



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

Ana Santacruz · Francisco Neptalí Morales-Serna · Mariana Leal-Cardín · Marta Barluenga · Gerardo Pérez-Ponce de León

Received: 18 September 2019 / Accepted: 11 January 2020 / Published online: 17 February 2020  
© Springer Nature B.V. 2020

**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 poeciliid *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 *cox1* 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.

This article was registered in the *Official Register of Zoological Nomenclature* (ZooBank)

urn:lsid:zoobank.org:pub:AC89566F-F860-4CB3-A7B7-A7FC86922A8F. This article was published as an Online First article on the online publication date shown on this page. The article should be cited by using the doi number. This is the Version of Record.

This article is part of the Topical Collection Arthropoda.

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s11230-020-09906-8>) contains supplementary material, which is available to authorized users.

A. Santacruz · G. Pérez-Ponce de León (✉)  
Instituto de Biología, Universidad Nacional Autónoma de México, Ap. Postal 70-153, C.P. 04510 Mexico City, Mexico  
e-mail: ppdleon@ib.unam.mx

F. N. Morales-Serna  
Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico

A. Santacruz  
Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Mexico City, Mexico

## Introduction

Copepods are the most abundant group of crustaceans, containing mainly free-living organisms, and a few parasitic lineages that infect predominantly fishes (Klompaker & 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 León 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 poeciliid, *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.

F. N. Morales-Serna  
Centro de Investigación en Alimentación y Desarrollo,  
A.C. Unidad Mazatlán en Acuicultura y Manejo  
Ambiental, Av. Sábalo-Cerritos s/n, Mazatlán 82112,  
Sinaloa, Mexico

M. Leal-Cardín · M. Barluenga  
Museo Nacional de Ciencias Naturales, CSIC, José  
Gutiérrez Abascal, 2, 28006 Madrid, Spain

### *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 manufacturers' instructions. The barcode region of the cytochrome *c* oxidase subunit 1 (*cox1*) 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 *cox1*) 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 (*cox1*).

*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.

