

Redescription of *Hatschekia exigua* Pearse, 1951 (Copepoda: Hatschekiidae), parasitic on the Squirrelfish *Holocentrus adscensionis* (Osbeck, 1765) (Actinopterygii: Holocentridae) off Ceará, Northeastern Brazil, with first description of the male

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Abstract The original description of *Hatschekia* exigua Pearse, 1951 neglected innumerous features of taxonomic value as well as morphometric data and illustrations. Posteriorly, other author tried to access the type material, but their poor state of preservation compromised a detailed redescription. Since then, this species is in need for new morphological data, mainly from fresh material. In the present work, three specimens of *Holocentrus adscensionis* from Mucuripe

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Bay, Fortaleza, State of Ceará, Northeastern Brazil, were parasitized by copepods on their gills. Parasites were fixed and preserved in 80% ethanol and cleared in 85% lactic acid for morphological observations using light microscopy. The females were identified as Hatschekia exigua by the cephalothorax representing about one-fourth of total body length, with lateral margins expanded into lateral lobes, first exopod with basal segment armed with one outer setae and terminal segment with three setae, first endopod with basal segment unarmed and terminal segment with five setae, leg 3 reduced to two setae and leg 4 reduced to single seta. Comparison with the type series revealed morphological differences in trunk and small appendages, which may be related to alterations in the specimens, caused by the mounting methodology and poor preservation. A detailed morphological analysis of the male revealed for the first time that they differ from their closest congeners by having five setae on the last endopodal segment of leg 1, by smooth intercoxal sclerites on legs 1 and 2 and by a proximolateral process on the third segment of antenna. Moreover, this work represents the first report of H. exigua in Brazil and the first hatschekild copepod found off the coast of Ceará, highlighting that the diversity of Hatschekiidae in this oceanographic region still needs further investigation.

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Fig. 1 *Hatschekia exigua* Pearse, 1951 (adult female). **A** habitus, dorsal, p1= leg 1, p2= leg 2, p3= leg 3, p4= leg 4; **B** detail of cephalothorax, dorsal, a1= antennule, a2= antenna, ch= dorsal chitinous

supporting frame, ma= maxilla; C posterior part of trunk, ventral, it= internal tissues; D caudal ramus, ventral; E egg sac, lateral. Scale bars: A= 300 μ m, B= 100 μ m, C= 40 μ m, D= 20 μ m, E= 400 μ m

Introduction

Sipohonostomatoid copepods of the family Hatschekiidae Kabata, 1979 represent one of the most diverse groups of parasitic copepods commonly found on the gills of marine actinopterygians (Boxshall & Halsey, 2004; Uyeno & Ali, 2013; Paschoal et al., 2022). Currently, this family comprises about 168 species, distributed within nine valid genera: *Bassettithia* Wilson, 1922, *Brachihatschekia* Castro-Romero & BaezaKuroki, 1989, *Congericola* Beneden, 1854, *Hatschekia* Poche, 1902, *Laminohatschekia* Boxshall, 1989; *Mihbaicola* Uyeno, 2013, *Prohatschekia* Nunes-Ruivo, 1954, *Pseudocongericola* Yü, 1933 and *Wynnowenia* Boxshall, 1987 (Walter & Boxshall, 2023).

Although Hatschekia represents the most speciose genus within Hatschekiidae, with about 149 species described worldwide (Paschoal et al., 2022; Walter & Boxshall, 2023), research on these copepods in the Southwestern Atlantic is scarce, with only three species reported so far, i.e., Hatschekia conifera Yamaguti, 1939 from the Atlantic Pomfret Brama brama (Bonnaterre) (Bramidae) off Mar del Plata and San Matías gulf, Argentina, Hatschekia nagasawai Paschoal, Couto, Pereira & Luque, 2022 from the Porkfish Anisotremus virginicus (Linnaeus) (Haemulidae) off Rio de Janeiro, Brazil and Hatschekia priacanthis Izawa, 2016, from the Atlantic Bigeye Priacanthus arenatus (Cuvier) (Priacanthidae) off Rio de Janeiro, Brazil (Luque & Tavares, 2007; Cantatore et al., 2012; Paschoal et al., 2022). Additionally, there are two undetermined species of Hatschekia, from the Coney Cephalopholis fulva (Linnaeus) (Serranidae) and the Bluewing *Prionotus punctatus* (Bloch) (Triglidae), off Rio de Janeiro, Brazil (Paschoal et al., 2022).

