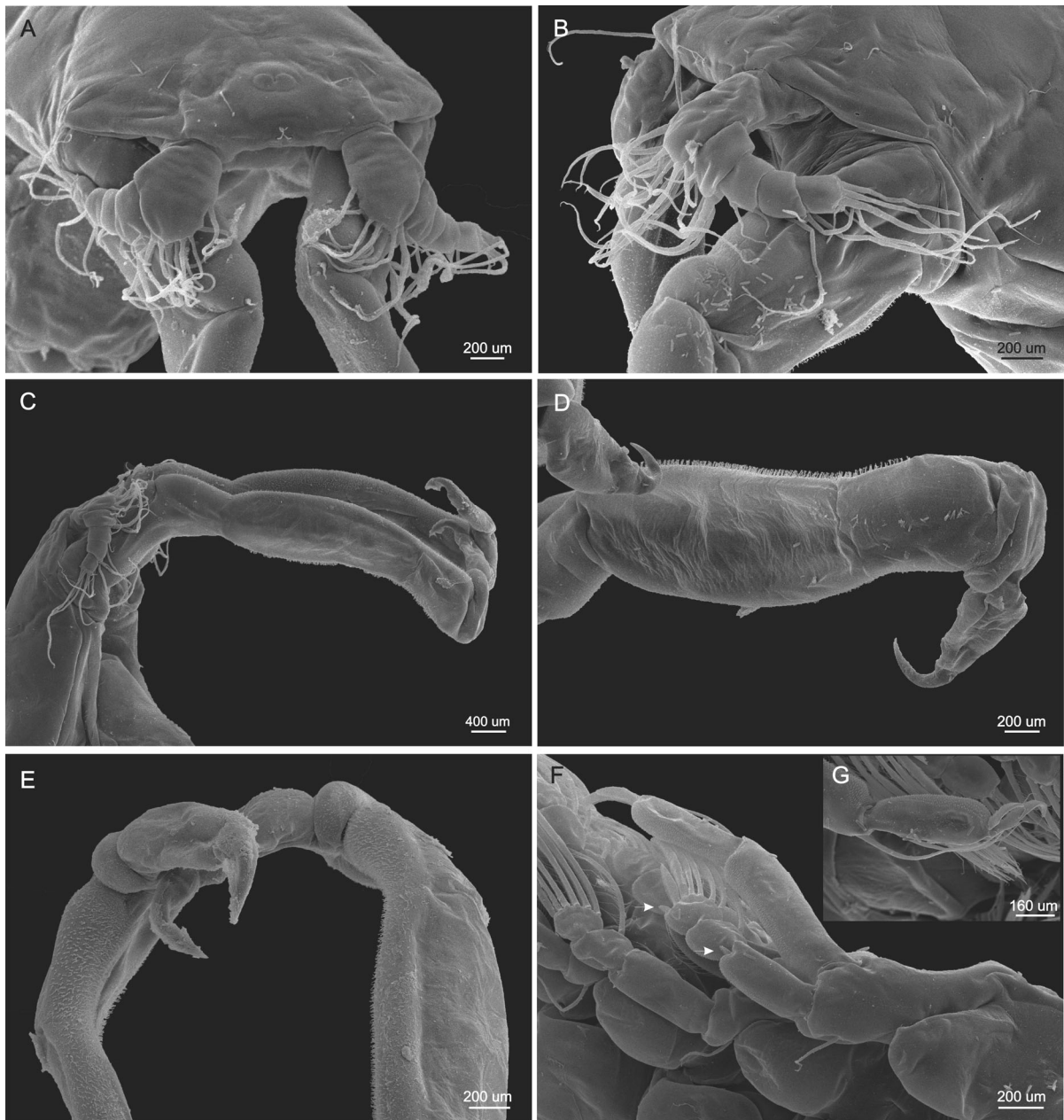
Fig. 5 SEM micrographs of adult female *Acusicola margulisae* n. sp. A, Antennulae, dorsal view; B, Antennule, lateral view; C, D, Antennae, lateral view; E, Antenna, dorsal view; F, Leg 1; G, Distal exopodal segment of Leg 1

of morphologically very similar, i.e. *Ergasilus*, *Pseudergasilus* Yamaguti, 1936, *Paraergasilus* Markewitsch, 1937, *Neoergasilus* Yin, 1956 and *Sinergasilus* Yin, 1942. The genus *Ergasilus* was not recovered as a monophyletic assemblage. The estimated divergence between *A. margulisae* n. sp. and other members of the family Ergasilidae using 18S