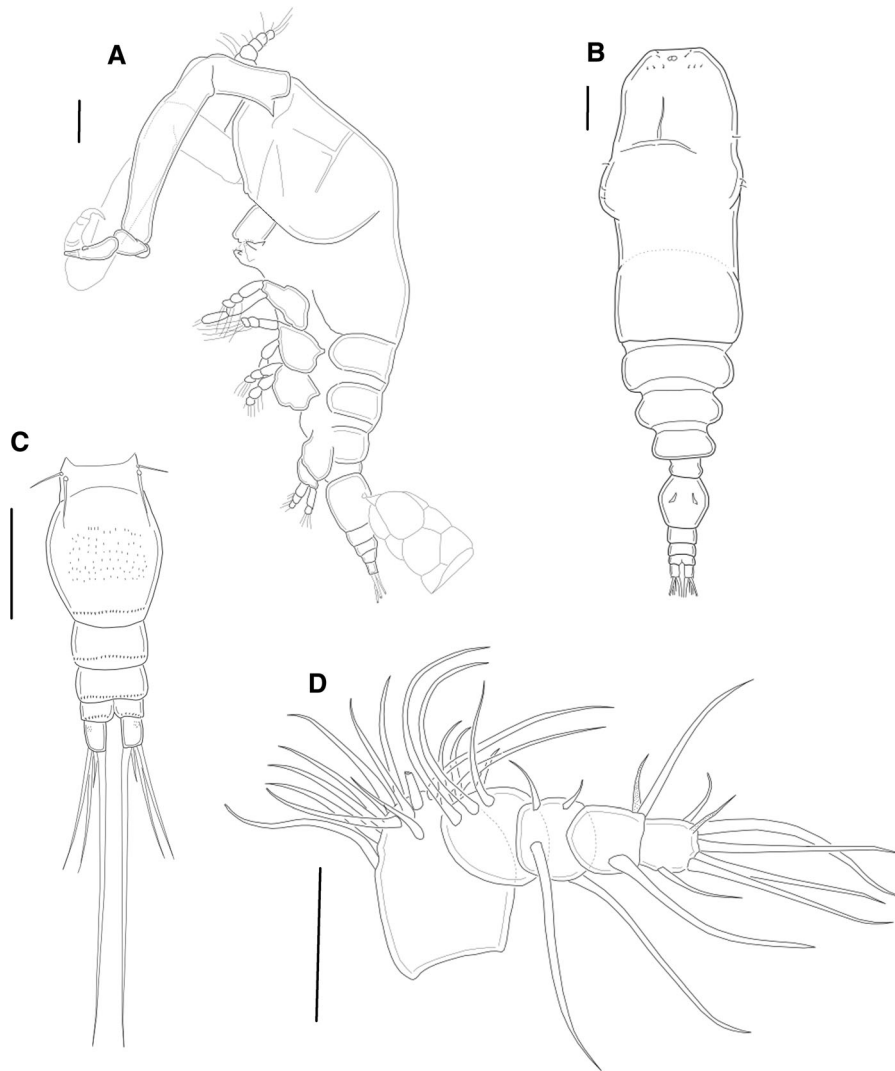
**Description (Figs. 1–5)**

*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

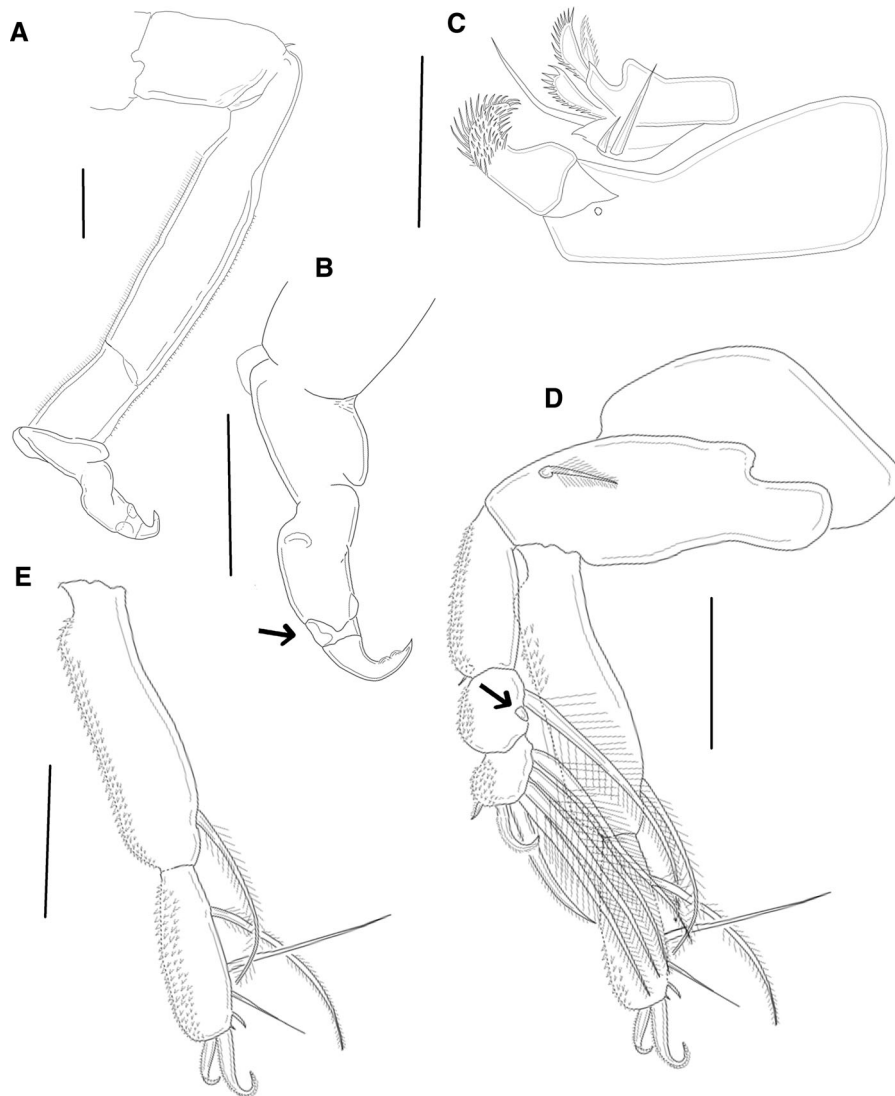


**Fig. 1** *Acusicola margulisae* n. sp., holotype female. A, Habitus, lateral view; B, Habitus, dorsal view; C, Urosome, ventral view; D, Antennule. Scale-bars: A–C, 100 µm; D, 50 µm