Hatschekia exigua Pearse, 1951 was originally described by Pearse (1951) based on three females collected from the gills of *Holocentrus adscensionis* Osbeck (Holocentridae), off Bimini, Bahamas. Despite the remarkable sampling effort and the relevance of his work, the original description of *H. exigua* is poorly detailed, lacking some structures with taxonomic importance and morphometric data, which can mislead the diagnosis and identification by other researchers (Jones, 1985). Subsequently, this species was revisited by Jones (1985) in a revision of the genus, pointing several morphological incongruences with the original description, as the segmentation and armature of the antennule and the morphology of leg 1 rami. Jones (1985) also affirmed that the type specimens were distorted and poorly preserved, compromising the observation of important morphological features and hindering a detailed redescription. Moreover, the author stated that a full redescription of *H. exigua* is needed, based on fresh material, collected from the type host (Jones, 1985).

During a survey of parasitic copepods on *Ho. adscensionis* from the Brazilian coastal zone, some specimens of *H. exigua* were recovered from the fish gills. In this paper, we provide a complete and detailed redescription of the female and the first description of the male of this species.

Materials and Methods

Three specimens of Ho. adscensionis (total length 25.3–29; mean \pm standard deviation 27.6 \pm 2) were bought from local fishermen in the Mucuripe Bay (3°43'18"S, 38°28'51"W), Fortaleza, State of Ceará, Northeastern Brazil. Copepods were collected from the gills, fixed, and preserved in 80% ethanol until morphological analysis. For microscopical observation, 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 Nikon Eclipse Ei (Nikon Instruments Inc, New York, USA) attached to an image capture system. Measurements were based on 14 adult females and six males, using the parameters proposed by Uyeno and Nagasawa (2009a; see Fig. 1), given as the range followed by mean and standard deviation in parentheses, all in micrometers. The type material of H. exigua deposited in the invertebrate collection of the National Museum of Natural History (accession code NMNH-88531), Smithsonian Institution of USA, studied by Pearse (1951) was also examined. The morphological terminology follows Huys and Boxshall (1991). Ecological terminology adopted for parasites is according to Bush et al. (1997). Host identification was based on Figueiredo and Menezes (1980), and 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: "H." for *Hatschekia* and "*Ho.*" for *Holocentrus*. Voucher specimens were deposited in the Coleção Carcinológica of the 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 the Brazilian Federal requirements.

Systematics

Order Siphonostomatoida Burmeister, 1835 Family Hatschekiidae Kabata, 1979 Genus *Hatschekia* Poche, 1902 *Hatschekia exigua* Pearse, 1951

Type-host and type-locality: The Squirrelfish *Holocentrus adscensionis* (Osbeck) (Actinopterygii: Holocentridae); Bimini, Bahamas, Caribbean Sea (geographical coordinates not available) (Pearse, 1951).

Host and locality of present material: Ho. adscensionis; Mucuripe Bay (3°43'18"S, 38°28'51"W), Fortaleza, State of Ceará, Northeastern Brazil.

Site on host: Gills.

Prevalence and mean intensity: 100% (three fish infested out of three analyzed); mean of 6 copepods per infested fish (range 4–9).

Voucher specimens: 14 females (MZUSP-45252) and six males (MZUSP-45253). Four specimens were dissected and kept in the personal collection of the first author.

