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Corresponding author:
Felipe Bisaggio Pereira;
Email: felipebisaggiop@hotmail.com

Acusicola rochai n. sp. (Copepoda: Ergasilidae) parasitizing *Anableps anableps* (Anablepidae) from the Amazon Coast, with a key for *Acusicola* spp.

João Victor Couto[1], Jorge Luiz Silva Nunes[2], Fabiano Paschoal[3] and Felipe Bisaggio Pereira[4]

[1]Programa de Pós-Graduação em Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; [2]Laboratório de Organismos Aquáticos, Departamento de Oceanografia e Limnologia, Universidade Federal do Maranhão, São Luís, MA, Brazil; [3]Programa de Pós-Graduação em Biodiversidade e Conservação, Universidade Federal do Maranhão, São Luís, MA, Brazil and [4]Departamento de Parasitologia, Instituto de Ciências Biológicas, Bloco L4 sala 252, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

Abstract

Ergasilid copepods represent one of the commonest groups of fish parasites in Brazil. Within Ergasilidae, three genera share a peculiar latching mechanism on the antenna that completely encircles the gill filament, one of which is *Acusicola* Cressey, 1970. During a survey of estuarine fish from the Brazilian Amazon Coast, a new species of *Acusicola* was found on the gills of the largescale foureyes *Anableps anableps* (Linnaeus, 1758) (Actinopterygii: Anablepidae) in São Marcos Bay, State of Maranhão. *Acusicola rochai* n. sp. can be distinguished from its closest congeners mainly by three protrusions on the dorsal surface of third and fourth pedigerous somites, and by smooth interpodal plates. This work is the first report of a parasitic copepod infesting a fish from Anablepidae and, consequently, the host *An. anableps*. The existing dichotomous key proposed for the genus *Acusicola* includes only ten species, excluding the eight species subsequently described. Therefore, in the present work, a new dichotomous key is provided based on reliable and well-documented features.

Introduction

The Amazon coast extends on the States of Amapá, Pará, and Maranhão, representing about 35% of the total Brazilian shore (Pereira *et al.*, 2009). The Amazon River mouth is one of the largest discharges of freshwater and sediment into the ocean, creating unique conditions for a massive animal fauna biodiversity, with more than 700 species currently reported (Tosetto *et al.*, 2022; Checon *et al.*, 2023). The notable biodiversity of the local ichthyofauna is reflected on its socioeconomic scenario, since fisheries represent an important economic, nutritional, and cultural activity, with fish being the main food resource for local populations (Tenório *et al.*, 2015; Jimenez *et al.*, 2019). Despite such an important role in Brazil, the Amazon coast has been constantly impacted by anthropogenic activities, with few effective governmental efforts for conservation, which has resulted in decline of fish stocks and direct impacts on coastal environments (Szlafsztein, 2012; Hayashi *et al.*, 2019).

Ergasilidae Burmeister, 1835 is a group of cosmopolitan parasitic crustaceans, commonly found on the gills, nostrils, fins, tegument and urinary bladder of fish, and rarely elasmobranchs and bivalve molluscs (Malta, 1993; Boxshall and Halsey, 2004; Couto *et al.*, 2023). This family is one of the most species rich within cyclopoid copepods, with 275 species from 30 genera currently known. These copepods represent the commonest taxon infesting fish in Brazil, totalling 77 species from 17 genera reported in the country. Despite its notable diversity, recent studies have stated that the knowledge related to the richness and distribution of Ergasilidae may be inaccurate, due to a low number of fish species investigated for parasitic copepods in Brazil (Luque *et al.*, 2013; Couto *et al.*, 2024a).

The genus *Acusicola* Cressey, 1970 was originally proposed to allocate *Acusicola cunula* Cressey, 1970, from the Needlefish *Pseudotylosurus angusticeps* (Günther, 1866) (Actinopterygii: Belonidae) in Brazil, and *Acusicola tenax* (Roberts, 1965), a parasite of the White crappie *Pomoxis annularis* Rafinesque, 1818 (Actinopterygii: Centrarchidae) collected in the USA, which was first assigned to *Ergasilus*. Currently, *Acusicola* comprises 17 species in which ten were reported from Brazil, mainly in the Amazon River Basin (Cressey and Collette, 1970; Couto *et al.*, 2023). This taxon belongs to a group of three genera within Ergasilidae that share a five-segmented antennule, and a latching mechanism on its antennae, which allow the copepod to completely encircle the gill filament of its host. This group is composed of *Acusicola*, *Miracetyma* Malta, 1994, and *Amplexibranchius* Thatcher & Paredes, 1835, which was believed to form a unique lineage in the family, supposedly supporting a subfamily named Acusicolinae Thatcher, 1984; however, such hypothesis was rejected because of the lack of robust phylogenetic evidence (Thatcher, 1984; Thatcher and Paredes, 1985; Boxshall and Halsey, 2004). Despite these similarities, *Acusicola* spp. have a two-segmented endopod armed with at least six elements on the first leg, which represents a particular pattern that distinguishes it from the latter two genera (Boxshall and Halsey, 2004).

During a survey of parasitic copepods from the Brazilian Amazon Coast, specimens of *Acusicola* were collected on the gills of the largescale foureyes *Anableps anableps* (Linnaeus, 1758) (Actinopterygii: Anablepidae). A detailed morphological study of these specimens revealed that they represent a new species, which is described herein.

Material and methods

Fish were caught in the São Marcos Bay (2°31′48″S, 44°20′28″W) (Figure 1), State of Maranhão, Brazilian Amazon Coast, and kept frozen at −20°C, prior to parasitological examination. Copepods were collected through washing of the gill filaments in flowing water, or detached using a needle, fixed and preserved in 70% ethanol. For observation using light microscopy, parasite specimens were cleared in 85% lactic acid, and the appendages were dissected and examined using the wooden slide procedure described by Humes and Gooding (1964). Drawings were made using a drawing tube attached to an Olympus CH2 microscope. Measurements were performed using an ocular micrometer and are presented as range, followed by mean and standard deviation in parentheses, all in micrometers. The descriptive terminology and classification of copepods followed Boxshall and Halsey (2004). Prevalence and mean intensity were given according to Bush *et al.* (1997). Host identification was based on Marceniuk *et al.* (2021) and their nomenclature and classification were updated according to Eschmeyer's Catalog of Fishes (Van der Laan *et al.*, 2023). To avoid ambiguity of some generic names, the following abbreviations were used: '*A.*' for *Acusicola* and '*An.*' for *Anableps*. Type-specimens were deposited in Coleção Carcinológica do Museu de Zoologia da Universidade de São Paulo (acronym MZUSP), Brazil. Access to genetic heritage was registered in the Sistema Nacional de Gestão do Patrimômio Genético e do Conhecimento Tradicional Associado (acronym SisGen), under the number A03E910, according to Brazilian Federal requirements.

Results

Sixty-two specimens of *An. anableps* were analysed, in which 21 were parasitized at least by one specimen of *Acusicola*. A total of 178 female copepods were collected, showing prevalence of 66% and mean intensity of 4.34 copepods per infected fish (range 1–16).