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  $\mu$ m; C–E, 50  $\mu$ m

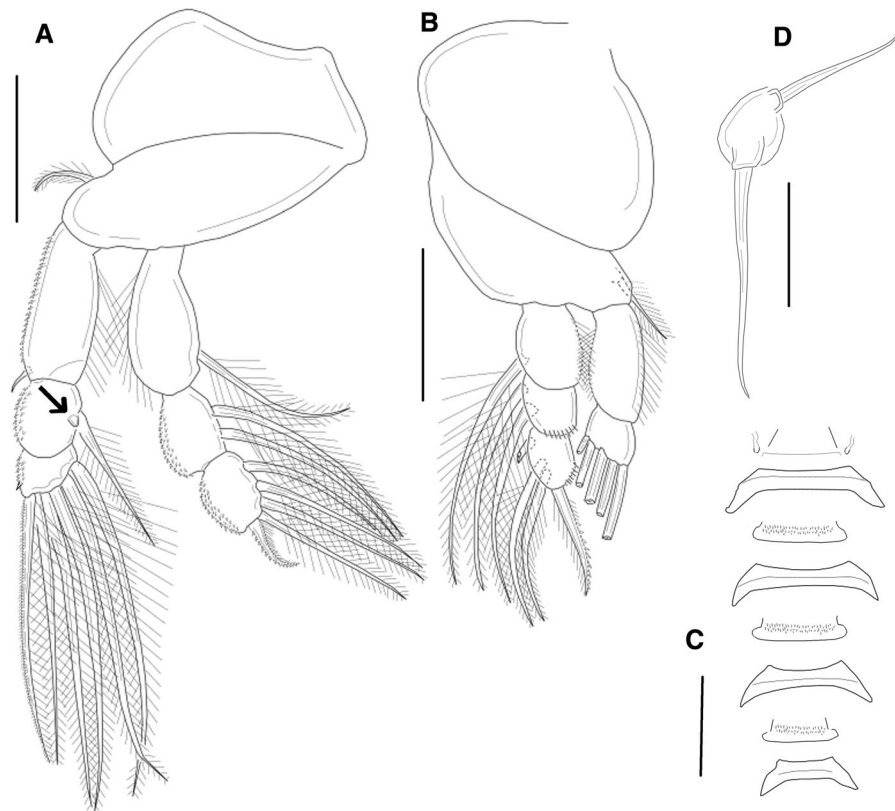
distal corner). Spines of both rami fringed with spinules on outer margin.

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 margulisiae* 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  $\mu$ m; C, 100  $\mu$ m; D, 25  $\mu$ m

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.

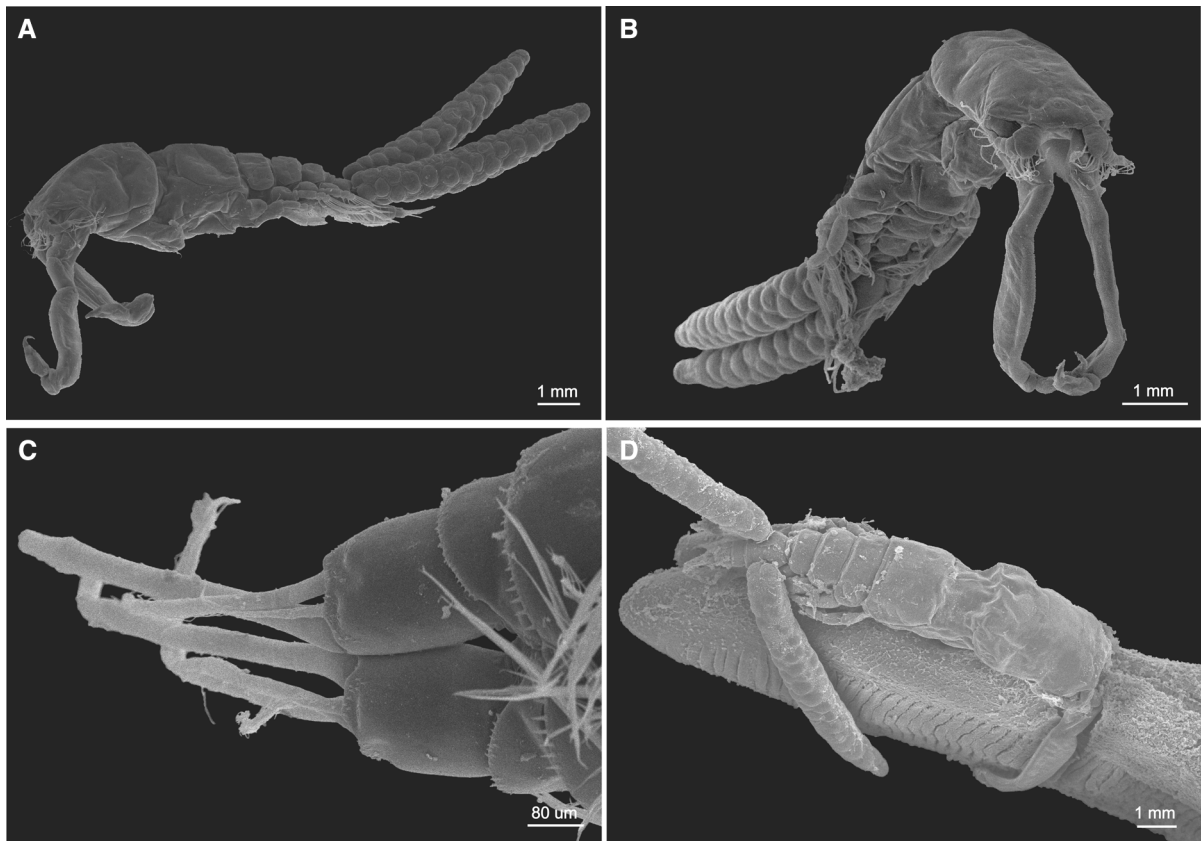
Leg 5 (Fig. 3D) represented by 2 setae; each carried on separate papilla.