Redescription of adult female [based on 18 specimens; Figs. 1-3]. Body elongate (Fig. 1A), comprising distinct cephalothoracic head, short neck and long cylindrical trunk; external cuticular layer smooth. Body length 690–1138 (1004 \pm 128.1), excluding caudal rami. Cephalothorax (Figs. 1A, B) forming octagonal to ovoid segment, expanded laterally forming blunt lobes and expanded posteriorly, shorter than wide 192–273 (218 \pm 23.5) \times 201–304 (260 \pm 30.2), representing about one-fourth of total body length; dorsal surface bearing chitinous supporting frame resembling key-hole shape, symmetrically with median longitudinal thick bar; each anterolateral corner with process anteriorly and two median transverse slender bars connecting to median thick one; posterior part deep (Fig. 1B). Trunk fusiform, longer than wide, $473-940 (770 \pm 129.7) \times 189-408 (276 \pm$ 60.9), lacking posterolateral lobes or processes, with anterior narrow and short "neck"; expanding to maximum width at region slightly posterior to level of first leg level and gradually narrowing towards posterior end; internal tissues separated from cuticle and apparently sheathed with cuticular membrane. Urosome (Fig. 1C) comprising fused genital complex and abdomen, wider than long 32–60 (48 ± 9.5) × 45–75 (60 ± 11.2). Caudal ramus (Fig. 1D) incompletely fused to urosome, longer than wide 24–35 (30 ± 3.3) × 12–18 (15 ± 1.7), with five naked setae: four distal and one lateral. Egg sacs (Fig. 1E) shorter than trunk, uniseriate, with mean of eight eggs per sac, range from six to ten eggs per sac (n = 5).

Rostrum absent. Antennule (Fig. 2A) indistinctly five segmented, $118-171 (152 \pm 15.1) \log$; armature formula: 6, 3, 3, 1, 7 + 2 aesthetascs. Antenna (Fig. 2B) with three segments: proximal (coxa) unarmed, mid (basis) ornamented with circular pit and terminal claw short and robust. Antenna 169-228 (204 ± 19.4) long; length of proximal, mid, and terminal segments 30-66 (53 \pm 10.6), 82-120 (103 \pm 12.2) and 40–57 (48 \pm 5.4), respectively. Parabasal papilla (Fig. 2B) blunt, thumb-like with annulated surface. Oral cone robust. Mandible (Fig. 2D) styliform tapering posteriorly. Maxillule (Fig. 2E) bilobate, both lobes armed with two tapering setae of unequal sizes. Maxilla (Fig. 2C) with four segments: proximal unarmed; second rod-like with one basal seta and row of blunt, fine spinules on middle outer surface; third elongate with one distal seta. Terminal segment small, with one short seta and bifid claw. Maxilliped absent.

Legs 1 and 2 biramous, each joined by bar-like smooth intercoxal sclerite (Fig. 3C). Exopods represented by two incompletely fused segments and twosegmented endopods. All setae on rami naked and thick. Armature formula as follow:

	Protopod	Exopod	Endopod
Leg 1	1-1	1–0; 4	0–0; 5
Leg 2	1–0	1–0; 4	0–1;4

Leg 1 (Fig. 3A) 92–114 (103 ± 6.7) long, with coxa and basis fused forming large protopod and retaining trace of suture. Protopod 56–65 (60 ± 2.9) long, with large inner seta and slender outer seta. Exopod longer than endopod, 35-51 (43 ± 5.7) and 29–38 (34 ± 3.2) long, respectively. Leg 2 (Fig. 3B)





Fig. 2 *Hatschekia exigua* Pearse, 1951 (adult female). A antennule, dorsal, arrows pointing to aesthetascs; B antenna, ventral, arrow pointing to parabasal papilla; C maxilla, ventral;

111–146 (131 \pm 11.4) long, with coxa and basis fused to form protopod, retaining trace of suture. Protopod 71–84 (76 \pm 4.6) long, with slender basal outer seta. Exopod longer than endopod, 40–65 (55 \pm 8.1) and 37–60 (52 \pm 5.5) long, respectively. Protopods and rami of legs 1 and 2 ornamented with rows of blunt and fine spinules on anterior surface.

Leg 3 (Fig. 3D) represented by two simple naked setae inserted in rounded papilla at middle of trunk. Leg 4 (Fig. 3E) represented by small naked seta located at posterior quarter of trunk with slightly swollen base.