Systematics

Class Copepoda Milne Edwards, 1840
Order Cyclopoida Burmeister, 1834
Family Ergasilidae Burmeister, 1835
Genus *Acusicola* Cressey, 1970
Type-species: *Acusicola cunula* Cressey, 1970 by original designation.
***Acusicola rochai* n. sp.**
ZooBank registration: urn:lsid:zoobank.org:pub:AD08F121-4561-4D0A-A620-37CA67A4EDD0
(Figures 2–4)

Material examined

Holotype female (MZUSP-45941) and nine paratype females (MZUSP-45942) collected on the gill filaments of the largescale

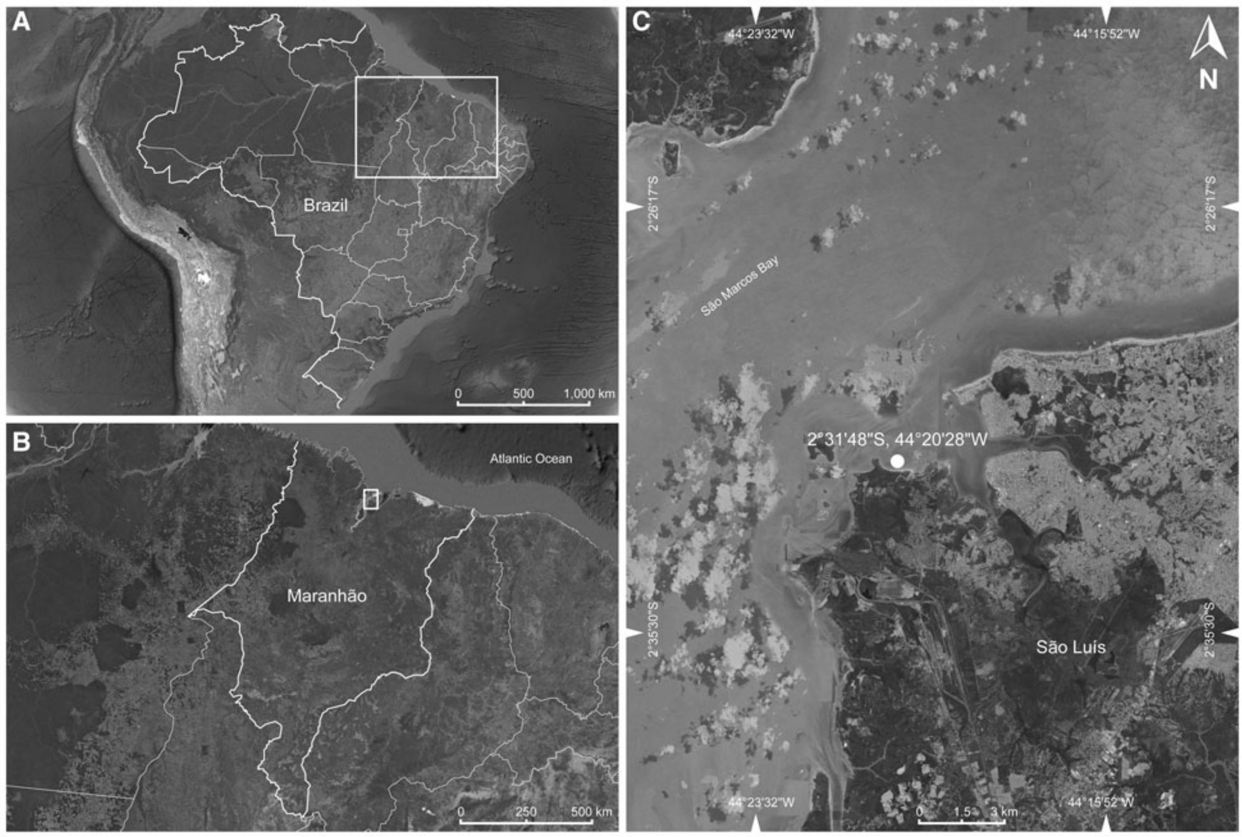

Figure 1. Map indicating the sampling site of the present study (modified from Google Earth): (A) Brazil, showing position of the State of Maranhão (at the centre of the white rectangle); (B) State of Maranhão, showing the municipality of São Luís (delimited by the white rectangle); (C) São Marcos Bay, with the white dot, georeferenced, showing sampling site.

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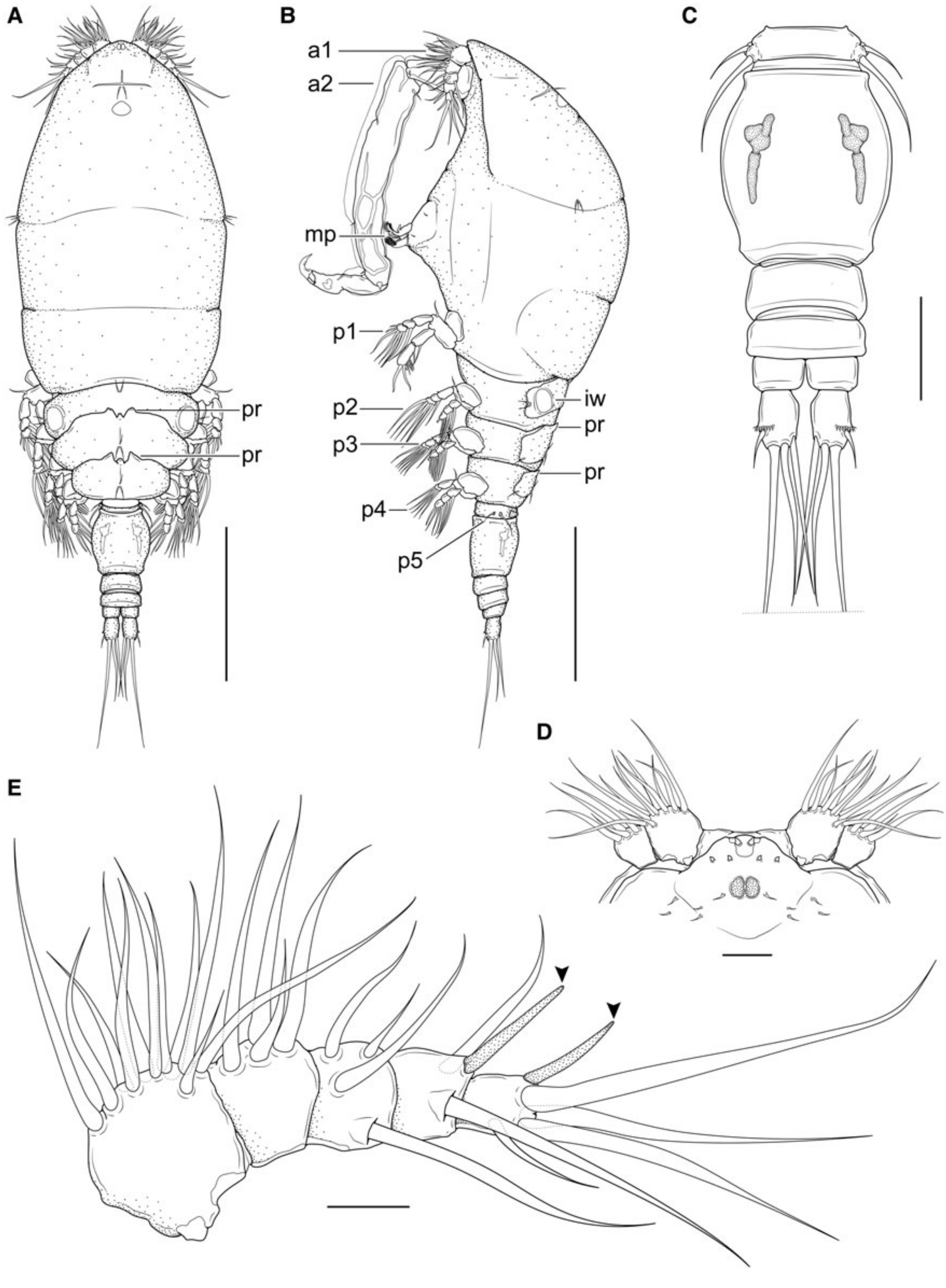