rDNA ranged between 2.3–5.1% (Supplementary Table S2), and for 28S rDNA divergence ranged between 10.46–18.04% (Supplementary Table S3). The mean intraspecific sequence divergence among 33 isolates of the new species based on *cox*1 sequences was very low (0.4%) indicating a low difference
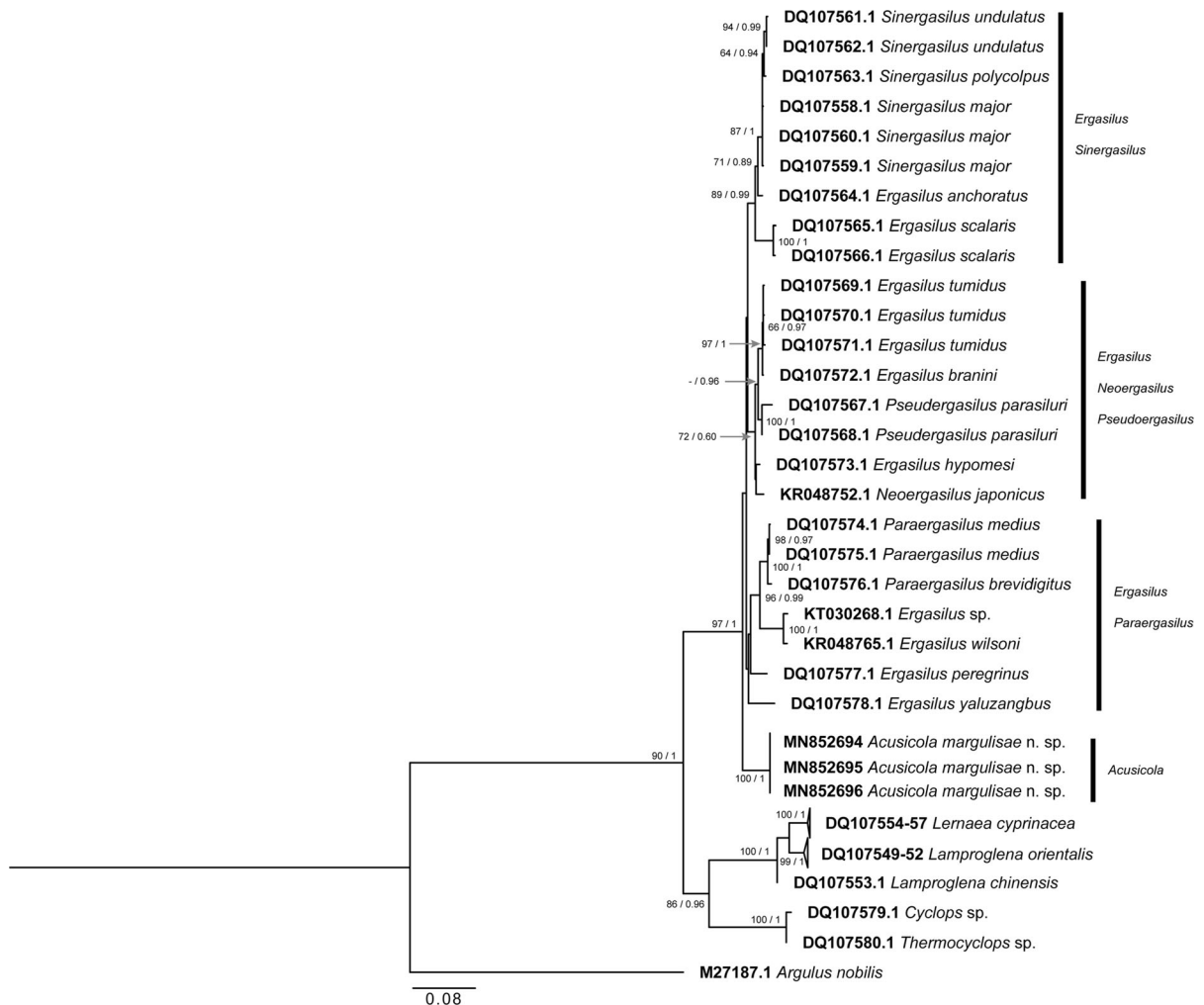
Fig. 6 Maximum likelihood tree for members of the family Ergasilidae, based on 18S rDNA sequences. Bootstrap support and posterior probabilities are displayed at the nodes only if either is over 60%. The scale-bar represents the number of nucleotide substitutions per site

among specimens collected from different host species.

Discussion

The genus *Acusicola* was proposed by Cressey & Collette (1970), with *A. tenax* as the type-species. The genus includes marine and freshwater representatives. Currently, 14 species of *Acusicola* are considered valid, i.e. *A. brasiliensis*, *A. cunula*, *A. joturicola*, *A. lycengraulidis* Thatcher & Boeger, 1983, *A. mazatlanensis*, *A. minuta*, *A. paracunula*, *A. pellonidis* Thatcher & Boeger, 1983, *A. rogeri* Motta Amado &

Rocha, 1996, *A. rotunda*, *A. spinuloderma*, *A. spinulosa*, *A. tenax*, and *A. tucunarense* Thatcher, 1984 (see Walter & Boxshall, 2018); all these species have been reported from a range of freshwater, brackish and marine fish hosts, as well as in plankton samples (Table 1). Species of *Amplexibranchius* Thatcher & Paredes, 1985 and *Acusicola* differ from the other ergasilids considered in the study of da Motta et al. (1996) in the structure of the antennae and legs 1-4.