Description of adult male [based on six specimens; Figs. 4, 5]. Body (Fig. 4A) comprising cephalothorax, short neck cylindrical trunk containing spermatophores, and with lateral seta representing leg

D mandible, ventral; **E** maxillule, ventral. Scale bars: A-B= 20 µm, C= 30 µm, D= 5 µm, E= 10 µm

6 (Figs. 4A, C) on genital operculum. Body length $396-450 (432 \pm 24.5)$, excluding caudal rami. Cephalothorax (Figs. 4A, B) longer than wide 140-153 $(149 \pm 6) \times 119 - 127 (123 \pm 3.3)$, representing about one-third of total body length; dorsal surface bearing chitinous supporting frame (Fig. 4B), symmetrical with median longitudinal thick bar; form somewhat trapezoidal, divided anteriorly, with two rounded processes in anterolateral corners; first third formed by square process inserted in transverse bars, each with one blunt process at anterior margin, and spatulashaped process deep in posterior margins; remaining part continuous, forming base of trapezium. Urosome (Fig. 4C) longer than wide $35-43 (40 \pm 3.6) \times 35-39$ (36 ± 1.7) . Caudal ramus (Fig. 4C) incompletely fused to urosome, longer than wide 41-50 (46 ± 3.9)



Fig. 3 *Hatschekia exigua* Pearse, 1951 (adult female). **A** leg 1, ventral; **B** leg 2, ventral; **C** intercoxal sclerites of legs 1 and 2, ventral; **D** leg 3, ventral; **E** leg 4, ventral. Scale bars: $A-B=20 \mu m$, $C=40 \mu m$, $D-E=20 \mu m$

 \times 13–16 (15 ± 1.5), with six naked setae: five distal and one lateral.

Rostrum (Fig. 5A) formed by triangular plate with pair of blunt teeth anteriorly, and middle pointed process; pair of robust blunt rostral processes laterally to plate. Antennule (Fig. 5B) five segmented, 123–142 (132 ± 8.2) long; armature formula: 10, 4, 5, 1, 8 + 2 aesthetascs. Antenna (Fig. 5C) with three segments; last segment with proximolateral process and armed with two setae, terminal claw short and robust. Antenna 111–126 (121 ± 6.8) long; length of proximal, mid, and terminal segments 20–36 (29 ± 8.5), 52–58 (55 ± 2.5) and 29–45 (37 ± 6.6), respectively. Parabasal papilla (Fig. 5C) blunt, thumb-like, with annulated surface. Oral cone, mandible, maxillulae and maxilla as in female. Maxilliped absent.

Legs 1 and 2 biramous, joined by bar-like smooth intercoxal sclerite (Fig. 3D). Exopods represented by

two incompletely fused segments and two-segmented endopods. All setae on rami naked and thick. Armature formula as follow:

	Protopod	Exopod	Endopod
Leg 1	1-1	1–0; 3	0–0; 5
Leg 2	1–0	1–0; 4	0–1;4

Leg 1 (Fig. 5D) 73–78 (75 \pm 2.2) long, with coxa and basis fused to form protopod, retaining trace of suture. Protopod, exopod and endopod length 38–42 (41 \pm 2), 31–40 (34 \pm 4.1) and 23–26 (25 \pm 1.4) long, respectively. Armature of leg 1 same as in female. Leg 2 (Fig. 5E) 82–94 (89 \pm 5) long, with coxa and basis fused to form protopod, retaining trace of suture. Protopod, exopod and endopod 38–42 (41 \pm 2), 31–40 (34 \pm 4.1) and



Fig. 4 *Hatschekia exigua* Pearse, 1951 (adult male). **A** habitus, dorsal, p1= leg 1, p2= leg 2, p3= leg 3, p4= leg 4, p6= leg 6; **B** detail of cephalothorax, dorsal, a1= antennule, a2=

23–26 (25 \pm 1.4) long, respectively. Protopods and rami of legs 1 and 2 ornamented with rows of blunt and fine spinules on anterior surface.

Leg 3 (Fig. 5G) represented by lateral lobe armed with five setae: two apical, two lateral and one near base. Leg 4 (Fig. 5H) represented by small naked seta located at posterior two thirds of trunk with slightly swollen base.

Discussion

The females analyzed in this work were identified as H. *exigua* because of the cephalothorax representing about one-fourth of total body length, with convex

antenna, ch= dorsal chitinous supporting frame, ma= maxilla; C posterior part of trunk, ventral. Scale bars: A= 50 μ m, C-D= 20 μ m

lateral margins and expanded into rounded lateral lobes, the first exopod armed with one seta on the basal segment and three setae on the terminal segment, as well as with basal segment unarmed and terminal segment with five setae, the leg 3 reduced to two setae and the leg 4 reduced to single seta (Pearse, 1951). Moreover, considering that species of *Hatschekia* are highly host-specific at family level, the present specimens were collected from *Ho. adscensionis*, type host of *H. exigua*, and the fact that no other congener has been reported infesting Holocentridae fish, the specific identification of the present material is strongly supported (Kabata, 1979; Uyeno & Ali, 2013).