Figure 2. *Acusicola rochai* n. sp., adult female: (A) habitus, dorsal, pr = protrusion; (B) habitus, lateral, a1 = antennule, a2 = antenna, mp = mouthparts, p1 = leg 1, iw = integumental window, p2 = leg 2, pr = protrusion, p3 = leg 3, p4 = leg 4, p5 = leg 5; (C) urosome and caudal rami, ventral; (D) rostral area, ventral; (E) antennule, ventral, arrows pointing aesthetascs. Scale bars: A–B, 150 µm; C, 30 µm; D, 35 µm; E, 20 µm.

foureyes *An. anableps* (Linnaeus, 1758) (Actinopterygii: Anablepidae) (type host) from São Marcos Bay (2°31′48″S, 44° 20′28″W), State of Maranhão, Brazil (type locality).

Etymology

The new species is named '*rochai*' in honour of Dr Carlos Eduardo Falavigna da Rocha for his contribution to the knowledge about the richness and diversity of the genus *Acusicola*.

Description

Adult female [based on 10 specimens]. Body length from anterior margin of prosome to posterior margin of caudal rami 595–889 (697 ± 101.4). Body comprising prosome and urosome (Figure 2A, B). Prosome consisting of cephalosome, with antennule visible in dorsal view, and four pedigerous somites. Cephalosome and first pedigerous somite fused (=cephalothorax), with boundary almost indistinct (Figure 2A, B). Cephalothorax (Figure 2A, B) bullet-shaped, longer than wide, 283–360 (319 ±

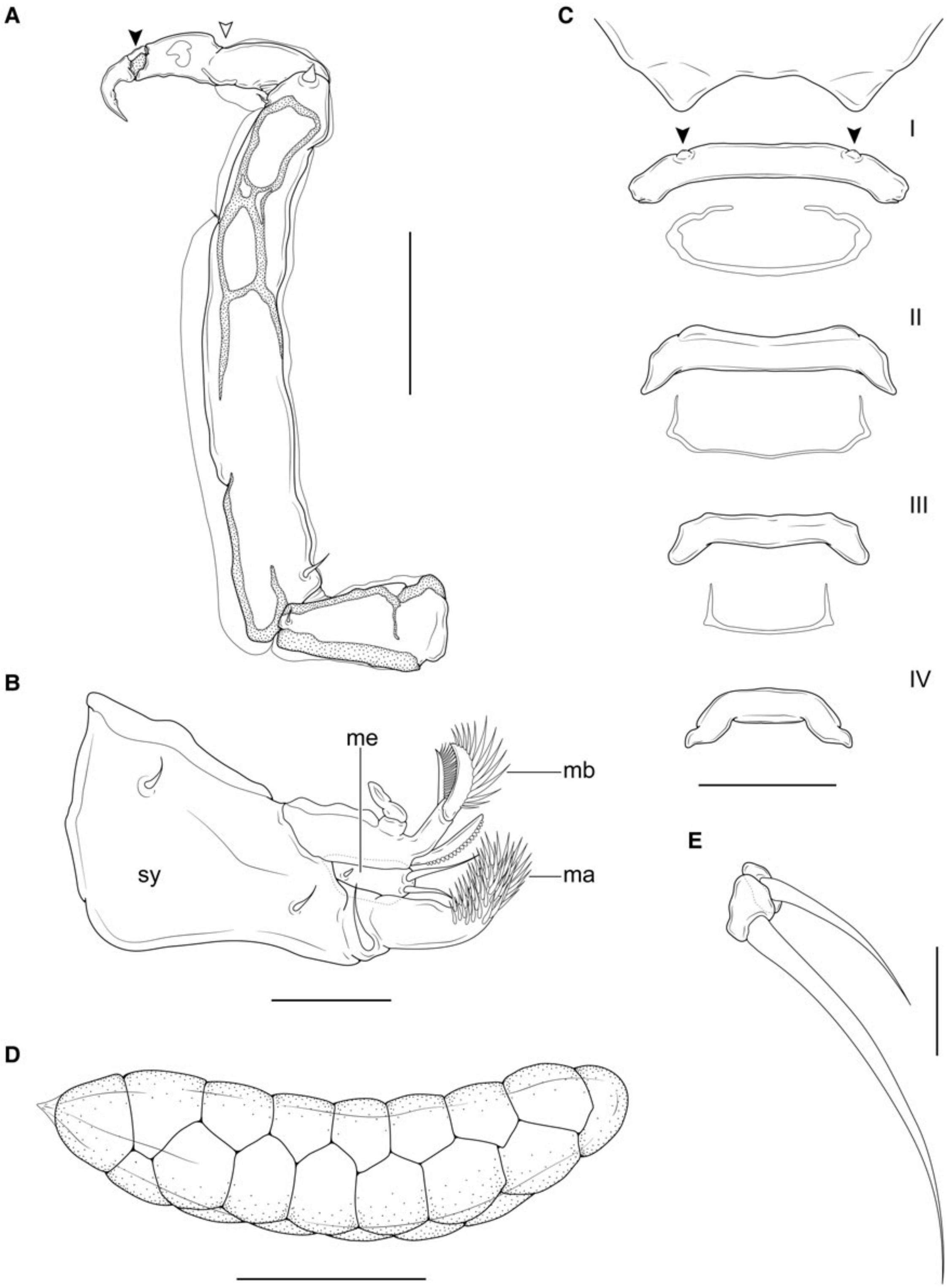

Figure 3. *Acusicola rochai* n. sp., adult female: (A) antenna, ventral, black arrow pointing third vestigial segment, white arrow pointing groove; (B) mouthparts, ventral, mb = mandible, me = maxillule, sy = syncoxa, ma = maxilla; (C) interpodal plates of legs 1–4, ventral, arrow pointing protrusions; (D) egg sac, dorsal; (E) leg 5, dorsal. Scale bars: A, 60 µm; B, 20 µm; C, 35 µm; D, 200 µm; E, 10 µm.