#### 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. mazatlanensis*, *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. margulisiae* 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. margulisiae* 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 margulisiae* 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).

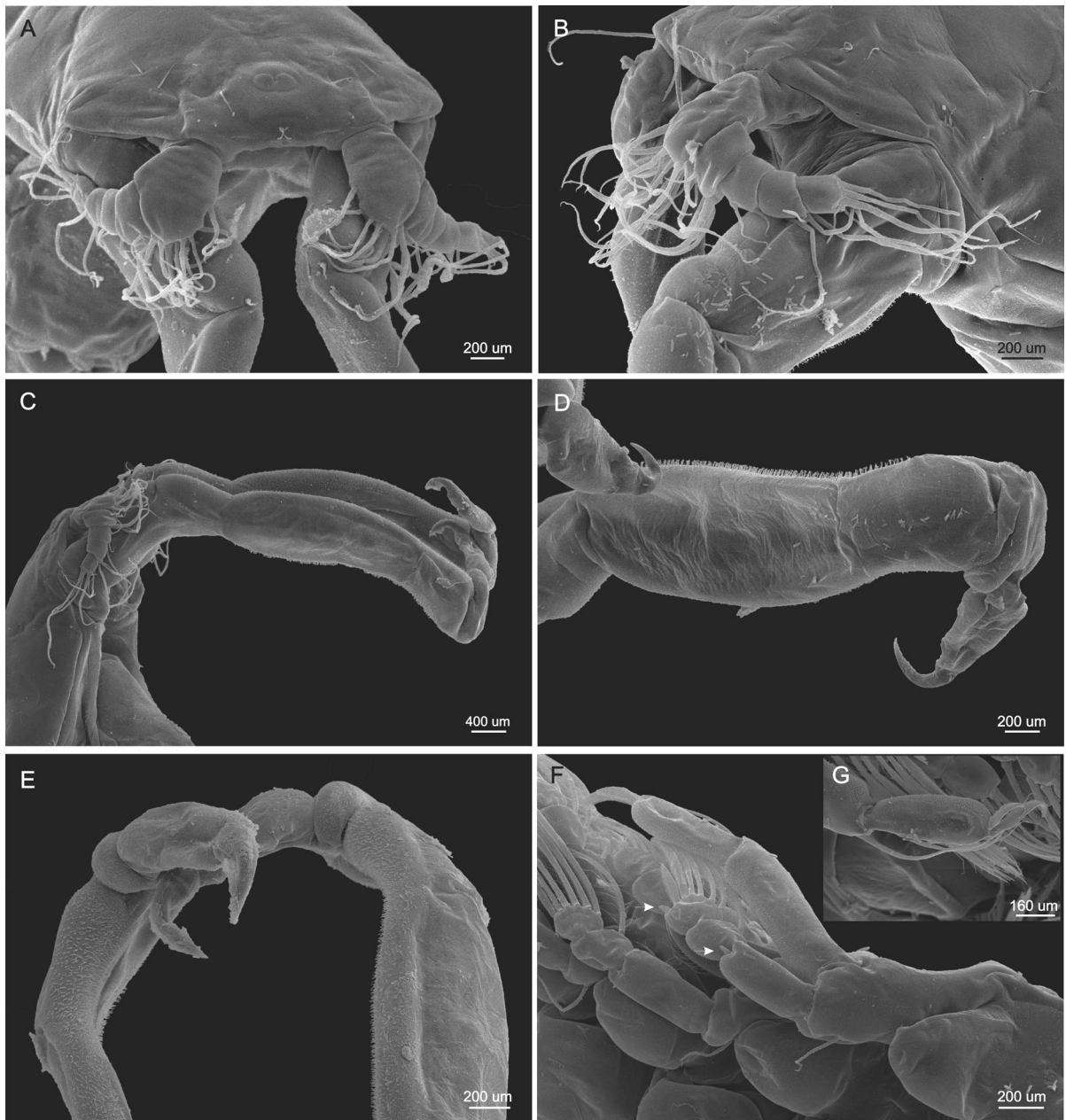
*Acusicola margulisae* n. sp. differs from *A. tenax* and *A. minuta* by having two considerably shorter inner apical setae on the second endopodal segment of leg 1. The new species differs further from *A. tenax* by