Acusicola margulisae n. sp. is the second species of the genus reported as an ectoparasite of cichlids and the first in poeciliids; *A. tucunarense* was already reported in cichlids from Brazil (Araujo et al., 2009). Members of the family Ergasilidae exhibit low levels

Table 1 Species of *Acusicola* (Copepoda: Ergasilidae) reported in the literature

Species	Host	Habitat	Reference
A. brasiliensis da Motta Amado & Rocha, 1996	*Atherinella brasiliensis* (Quoy & Gaimard) (Atherinopsidae); *Lile piquitinga* (Schreiner, Miranda & Ribeiro) (Clupeidae)	Brackish and marine	da Motta Amado & da Rocha Falavigna (1996)
A. cunula Cressey, 1970	*Pseudotylosurus angusticeps* (Günther) (Belonidae)	Freshwater	Cressey & Collette (1970)
A. joturicola El-Rashidy & Boxshall, 1999	*Joturus pichardi* Poey (Mugilidae)	Brackish	El-Rashidy & Boxshall (1999)
A. lycengraulidis Thatcher & Boeger, 1983	*Lycengraulis grossidens* (Spix & Agassiz) (Engraulidae)	Freshwater	Thatcher & Boeger (1983a)
A. margulisae n. sp.	*Amphilophus citrinellus* (Günther), *Parachromis managuensis* (Günther), *Oreochromis* sp. (Cichlidae); *Poecilia mexicana* (Steindachner) (Poeciliidae)	Freshwater	This study
A. mazatlanensis El-Rashidy & Boxshall, 1999	*Agonostomus monticola* (Bancroft) (Mugilidae)	Brackish	El-Rashidy & Boxshall (1999)
A. minuta Araujo & Boxshall, 2001	Plankton samples	Brackish	Araujo & Boxshall (2001)
A. paracunula da Motta Amado & Rocha, 1996	*Pellona flavipinnis* (Valenciennes) (Pristigasteridae); *Pseudotylosurus microps* (Günther) (Belonidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
A. pellonidis Thatcher & Boeger, 1983	*Pellona castelnaeana* (Valenciennes) (Pristigasteridae)	Freshwater	Thatcher & Boeger (1983b)
A. rogeri da Motta Amado & Rocha, 1996	*Strongylura marina* (Walbaum) (Belonidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
A. rotunda da Motta Amado & Rocha, 1996	*Lycengraulis batesii* (Günther) (Engraulidae)	Freshwater and brackish	da Motta Amado & da Rocha Falavigna (1996)
A. spinuloderma El-Rashidy & Boxshall, 1999	*Agonostomus monticola* (Bancroft), *Joturus pichardi* Poey (Mugilidae)	Freshwater and brackish	El-Rashidy & Boxshall (1999)
A. spinulosa da Motta Amado & Rocha, 1996	*Lycengraulis batesii* (Günther) (Engraulidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
A. tenax (Roberts, 1965)	*Pomoxis annularis* Rafinesque (Centrarchidae)	Freshwater	Roberts (1965)
A. tucunarense Thatcher, 1984	*Cichla ocellaris* Bloch & Schneider (Cichlidae)	Freshwater	Thatcher (1984)

Note: Host names have been updated according to Froese & Pauly (2019)

of host specificity and this is probably the reason why *Acusicola margulisae* n. sp. infects unrelated hosts. The systematics and classification of the genus *Acusicola* have been scarcely studied. da Motta Amado et al. (1995) conducted a cladistic analysis of the family Ergasilidae based on morphological characters. In their study, *Acusicola* was nested as the sister group of the genera *Amplexibranchius* and *Prehendorastrus* Boeger & Thatcher, 1990. More recently, Song

et al. (2008) assessed the phylogenetic relationships of 14 species allocated in four of the 24 valid genera of the Ergasilidae; using Bayesian inference in the ribosomal gene 18S. In this study, no representative of *Acusicola* was included. These authors recovered *Ergasilus*, the type-genus of the family as paraphyletic, a result corroborated here. Our study provides the first genetic information and ultrastructural data on the morphology for a species of *Acusicola*.

Yet, the phylogenetic relationships and the current classification of the family Ergasilidae still requires a more comprehensive taxon sampling and a detailed study of the morphology using SEM. More sequences of the 18S rRNA gene, and ideally other ribosomal genes such as 28S rRNA, will be required to accomplish a robust classification system for this important group of parasitic copepods.

Compliance with ethical standards

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

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

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