37) × 129–261 (192 ± 47), not inflated and slightly constricted, representing 45% of body length; dorsal surface with anterior naupliar eye bearing five sensilla on each side, inverted T-shaped mark with two sensilla and circular mark posteriorly; three sensilla on each lateral edge (Figure 2A). Second pedigerous somite with two dorsal and two lateral sensilla; third and fourth pedigerous somites with three dorsal and two lateral sensilla each (Figure 2A, B). Second pedigerous somite bearing pair of rounded integumental windows laterally (Figure 2A, B). Third and fourth pedigerous somite with three anterior protrusions (Figure 2A, B). Urosome consisting of fifth pedigerous somite, genital double-somite, and three free abdominal somites; third abdominal somite (= anal somite) bipartite (medially incised). Fifth pedigerous somite short (Figure 2C). Genital double-somite globular, slightly longer than wide 61–77 (72.6 ± 4.6) × 60–75 (68.6 ± 5) (Figure 2C). Free abdominal somites wider than long (Figure 2C); first somite longer than second; anal somite shorter than previous two. Caudal rami 1.5× longer than wide and longer

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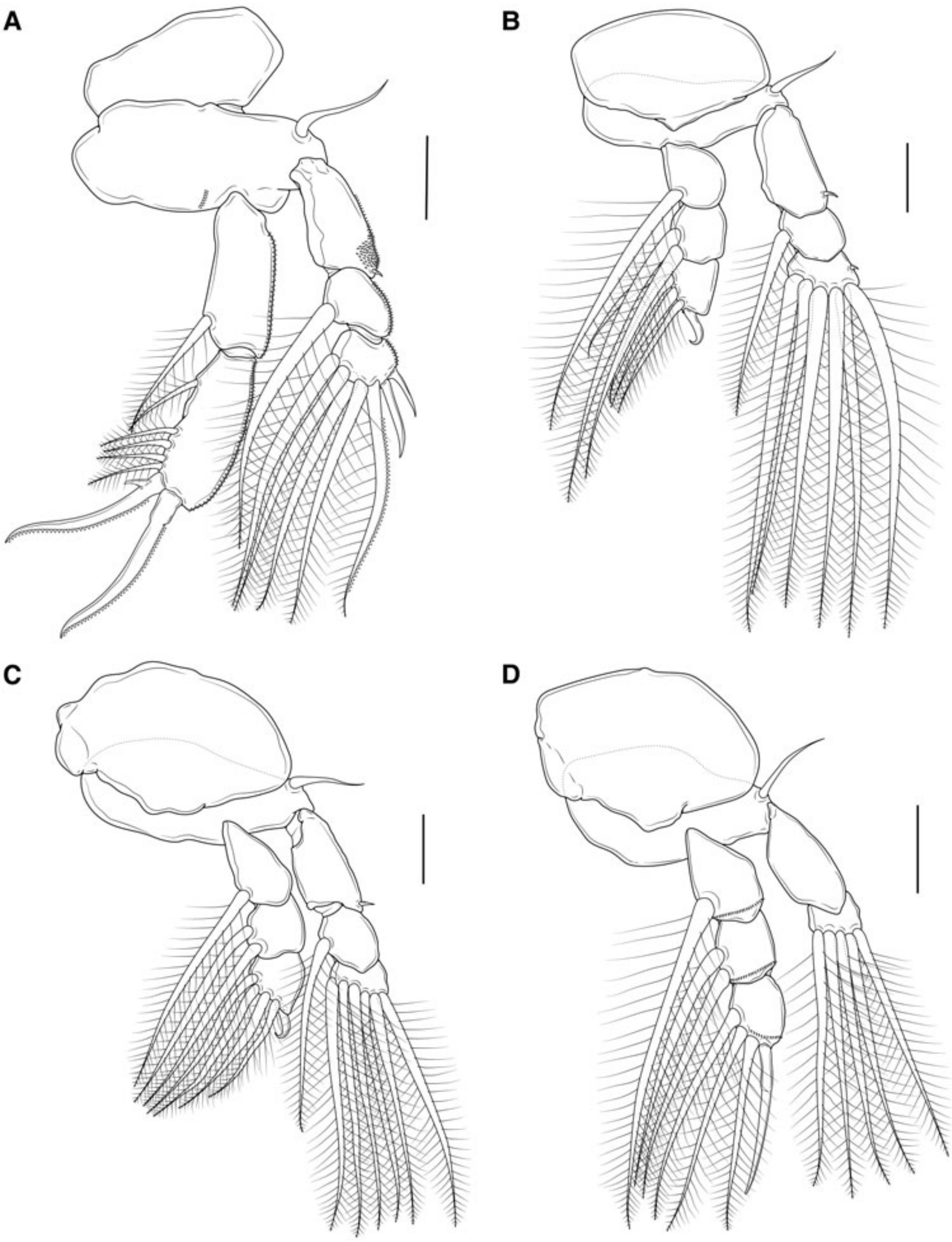

Figure 4. *Acusicola rochai* n. sp., adult female: (A) leg 1, ventral; (B) leg 2, ventral; (C) leg 3, ventral; (D) leg 4, ventral. Scale bars: A–D, 20 μm.

than anal somite, with row of spinules on posterolateral margin near insertion of minor seta; each ramus armed with large apical seta, two medial apical setae and minor ventral seta (Figure 2C). Paired egg-sacs (Figure 3D) longer than wide, each composed of 1–2 rows of eggs.

Rostrum (Figure 2D) with two anterior and four posterior blunt elements. Antennule five-segmented (Figure 2E), tapering distally, aesthetascs present on fourth and fifth segments; setal formula as follows: 11: 4: 4: 2 + ae: 4 + ae: all setae naked. Antenna (Figure 3A) comprising coxobasis and three-segmented endopod with terminal claw. Coxobasis, first endopodal segment and the first half of the second endopodal segment with hyaline processes on inner margins and enclosed by membranous sheath. Coxobasis short, proximally longer, armed with distally naked seta; membrane between coxobasis and first endopodal segment not inflated. First endopodal segment longest, nearly 6.9× longer than wide, armed with one posterior blunt element and three spiniform elements: one anterior, one medio-lateral, and one posterior, near insertion of the second segment; all elements inserted on cuticular elevations; second endopodal segment longer than wide, representing 35% of previous segment length; third endopodal segment vestigial bearing short, curved claw with fossa on inner margin near tip.

Mouthparts (Figure 3B) include mandible, maxillule, and maxilla; maxilliped absent. Mandible unsegmented bearing palp, anterior, mid, and posterior blades; palp small and naked; anterior blade with small spinules on outer margin; mid blade with long spinules on outer margin; posterior blade with smooth teeth along posterior margin. Maxillule small, bearing inner minute spiniform element and two outer setae. Maxilla comprising large syncoxa with two small setae, one on posterior outer margin and one on inner margin, and naked seta near teeth; second segment (basis) bearing long and sharp anterior teeth with long spinules along anterior, ventral, and apical margins.

Swimming legs 1–4 biramous (Figure 4A–D), each with two-segmented protopod comprising coxa and basis; interpodal plates (Figure 3C) smooth; first intercoxal sclerite with a pair of

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protrusions anteriorly. Armature of legs (spines, Roman numerals; setae, Arabic numerals) as follows:

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

Leg 1 (Figure 4A) coxa unarmed. Basis with outer naked seta and row of spinules on posterior margin, near endopod insertion. Exopod three-segmented, with rows of spinules on outer margin of all segments; first segment with small outer spine; second segment with inner plumose seta; third segment with two unequal subapical spines, long apical semi-pinnate seta, and four plumose setae. Endopod two-segmented, both segments with rows of spinules on outer margin; first segment representing 70% of exopodal ramus length, with plumose inner seta; second segment with two apical pectinate spines, innerspine falciform, and five plumose setae.