Fig. 5 Hatschekia exigua Pearse, 1951 (adult male). A detail of rostrum, ventral; B antennule, dorsal, arrows pointing to aesthetascs; C antenna, ventral, arrow pointing to parabasal

papilla; **D** leg 1, ventral; **E** leg 2, ventral; **F** intercoxal sclerites of legs 1 and 2, ventral; **G** leg 3, ventral; **H** leg 4, ventral. Scale bars: $A-B=30 \mu m$, $C=20 \mu m$, $C-H=10 \mu m$

Examination of *H. exigua* type series (NMNH-88531) revealed the poor condition of material. It is represented by three copepods mounted on the same slide, in which the specimens are distorted and none of them show all the diagnostic features clearly. Based on the comparative analysis between the present specimens and those studied by Pearse (1951), it was possible to observe that the antennule of H. exigua was not 3-segmented, but at least 5-segmented. Further morphological comparisons were also compromised by the poorly detailed description of the type material (Pearse, 1951). As previously commented, Jones (1985) provided additional data and illustrations of the types of H. exigua but could not adequately redescribe the species. According to Jones (1985), the cephalothorax of *H. exigua* has lateral margins expanded into blunt lobes and the posterior margin of the trunk has small lobes on either side. Nevertheless, the cephalothorax of the present species has lateral blunt lobes, but not that prominent as illustrated by Jones (1985), and the posterior end of trunk is smooth (see Figs. 7L, M in Jones, 1985). These differences may be accounted by the mounting methodology and the poor conservation of the type material, which is distorted and compressed, compromising a detailed observation of body and appendages by Jones (1985). These new observations on the females, contribute to a better understanding H. exigua morphology, preventing further taxonomic confusion.

The small appendages of Hatschekia spp. are structures of great complexity, some apparently stable within conspecific individuals and others with intraspecific variability. These different levels of variability make several authors concern about which features should be used in the specific diagnosis and differentiation of species (Kabata, 1991; Uyeno & Nagasawa, 2009a; 2013; Paschoal et al., 2022). In this context, *H. exigua* presented intraspecific variability in the setation pattern of leg 2 when compared with the observations by Jones (1985), since the leg 2 was described with four and six apical setae on the terminal segments of exopod and endopod, respectively, while in the present study these segments were armed with three and four setae. The present specimens also differed from those described by Jones (1985) in the armature of the caudal ramus, while the author described at least four setae in this appendage, it was armed with six setae in the present material. Therefore, it is reasonable to consider that these differences could be related to difficulties in the observation of the appendages or due to the loss of some setae by preservation issues (see paragraph above), mainly on the caudal ramus. However, the armature on the rami of legs 1 and 2 should be treated with caution because of its high intraspecific variability in the genus Hatschekia (Jones, 1985; Kabata, 1991; Uyeno & Nagasawa, 2013). In this sense, we reinforce that the general body plan and morphometric data should has preference in the specific diagnosis and differentiation, as well as the use of small appendages with great stability to support these analyses. At the time Pearse (1951) described *H. exigua*, there were only 37 valid congeneric species, and 75% (112 species) of the current richness of the genus was known posteriorly. Therefore, Hatschekia currently contains more than 140 valid species worldwide, 30 of which have a bar-like smooth intercoxal sclerite, and trunk lacking expansions, swellings, or processes as in the present females. Of these, only four have the same proportion of cephalothorax in relation to total body length as in H. exigua: Hatschekia aulacocephalis Izawa, 2016, Hatschekia cirrhitichthysicola Izawa, 2016, Hatschekia euanus Lee, Lee & Boxshall, 2013 and Hatschekia hoplobrotulae Izawa, 2015 (Lee et al., 2013; Izawa, 2015; 2016a). However, H. exigua can be distinguished from all these previously mentioned congeners because it has three setae on the last exopodal segment of leg 1 (vs. five setae in H. aulacocephalis; six in H. cirrhitichthysicola, H. euanus and H. hoplobrotulae) and five setae in the caudal rami (vs. six setae in the latter four species). Hatschekia exigua also differs from H. aulacocephalis based on the fourth segment of antennule lacking an enlarged ventral setae and the second segment of antenna unarmed (vs. enlarged seta present and second segment of antenna with proximoventral seta in the latter); from *H. cirrhitichthysicola* by rostral area lacking processes (vs. pointed processes in the latter); from H. cirrhitichthysicola and H. euanus by leg 3 reduced to two setae (vs. single seta in the latter two species); and from H. hoplobrotulae by leg 2 with three and four setae on the last exopodal and endopodal segment, respectively, and trunk 2.8 times longer than wide (vs. five setae on the last segment of both rami and trunk more than 3 times longer in the latter) (Lee et al., 2013; Izawa, 2015; 2016a).