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

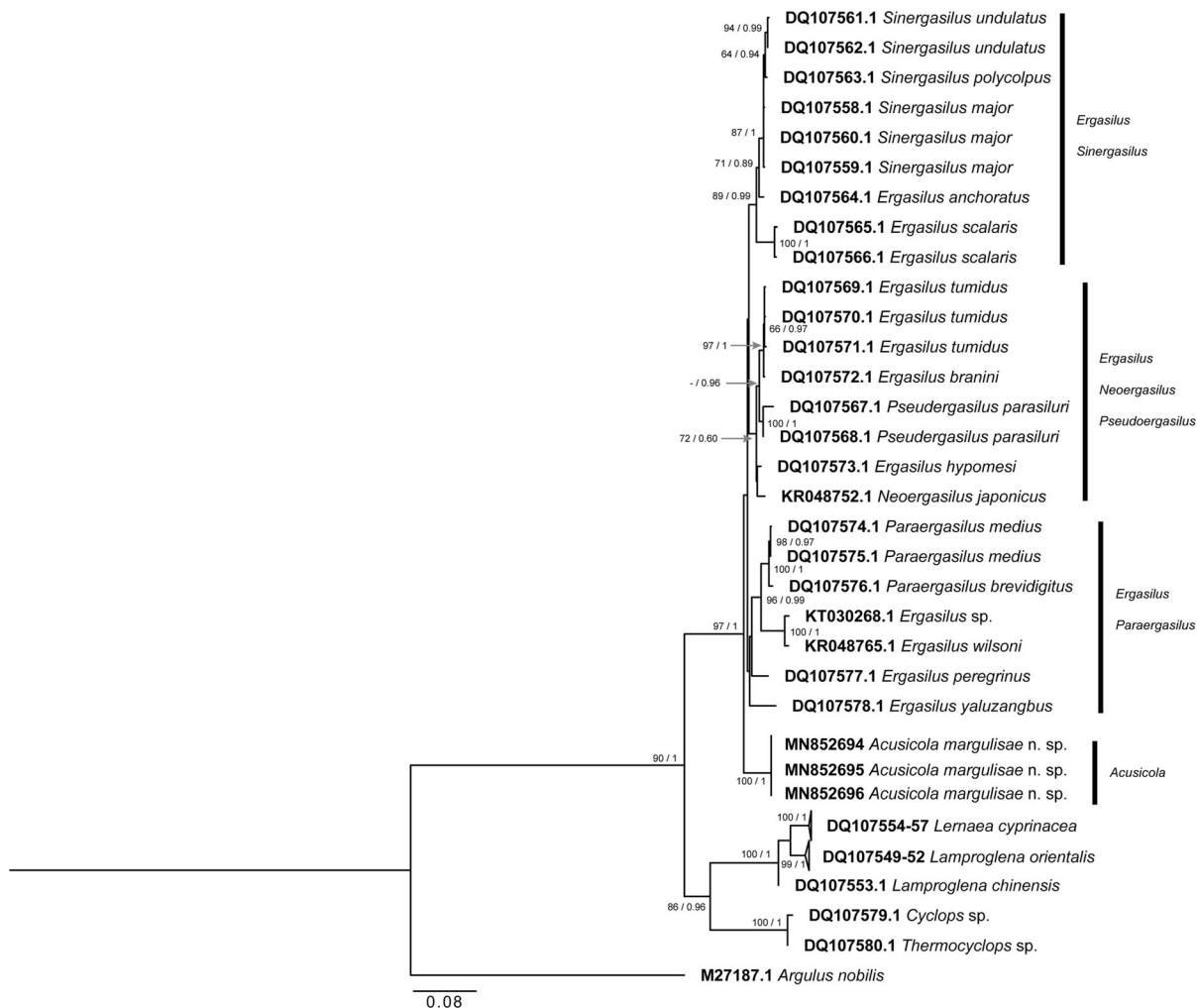




**Fig. 5** SEM micrographs of adult female *Acusicola margulisae* n. sp. A, Antennulae, dorsal view; B, Antennule, lateral view; C, D, Antennae, lateral view; E, Antenna, dorsal view; F, Leg I; G, Distal exopodal segment of Leg I

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 *cox1* 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. tucunarensis* 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. tucunarensis* 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
<i>A. brasiliensis</i> da Motta Amado & Rocha, 1996	<i>Atherinella brasiliensis</i> (Quoy & Gaimard) (Atherinopsidae); <i>Lile piquitinga</i> (Schreiner, Miranda & Ribeiro) (Clupeidae)	Brackish and marine	da Motta Amado & da Rocha Falavigna (1996)
<i>A. cunula</i> Cressey, 1970	<i>Pseudotylosurus angusticeps</i> (Günther) (Belonidae)	Freshwater	Cressey & Collette (1970)
<i>A. joturicola</i> El-Rashidy & Boxshall, 1999	<i>Joturus pichardi</i> Poey (Mugilidae)	Brackish	El-Rashidy & Boxshall (1999)
<i>A. lycengraulidis</i> Thatcher & Boeger, 1983	<i>Lycengraulis grossidens</i> (Spix & Agassiz) (Engraulidae)	Freshwater	Thatcher & Boeger (1983a)
<i>A. margulisae</i> n. sp.	<i>Amphilophus citrinellus</i> (Günther), <i>Parachromis managuensis</i> (Günther), <i>Oreochromis</i> sp. (Cichlidae); <i>Poecilia mexicana</i> (Steindachner) (Poeciliidae)	Freshwater	This study