Leg 2 (Figure 4B) coxa with protrusion on posterior margin. Basis with outer naked seta. Exopod three-segmented, all segments smooth; first segment longest, with small outer spine; second segment with inner plumose seta; third segment, with six apical plumose setae and small subapical outer spine. Endopod three-segmented, all segments smooth; first segment with plumose inner seta; second segment with two plumose inner setae; third segment with apical curved spine, and five plumose setae.

Leg 3 (Figure 4C) similar to leg 2, except for absence of outer spine on last exopodal segment.

Leg 4 (Figure 4D) coxa with protrusion on posterior margin. Basis with outer naked seta. Exopod two-segmented, both segments smooth; first segment unarmed; second segment with five plumose setae. Endopod three-segmented, all segments with row of spinules on posteroventral margin; first segment with inner plumose seta; second segment with two plumose setae; third segment with long apical spine, representing 62% of endopodal ramus length, and three plumose setae.

Leg 5 (Figure 3E) represented by two unequal naked setae carried on rounded papilla.

Remarks

Representatives of Ergasilidae are characterized by the second antenna modified in a robust prehensile organ, mandibles with two or three spinulate blades, lack of maxilliped in adult females, and leg 4 with one or two segments, or rarely absent (Boxshall and Halsey, 2004). Among the 30 genera hitherto described in the family, *Acusicola* can be identified based on a five-segmented antennule, a groove on the second endopodal segment of the antennae that latches the claw of the opposite side, allowing a complete encircling of the gill filament, and leg 1 with two-segmented endopod armed with at least six elements (and rarely three) (Amado and Rocha, 1996; Boxshall and Halsey, 2004; Couto *et al.*, 2023; Walter and Boxshall, 2024). Therefore, the present parasitic copepods have all the characters previously mentioned, clearly justifying their allocation in Ergasilidae and *Acusicola*.

Currently, *Acusicola* comprises 17 nominal species, but only the following six have the first leg with one spine on the first exopodal segment and five setae on the last endopodal segment as in the new species: *Acusicola brasiliensis* Amado & Rocha, 1996, *Acusicola joturicola* El-Rashidy & Boxshall, 1999, *Acusicola margulisae* Santacruz, Morales-Serna, Leal-Cardín, Barluenga & de León, 2020, *Acusicola mazatlanesis* El-Rashidy & Boxshall, 1999, *Acusicola pellonidis* Thatcher & Boeger, 1983, and *Acusicola spinuloderma* El-Rashidy & Boxshall, 1999 (Thatcher and Boeger, 1983a, 1983b; Amado and Rocha, 1996; El-Rashidy and Boxshall, 1999; Santacruz *et al.*, 2020; Walter and Boxshall, 2024). However, *A. rochai* n. sp. can be easily differentiated from these congeners by the presence of three anterior protrusions on the dorsal margins of third and fourth pedigerous somites (*vs* absent on the congeners listed above) and by the smooth urosome (*vs* with rows of spinules on *A. brasiliensis*, *A. joturicola*, *A. margulisae*, *A. mazatlanensis*, and *A. spinuloderma*; and small posterolateral spine on the last abdominal segment in *A. pellonidis*) (Thatcher and Boeger, 1983a, 1983b; Amado and Rocha, 1996; El-Rashidy and Boxshall, 1999; Santacruz *et al.*, 2020).

The new species also differs from *A. brasiliensis* because its cephalosome is fused to the first pedigerous somite (*vs* first pedigerous somite free in the latter species); from *A. brasiliensis* and *A. spinuloderma* by the absence of a spine on the last exopodal segment of leg 3 (*vs* spine present in the latter two species); from *A. joturicola*, *A. spinuloderma*, *A. margulisae*, and *A. mazatlanensis* because its proximal endopodal segment of antenna is smooth (*vs* with spinules in *A. joturicola*, *A. spinuloderma*, and *A. mazatlanensis*; and striations and setules in *A. margulisae*); and from *A. pellonidis* because it has six setae on the last exopodal segment of leg 2 (*vs* four setae in the latter species) (Thatcher and Boeger, 1983a, 1983b; Amado and Rocha, 1996; El-Rashidy and Boxshall, 1999; Santacruz *et al.*, 2020).

Additionally, there is only an additional species of *Acusicola* that has dorsal protrusions on the pedigerous somites as in the new species, i.e. *Acusicola iamarinoi* Couto, Pereira, Luque, Paschoal & Pereira, 2022. Nevertheless, *A. rochai* n. sp. has three protrusions on the third and fourth pedigerous somites, while in *A. iamarinoi* the third pedigerous somite is smooth and the fourth pedigerous somite has only two protrusions (Couto *et al.*, 2023). Furthermore, *A. rochai* n. sp. can be distinguished from *A. iamarinoi* because its cephalosome is fused to the first pedigerous somite (*vs* first pedigerous somite free in the latter), it has five setae on the last endopodal segment of leg 1 (*vs* four setae in the latter), the second and third interpodal plates are smooth (*vs* with row of spinules in the latter), and its urosome is smooth (*vs* with rows and patches of spinules in the latter) (Couto *et al.*, 2023).

Discussion

In South America, the family Anablepidae is represented by only two species, namely, *An. anableps* and *Anableps microlepis* Müller & Troschel, 1844. Species of this genus are commonly known as 'foureyes' fish due to their unusual morphology: eyes divided into two portions, each with an individual pupil, enabling surface-swimming individuals to simultaneously focus on images above and below water. *Anableps anableps* is commonly found inhabiting freshwater and mangrove coastlines in Brazil, primarily in the Amazon River Delta, Northeast coast, and is frequently used as a subsistence resource by some populations (Nelson *et al.*, 2016; Figueiredo *et al.*, 2019; Rodrigues *et al.*, 2021; Froese and Pauly, 2024). Despite its local importance and peculiar aspect, only five species of parasitic crustaceans have been reported on *An. anableps*: *Gnathia* sp. (praniza larvae) in the State of Pará, *Excorallana longicornis* Lemos de Castro, 1960 (Corallanidae), and *Nerocila acuminata* Schiödte & Meinert, 1881 (Cymothoidae), both from the State of Amapá, *Cymothoa curta* Schioedte & Meinert, 1884 in an unspecified locality, and *Cymothoa* sp. (both Cymothoidae) off the State of Pará, Brazil (Schioedte and Meinert, 1884; Diniz *et al.*, 2008; Esteves-Silva *et al.*, 2020; Loureiro *et al.*, 2021). In this sense, the present study represents the first report of a parasitic copepod infesting an Anablepidae fish in Brazil and, consequently, the host *An. anableps*. Additionally, considering the potential for parasitic crustaceans observed in this host species, it is reasonable to consider

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this fish as a potential host for other copepods, which demands further investigations to better understand its parasitic fauna and ecological interactions. Moreover, this work also underscores the high biodiversity potential of parasitic copepods in fish with little commercial importance, which are frequently neglected due to the historically uneven research, widely documented in Brazil (Luque and Poulin, 2007; Luque and Tavares, 2007; Luque *et al*., 2013; Couto *et al*., 2023, 2024a, 2024b).