Males of only 25 species of *Hatschekia* are currently known, indicating a scarce knowledge on such representatives (Wilson, 1913; Pearse, 1947; Nuñes-Ruivo, 1954; Scram & Aspholm, 1997; Uyeno & Nagasawa 2009a; b; El-Rashidy & Boxhall, 2011; Uyeno & Nagasawa, 2012; Izawa, 2015; 2016a; b; c; 2018). The present males differ from all congeners because they possesses leg 3 armed with five setae, feature that has never been reported in the genus. It should be mentioned that H. exigua shares the last exopodal segment of leg 1 armed with only three setae with two other congeners, i.e., Hatschekia curvata Yamaguti & Yamasu, 1959 and Hatschekia nemipteri Izawa, 2016 (Izawa, 2016a). However, H. exigua can be clearly differentiated from these species by having five setae on the last endopodal segment of leg 1 (vs. two setae on the latter two species). It also can be distinguished from *H. curvata* by smooth intercoxal sclerites on legs 1 and 2 (vs. intercoxal sclerites with processes in the latter); and from H. nemitpteri by a proximolateral process on the third segment of antenna (vs. absent in the latter) (Izawa, 2016a).

Since the establishment of *Hatschekia* in the 19th century, the differential diagnosis of species mainly relies on the morphology of females (Paschoal et al., 2022). Nevertheless, the morphological diversity of males, as presented here, was also important to support the specific diagnosis of *H. exigua*. Even though comparisons among males are less common in *Hatschekia*, it appears to be valuable for specific diagnosis and demonstrates that the body plan of males can be more diverse and useful than previously believed. Therefore, studies including males of *Hatschekia* should be better explored in future taxonomic studies, to contribute even more to the knowledge related to the genus and prevent systematics uncertainties.

Brazil has the longest coastline along the West Atlantic Ocean, extending for about 8,500 km, and supporting one of the richest ichthyofaunas of the world (Fernandez et al., 2019; Froese & Pauly, 2023). Despite being a hotspot of biodiversity for parasitic copepods, the regional diversity of hatschekiids appears to be underestimated due to uneven distribution of research effort, regarding the study of these organisms (Luque et al., 2013; Uyeno & Nagasawa, 2013; Paschoal et al., 2022). Such scarcity of studies becomes particularly evident in the State of Ceará, Northeastern Brazil, where only the fossil copepod *Kabatarina pattersoni* Cressey & Boxshall, 1989 † (Copepoda: Dichelesthiidae), from *Cladocyclus gardneri* Agassiz † (Cladocyclidae), in the Araripe Plateau, has been reported (Cressey & Boxshall, 1989). In this sense, the present study represents not only the first report of *H. exigua* in Brazil but also the first hatschekiid copepod reported off the coast of Ceará. Therefore, the present results highlight the underestimated status of the richness associated with Hatschekiidae in Brazil, as well as with parasitic copepods in general, emphasizing the need for further investigations to improve the knowledge on their diversity and geographic distribution in the country.

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Author contributions JVC and CVF performed field collections; JVC and FP analyzed the copepods and prepared the illustrations; JVC and AJP prepared the main manuscript; FBP and FP supervised the study; all authors reviewed the manuscript & approved the final version.

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Data availability All data used analysis is fully availabe in the manuscript.

Declarations

Competing interests The authors declare no competing interests.

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

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

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