<i>A. mazatlanensis</i> El-Rashidy & Boxshall, 1999	<i>Agonostomus monticola</i> (Bancroft) (Mugilidae)	Brackish	El-Rashidy & Boxshall (1999)
<i>A. minuta</i> Araujo & Boxshall, 2001	Plankton samples	Brackish	Araujo & Boxshall (2001)
<i>A. paracunula</i> da Motta Amado & Rocha, 1996	<i>Pellona flavipinnis</i> (Valenciennes) (Pristigasteridae); <i>Pseudotylosurus microps</i> (Günther) (Belonidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
<i>A. pellonidis</i> Thatcher & Boeger, 1983	<i>Pellona castelnaeana</i> (Valenciennes) (Pristigasteridae)	Freshwater	Thatcher & Boeger (1983b)
<i>A. rogeri</i> da Motta Amado & Rocha, 1996	<i>Strongylura marina</i> (Walbaum) (Belonidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
<i>A. rotunda</i> da Motta Amado & Rocha, 1996	<i>Lycengraulis batesii</i> (Günther) (Engraulidae)	Freshwater and brackish	da Motta Amado & da Rocha Falavigna (1996)
<i>A. spinuloderma</i> El-Rashidy & Boxshall, 1999	<i>Agonostomus monticola</i> (Bancroft), <i>Joturus pichardi</i> Poey (Mugilidae)	Freshwater and brackish	El-Rashidy & Boxshall (1999)
<i>A. spinulosa</i> da Motta Amado & Rocha, 1996	<i>Lycengraulis batesii</i> (Günther) (Engraulidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
<i>A. tenax</i> (Roberts, 1965)	<i>Pomoxis annularis</i> Rafinesque (Centrarchidae)	Freshwater	Roberts (1965)
<i>A. tucunarensis</i> Thatcher, 1984	<i>Cichla ocellaris</i> 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.