When analysing the descriptions of new species within *Acusicola*, it is common to use armature of legs, proportion of segments in the antennae and leg 1, body size, and other morphometric data of females for diagnosing the species (Roberts, 1965; Cressey and Collette, 1970; Thatcher and Boeger, 1983a, 1983b; Thatcher, 1984; Amado and Rocha, 1996; El-Rashidy and Boxshall, 1999; Araújo and Boxshall, 2001; Santacruz *et al*., 2020; Couto *et al*., 2023). Boxshall (2016) asserted that in many genera of Ergasilidae, the armature of swimming legs should be used with caution due to imprecise descriptions, particularly in older studies. Although we agree with this author in respect to many genera of ergasilids, in regard to *Acusicola*, this feature appears to be well-documented and reliable for species differentiation. Therefore, we encourage authors to use these characteristics for species diagnosis in the particular case of *Acusicola*.

Morphometric data and ratios of body to appendage segment lengths have proven to be very useful across many families of cyclopoid copepods, including Ergasilidae (Suárez-Morales *et al*., 2008; Oliveira *et al*., 2021; Uyeno and Nagasawa, 2021; Paschoal *et al*., 2022, 2023). Despite such an importance, the way that the information is described varies considerably in *Acusicola*, with numerous species lacking complete information about their measurements or proportions (Cressey and Collette, 1970; Amado and Rocha, 1996; El-Rashidy and Boxshall, 1999; Araújo and Boxshall, 2001; Santacruz *et al*., 2020). Therefore, it is highly recommended that further studies provide descriptions with most detailed morphometric data possible, to facilitate the intraspecific comparisons among congeners.

The use of body size as a diagnostic feature for parasitic copepods is also contradictory, since it can be heavily influenced by host–parasite interactions, as highlighted by Araújo and Boxshall (2001) and that we agree. Therefore, authors should prioritize other characters whenever feasible, as previously commented. Additionally, in the present study, the ornamentation of body segments (presence and absence of spinules, protrusions, etc.), interpodal plates and appendages, has proven to be useful for supplementing the specific diagnosis of the new species, and differentiates it from the closest congeners. This approach is common regarding other ergasilid genera (e.g. *Ergasilus*), but not that frequent in *Acusicola* (Boxshall, 2016; Taborda *et al*., 2016; Marques *et al*., 2017; Couto *et al*., 2024a). In this sense, despite the small size and occasional difficult visualization, it is fundamental for future studies not to neglect these features and provide more detailed descriptions, in order to enhance the knowledge on the morphological diversity of *Acusicola* spp., as well as of ergasilid copepods in general.

Most of the evolutionary modifications observed in parasitic copepods are reflected in their attachment apparatus, which influences the interaction with their hosts and the pathology they cause (Boxshall and Halsey, 2004; Pádua *et al*., 2015). In Ergasilidae, the prehensile antenna is usually the main appendage responsible for attachment, and varies greatly among genera. A judicious morphological analysis of the antennae from the species of *Acusicola* indicates great diversity of adaptations, which can be extremely informative for taxonomists. For example, the antennae of the three species described by El-Rashidy and Boxshall (1999) differ considerably in length, width, proportion and ornamentation of the first endopodal segment, and in relation to the membranous sheath: in *A. mazatlanensis*, this segment is 6.5× longer than wide, with small conical spinules, and the membranous sheath reaches the half of the second endopodal segment; in *A. joturicola*, it is also 6.5× longer than wide and share small conical spinules, but the membranous sheath reaches the first half of the outer margin of the claw; and *A. spinuloderma* has this segment 5.5× longer than wide, with large conical spines that decrease in size proximally and distally, and the membranous sheath reaches the vestigial third endopodal segment. In the study by Amado and Rocha (1996), the species also shows variation in the length-to-width ratio of the first endopodal segment, in the proportion of the second endopodal segment compared to the anterior segment, and in the ornamentation of both segments. Moreover, other notable variations can be observed in the descriptions of *A. pasternakae* and *A. minuta* (Araújo and Boxshall, 2001; Couto *et al*., 2023). Nevertheless, as the antennae represent the main attachment structure in *Acusicola*, it is reasonable to assume that it exhibits many of the adaptations acquired during its evolutionary history and holds taxonomic value, as previously discussed. In conclusion, it is recommended to consider the modifications of the antennae together with the leg armature, as important diagnostic features when dealing with these parasitic copepods.

Since the erection of *Acusicola*, only one dichotomous key has been provided for species identification (Amado and Rocha, 1996). Amado and Rocha (1996) relied primarily on the armature and proportion of leg elements of the ten known species at that time. Subsequently, eight additional species have been described, and new morphological traits have been documented in the genus, highlighting the necessity of updating this important taxonomic tool (Couto *et al*., 2023; Walter and Boxshall, 2024). Recently, Couto *et al*. (2024b) observed that mounting copepods in permanent slides can compress and distort body shape. Although the shape of cephalosome seems to be taxonomically informative in Ergasilidae, we are cautious when using this feature here, since *A. tucunarense*, *A. pellonidis*, and *A. lycengraulidis* were described based on permanent slides and could suffer from the same problems observed in previous works (Thatcher and Boeger, 1983a, 1983b; Thatcher, 1984; Couto *et al*., 2024b). Morphometric data and proportions of body, and leg segments and elements were used only as supporting data for identifying only certain species, since not all species of *Acusicola* has this information available. The prehensile antenna was also used here as an important diagnostic feature, since it appears to be highly informative in the genus (see above). The key provided herein was primarily developed in accordance with the features used by Amado and Rocha (1996), and supplemented with other reliable, easily observable and well-documented characters. The objective of this key is to be reliable and straightforward, as well as to be used by specialists on copepod taxonomy and other parasitologists alike. The key is provided as follows:

Key for species of *Acusicola*

(1) Cephalosome fused to first pedigerous somite . . . . (2)
Cephalosome not fused to first pedigerous somite . . . . (8)

(2) Second endopodal segment of leg 1 with two spines and five setae; third exopodal segment of leg 2 with outer spine . . . . (3)

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Second endopodal segment of leg 1 with two spines and three setae; third exopodal segment of leg 2 without outer spine . . . . *A. lycengraulidis*

(3) First exopodal segment of leg 1 with outer spine; third exopodal segment of leg 1 with two spines and five setae . . . . (4)
First exopodal segment of leg 1 unarmed; third exopodal segment of leg 1 with three spines and four setae . . . . *A. tenax*

(4) Third and fourth pedigerous somites with dorsal surface smooth . . . . (5)
Third and fourth pedigerous somites with three anterior protrusions on dorsal surface each . . . . *A. rochai* n. sp.

(5) Claw of antenna not enclosed by membranous sheath . . . . (6)
Claw of antennae enclosed by membranous sheath on outer margin . . . . *A. joturicola*

(6) First endopodal segment of antenna smooth or ornamented with small conical spinules . . . . (7)
First endopodal segment ornamented with large conical spinules decreasing in size proximally and distally . . . . *A. spinuloderma*

(7) First endopodal segment of antenna smooth; this segment is about 4.9× longer than wide . . . . *A. pellonidis*
First endopodal segment of antenna ornamented with small cone-shaped spinules; this segment is about 6.5× longer than wide . . . . *A. mazatlanensis*

(8) Second endopodal segment of leg 1 with at least six elements . . . . (9)
Second endopodal segment of leg 1 with three minute spine . . . . *A. spinulosa*

(9) First exopodal segment of leg 1 with outer spine . . . . (10)
First exopodal segment of leg 1 unarmed . . . . *A. tucunarense*

(10) Third endopodal segment of leg 1 with two spines and four or five setae; first endopodal segment of leg 4 with one seta . . . . (11)
Third endopodal segment of leg 1 with one spine and four setae; first endopodal segment of leg 4 unarmed . . . . . . *A. rogeri*

(11) Leg 5 reduced to single seta . . . . (12)
Leg 5 reduced to two setae . . . . (13)

(12) Third exopodal segment of legs 2 and 3 with outer spine; second endopodal segment of antenna not enclosed by dark membrane . . . . *A. cunula*
Third exopodal segment of legs 2 and 3 without outer spine; second endopodal segment of antenna enclosed by dark membrane . . . . *A. paracunula*

(13) Second endopodal segment of leg 1 with two spines and four setae . . . . (14)
Second endopodal segment of leg 1 with two spines and five setae . . . . (16)

(14) Fourth pedigerous somites with dorsal surface smooth . . . . (15)
Fourth pedigerous somites with two anterior protrusions on dorsal surface . . . . *A. iamarinoi*

(15) Cephalosome inflated, with antennal area projected forwards; first exopodal segment of legs 2 and 3 unarmed . . . . *A. rotunda*
Cephalosome not inflated, antennal area not projected forwards; first exopodal segment of legs 2 and 3 with outer spine . . . . *A. pasternakae*

(16) Second endopodal segment of antenna without inner membranous expansions; first endopodal segment of leg 1 longer than whole exopod . . . . (17)
Second endopodal segment of antenna with two inner membranous expansions; first endopodal segment of leg 1 shorter than whole exopod*****A. minuta*

(17) Second exopodal segment of legs 1–3 with small inner process near setae . . . . *A. margulisae*
Second exopodal segment of legs 1–3 without small inner process . . . . *A. brasiliensis*

Data. The authors confirm that the data supporting the findings of this study are available within the article.

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References

Amado MAPM and Rocha CEF (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.

Araújo HMP and Boxshall GA (2001) A new species of *Acusicola* Cressey (Copepoda: Ergasilidae) from Northeastern Brazil. *Systematic Parasitology* **49**, 149–157.

Boxshall GA (2016) A new species of *Ergasilus* von Nordmann, 1832 (Copepoda: Cyclopoida) from the gills of a Dasyatid ray, *Himantura oxyrhyncha* (Sauvage, 1878) from West Kalimantan, Indonesia. *Zootaxa* **4174**, 93–103.

Boxshall GA and Halsey SH (2004) *An Introduction to Copepod Diversity*. London: The Ray Society.

Bush JO, Lafferty KD, Lotz JM and Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. Revisited. *Journal of Parasitology* **83**, 575–583.

Checon HH, Costa HHR, Corte GN, Souza FM and Pombo M (2023) Rainfall influences the patterns of diversity and species distribution in sandy beaches of the Amazon coast. *Sustainability* **15**, 5417.

Couto JV, Nunes JLS, Rincon G, Paschoal F and Pereira FB (2024a) *Ergasilus lyraephorus* n. sp. (Copepoda: Cyclopoida:Ergasilidae) parasitic on the longtail knifefish *Sternopygus macrurus* (Bloch & Schneider, 1801) (Actinopterygii: Sternopygidae) from Northeast Brazil. *Systematic Parasitology* **101**, 6.

Couto JV, Pereira AN, Luque JL, Paschoal F and Pereira FB (2023) Two new species of *Acusicola* Cressey, 1970 (Copepoda: Cyclopoida: Ergasilidae) parasitic on the gills of two estuarine actinopterygians off Brazil. *Systematic Parasitology* **100**, 133–148.

Couto JV, Potes AJ, Feitosa CV, Pereira FB and Paschoal F (2024b) Redescription of *Hatschekia exigua* Pearse, 1951 (Copepoda: Hatschekiidae), parasitic on the Squirrelfish *Holocentrus adscensionis* (Osbeck) (Actinopterygii: Holocentridae) off Ceará, Northeastern Brazil, with first description of the male. *Systematic Parasitology* **101**, 27.

Cressey R and Collette B (1970) Copepods and needlefishes: a study in host-parasite relationships. *Fishery Bulletin* **68**, 347–432.

Diniz DG, Varella JEA, Guimarães MDF, Santos AFL, Fujimoto RY, Monfort KCF, Pires MAB, Martins ML and Eiras JC (2008) A note on the occurrence of praniza larvae of Gnathiidae (Crustacea, Isopoda) on fishes from northeast of Pará, Brazil. *Anais Da Academia Brasileira de Ciências* **80**, 657–664.

El-Rashidy RH and Boxshall GA (1999) Ergasilid copepods (Poecilostomatoida) from the gills of primitive Mugilidae (grey mullets). *Systematic Parasitology* **42**, 161–186.

Esteves-Silva PH, Oliveira MSB, Gentil-Vasconcelos HC, Costa-Campos CE and Tavares-Dias M (2020) New records of hosts for *Excorallana longicornis* and *Nerocila acuminata* (Crustacea: Isopoda) in brackish fish from the coast of the State of Amapá (Brazil), with an update on the geographic distribution of *Nerocila acuminata*. *Journal of Parasitic Diseases* **44**, 420–428.

Figueiredo M, Nunes J, Almeida Z, Paz A, Piorski N and Reis M (2019) Feeding ecology of *Anableps anableps* (Actinopterygii: Cyprinodontiformes: Anablepidae) off the north-eastern coast of Brazil. *Acta Ichthyologica et Piscatoria* **49**, 213–219.

Froese R and Pauly D (2024) *FishBase*. Available at http://www.fishbase.org (Accessed online 4 March 2024).

Hayashi SN, Souza-Filho PWM, Nascimento Júnior WR and Fernandes MEB (2019) The effect of anthropogenic drivers on spatial patterns of mangrove land use on the Amazon coast. *PLoS ONE* **14**, e0217754.

Humes A and Gooding R (1964) A method for studying the external anatomy of copepods. *Crustaceana* **6**, 238–240.

Jimenez EA, Barboza RSL, Amaral MT and Frédou FL (2019) Understanding changes to fish stock abundance and associated conflicts: perceptions of small-scale fishers from the Amazon coast of Brazil. *Ocean and Coastal Management* **182**, 104954.

Loureiro SN, Trindade P, Diniz DG, Vallinoto M, Diniz JAP and Giarrizzo T (2021) First record of *Cymothoa* sp. Fabricius, 1793 (Isopoda: Cymothoidae) parasitizing the four-eyed fish, *Anableps anableps* (Cyprinodontiformes: Anablepidae), on the northern Brazilian coast. *Biota Amazônia* **11**, 68–70.

Luque JL and Poulin R (2007) Metazoan parasite species richness in Neotropical fishes: hotspots and the geography of biodiversity. *Parasitology* **134**, 865–878.

Luque JL and Tavares LER (2007) Checklist of Copepoda associated with fishes from Brazil. *Zootaxa* **1579**, 1–39.