**Acknowledgements** We thank Brenda Solórzano and the Universidad Centroamericana in Managua, Nicaragua, for help in the field. The Ministry of Natural Resources (MARENA) in Nicaragua provided permission for collection permits (No. 001-012015). We also thank Berenit Mendoza Garfías for her help taking SEM micrographs in the Laboratorio de Microscopia Electrónica, UNAM, Mexico. Laura Márquez, Nelly López, Maria Luisa del Pozo and Iván Acevedo helped during molecular work. We thank curators of the collections where the type-material was deposited, in the Colección de Parásitos de Peces (CPPNP) at CIAD-Mazatlán, Sinaloa, Mexico, and the Colección Nacional de Crustáceos (CNCR) at Instituto de Biología, UNAM, Mexico.

**Funding** The work was funded by grants of the Spanish Ministerio de Ciencia e Innovación (grant CGL2013-42462-P and CGL2017-82986-C2-1-P) to MB. This study was also partially funded by a grant from the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM) IN202617 to GPPL.

#### 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.

## References

- Araujo, H. M. P., & Boxshall, G. A. (2001). A new species of *Acusicola* Cressey (Copepoda: Ergasilidae) from north-eastern Brazil. *Systematic Parasitology*, *49*, 149–157.
- Araujo, C. S. O. D., Barros, M. C., Gomes, A. L. D. S., Varella, A. M. B., Viana, G. D. M., Silva, N. P. D., et al. (2009). Parasites of natural and artificial populations of *Cichla* spp. *Revista Brasileira de Parasitologia Veterinária*, *18*, 34–38.
- Barluenga, M., & Meyer, A. (2004). The Midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes? *Molecular Ecology*, *13*, 2061–2076.
- Barluenga, M., & Meyer, A. (2010). Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evolutionary Biology*, *10*, 326.
- Boxshall, G. A., & Defaye, D. (2007). Global diversity of copepods (Crustacea: Copepoda) in freshwater. *Hydrobiologia*, *595*, 195–207.
- Bussing, W. A. (1976). Geographic distribution of the San Juan ichthyofauna of Central America with remarks on its origin and ecology. In T. B. Thorson (Ed.), *Investigations of the Ichthyofauna of Nicaraguan Lakes* (pp. 157–175). Lincoln, NE, USA: University of Nebraska Press.
- Cressey, R. F., & Collette, B. B. (1970). Copepods and needlefishes: a study in host-parasite relationships. *Fishery Bulletin*, *68*, 347–432.
- da Motta Amado, M. A. P., Ho, J. S., & da Rocha, C. E. F. (1995). Phylogeny and biogeography of the Ergasilidae (Copepoda, Poecilostomatoida), with reconsideration of the taxonomic status of the Vaigamidae. *Contributions to Zoology*, *65*, 233–243.
- da Motta Amado, M. A. P., & da Rocha Falavigna, C. E. (1996). New species of parasitic copepods of the genus *Acusicola* (Poecilostomatoida: Ergasilidae) from gill filaments of coastal and freshwater Brazilian fishes, and proposition of *Acusicola rogeri* n. sp. for *A. tenax sensu* Cressey & Collette (1970). *Hydrobiologia*, *324*, 183–193.
- Elmer, K. R., Kusche, H., Lehtonen, T. K., & Meyer, A. (2010). Local variation and parallel evolution: morphological and genetic diversity across a species complex of Neotropical crater lake cichlid fishes. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365*, 1763–1782.
- El-Rashidy, H., & Boxshall, G. A. (1999). Ergasilid copepods (Poecilostomatoida) from the gills of primitive Mugilidae (grey mullets). *Systematic Parasitology*, *42*, 161–186.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–297.
- Froese, R. & Pauly, D. Editors. (2019). FishBase. World Wide Web electronic publication. [www.fishbase.org](http://www.fishbase.org). Accessed 4 December 2019.
- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, *59*, 307–21.
- Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, *17*, 754–755.
- Huys, R., & Boxshall, G. A. (1991). *Copepod evolution*. London: Ray Society, 468 pp.
- ICZN (2012). *International Commission on Zoological Nomenclature: Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication*. *Bulletin of Zoological Nomenclature*, *69*, 161–169.
- Kearn, G. C. (2005). *Leeches, lice and lampreys: a natural history of skin and gill parasites of fishes*. Berlin: Springer Science & Business Media.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the

- organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649.
- Klompaker, A. A., & Boxshall, G. A. (2015). Fossil crustaceans as parasites and hosts. *Advances in Parasitology*, 90, 233–289.
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
- Lefort, V. L., Longueville, J. E., & Gascuel, O. (2017). SMS: Smart Model Selection in PhyML. *Molecular Biology and Evolution*, 34, 2422–2424.
- Luque, J. L., & Tavares, L. E. (2007). Checklist of Copepoda associated with fishes from Brazil. *Zootaxa*, 1579, 1–39.
- Luque, J. L., Vieira, F. M., Takemoto, R. M., Pavanelli, G. C., & Eiras, J. C. (2013). Checklist of Crustacea parasitizing fishes from Brazil. *Check List*, 9, 1449–1470.
- Maddison, W. P., & Maddison D. R. (2016). Mesquite: a modular system for evolutionary analysis. Version 3.61 <http://mesquiteproject.org>
- McCrary, J. K., Murphy, B. R., Stauffer, J. R., & Hendrix, S. S. (2007). Tilapia (Teleostei: Cichlidae) status in Nicaraguan natural waters. *Environmental Biology of Fishes*, 78, 107–114.
- Miller, M.A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA. pp. 1–8.
- Nadler, S. A., Bolotin, E., & Stock, S. P. (2006). Phylogenetic relationships of *Steinernema* Travassos, 1927 (Nematoda: Cephalobine: Steinernematidae) based on nuclear, mitochondrial and morphological data. *Systematic Parasitology*, 63, 161–181.
- Rambaut, A. (2012). FigTree v1. 4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. <http://tree.bio.ed.ac.uk/software/figtree/>.
- Roberts, L. S. (1965). *Ergasilus tenax* sp. n. (Copepoda: Cyclopoida) from the white crappie, *Pomoxis annularis* Rafinesque. *Journal of Parasitology*, 51, 987–989.
- Siebert, L., & Simkin, T. (2002). Momotombo. Volcanoes of the world: an illustrated catalog of Holocene volcanoes and their eruptions. Global Volcanism Program Digital Information Series, GVP-3. Washington, DC: Smithsonian Institution. <http://www.volcano.si.edu/world/volcano.cfm?vnum=1404-09>. Accessed 5 February 2019.
- Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., et al. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, 7, 539.
- Song, Y., Wang, G. T., Yao, W. J., Gao, Q., & Nie, P. (2008). Phylogeny of freshwater parasitic copepods in the Ergasilidae (Copepoda: Poecilostomatoida) based on 18S and 28S rDNA sequences. *Parasitology Research*, 102, 299–306.
- Thatcher, V. E. (1984). The parasitic crustaceans of fishes from the Brazilian Amazon. 7. *Acusicola tucunarensis*, n. sp. (Copepoda: Cyclopidea) from *Cichla ocellaris*, with an emendation of the genus and the proposal of *Acusicoliinae* [sic] subfam. nov. *Revista Brasileira de Biologia*, 44, 181–189.
- Thatcher, V. E., & Boeger, W. A. (1983a). The parasitic crustaceans of fishes from the Brazilian Amazon. 8. *Acusicola lycengraulidis* n. sp. (Copepoda: Cyclopoida) from *Lycengraulis grossidens* Cuvier. *Acta Amazonica*, 13, 943–951.
- Thatcher, V. E., & Boeger, W. A. (1983b). The parasitic crustaceans of fishes from the Brazilian Amazon. 10. *Acusicola pellowidii* n. sp. (Copepoda: Cyclopidea) from *Pellona castelnaeana* (Valenciennes). *Amazoniana*, 8, 273–279.
- Waid, R. M., Raesly, R. L., McKaye, K. R., & McCrary, J. K. (1999). Zoogeografía íctica de lagunas cratéricas de Nicaragua. *Encuentro*, 51, 65–80.
- Walter, T.C., & Boxshall, G. (2018). World of Copepods database. *Acusicola* Cressey, 1970. <http://www.marinespecies.org/copepoda/aphia.php?p=taxdetails&id=346698> on 2019–01–22. Accessed 1 June, 2019.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.