Luque JL, Vieira FM, Takemoto RM, Pavanelli GC and Eiras JC (2013) Checklist of Crustacea parasitizing fishes from Brazil. *Check List* **9**, 1449.

Malta JCO (1993) *Myracetima piraya* sp. nov. (Copepoda, Ergasilidae) das Brânquias de *Pygocentrus nattereri* (Kner, 1860) (Characiformes: Serrasalmidae) da Amazônia Brasileira. *Acta Amazonica* **23**, 261–269.

Marceniuk AP, Caires RA, Carvalho-Filho A, Rotundo MM, Santos WCRD and Klautau AGCDM (2021) *Peixes teleósteos da costa norte do Brasil*. Belém: Museu Paraense Emílio Goeldi, 775p.

Marques TM, Clebsh L, Córdova L and Boeger WA (2017) *Ergasilus turkayi* n. sp. (Copepoda, Cyclopoida, Ergasilidae): a gill parasite of *Serrasalmus hollandi* Jégu, 2003 (Characiformes, Serrasalmidae) from the Paragua River, Bolivia. *Nauplius* **25**, e2017020.

Nelson JS, Grande TC and Wilson MVH (2016) *Fishes of the World*, 5th Edn. New Jersey: John Wiley & Sons.

Oliveira MSB, Corrêa LL, Adriano EA and Tavares-Dias M (2021) Integrative taxonomy of a new species of *Therodamas* (Ergasilidae) infecting the Amazonian freshwater fish *Leporinus fasciatus* (Anostomidae). *Parasitology Research* **120**, 3137–3147.

Pádua SB, Jerônimo GT, Menezes-Filho RN, Taboga SR, Martins ML and Andrade Belo MA (2015) Pathological assessment of farmed yellowtail tetra *Astyanax altiparanae* infested by *Acusicola* sp. (Ergasilidae). *Aquaculture Reports* **2**, 63–66.

Paschoal F, Couto JV, Pereira FB and Luque JL (2022) A new species of Hatschekiid copepod (Crustacea: Hatschekiidae) parasitic on the porkfish *Anisotremus virginicus* (Linnaeus, 1758) (Actinopterygii: Haemulidae), with notes on previously known species of *Hatschekia* Poche, 1902 collected from actinopterygians off Brazil. *Acta Parasitologica* **67**, 1126–1135.

Paschoal F, Couto JV, Pereira FB and Luque JL (2023) A new species of *Colobomatus* Hesse, 1873 (Copepoda: Philichthyidae) parasitic in the inter-orbital canals of the Caitipa mojarra *Diapterus rhombeus* (Cuvier, 1829) (Actinopterygii: Gerreidae) from Sepetiba Bay, Southeastern Brazil. *Systematic Parasitology* **100**, 111–120.

Pereira LCC, Dias JA, Carmo JA and Polette M (2009) Preface: the Brazilian Amazon coastal zone. *Revista de Gestão Costeira Integrada* **9**, 3–7.

Roberts LS (1965) *Ergasilus tenax* sp. n. (Copepoda: Cyclopoida) from the white crappie, *Pomoxis annularis* Rafinesque. *The Journal of Parasitology* **51**, 987–989.

Rodrigues E, Machado F, Oliveira R and Andrade M (2021) Target fishes from subsistence fishing in a riverine community from lower Pará River, Northern Amazonia. *International Journal of Fisheries and Aquatic Studies* **9**, 54–59.

Santacruz A, Morales-Serna FN, Leal-Cardín M, Barluenga M and Léon GP (2020) *Acusicola margulisae* n. sp. (Copepoda: Ergasilidae) fom freshwater fishes in a Nicaraguan crater lake based on morphological and molecular evidence. *Systematic Parasitology* **97**, 165–177.

Schioedte JC and Meinert F (1884) Symbolæ ad Monographium Cymothoarum Crustaceorum Isopodum Familiæ. IV. Cymothoidæ Trib. II. Cymothoinæ. Trib. III: Lironecinæ. *Naturhistorisk Tidsskrift* **14**, 221–454.

Suárez-Morales E, Santana-Piñeros A and González-Solís D (2008) A new species and host range of *Therodamas* (Copepoda, Ergasilidae) from the Eastern Tropical Pacific. *Crustaceana* **81**, 1107–1117.

Szlafsztein CF (2012) The Brazilian Amazon coastal zone management: implementation and development obstacles. *Journal of Coast Conservation* **16**, 335–343.

Taborda NL, Paschoal F and Luque JL (2016) A new species of *Ergasilus* (Copepoda: Ergasilidae) from *Geophagus altifrons* and *G. argyrostictus* (Perciformes: Cichlidae) in the Brazilian Amazon. *Acta Parasitologica* **61**, 549–555.

Tenório GE, Souza-Filho PWM, Ramos EMLS and Alves PJO (2015) Mangrove shrimp farm mapping and productivity on the Brazilian Amazon coast: environmental and economic reasons for coastal conservation. *Ocean & Coastal Management* **104**, 65–77.

Thatcher VE (1984) The parasitic crustaceans of fishes from the Brazilian Amazon, 7, *Acusicola tucunarense* n. sp. (Copepoda: Cyclopoidea) from *Cichla ocellaris*, with an emendation of the genus and the proposal of Acusicolinae Subfam. Nov. *Revista Brasileira de Biologia* **44**, 181–189.

Thatcher VE and Boeger W (1983a) The parasitic crustaceans of fishes from the Brazilian Amazon, 10, *Acusicola pellonidis* n. sp. (Copepoda: Cyclopidea) from *Pellona castelnaeana* (Valenciennes). *Amazoniana* **8**, 273–279.

Thatcher VE and Boeger W (1983b) The parasitic crustaceans of fishes from the Brazilian Amazon, 8, *Acusicola lycengraulidis* n. sp. (Copepoda: Cyclopidea) from *Lycengraulis grossidens* Cuvier. *Acta Amazonica* **13**, 943–951.

Thatcher VE and Paredes V (1985) A parasitic copepod, *Amplexibranchius bryconis* gen. et sp. nov. (Ergasilidae: Acusicolinae), from an Amazonian fish and remarks on the importance of leg morphology in this subfamily. *Amazoniana* **9**, 205–214.

Tosetto EG, Bertrand A, Neumann-Leitão S and Nogueira Júnior M (2022) The Amazon River plume, a barrier to animal dispersal in the Western Tropical Atlantic. *Scientific Reports* **12**, 537.

Uyeno D and Nagasawa K (2021) Three species of copepods parasitic on the blue mackerel *Scomber australasicus* Cuvier (Actinopterygii: Perciformes: Scombridae) from Southern Japan, with description of a new species *Colobomatus itoui* n. sp. (Cyclopoida: Philichthyidae). *Systematic Parasitology* **98**, 625–640.

Van der Laan R, Fricke R and Eschmeyer WN (2023) *Eschmeyer's Catalog of Fishes. Classification*. Available at www.calacademy.org/scientists/catalog-of-fishes-classification (Accessed online 4 March 2024).

Walter TC and Boxshall GA (2024) *World of Copepods Database. Ergasilidae Burmeister, 1835. WoRMS*. Available at https://www.marinespecies.org/copepoda/aphia.php?p=taxdetails&id=128571 (Accessed online 4 March 2024).