

Two new species of *Colobomatus* Hesse, 1873 (Crustacea: Philichthyidae) parasitic in the interorbital canals of *Holocentrus* spp. (Holocentriformes: Holocentridae) in the South Atlantic Ocean

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Abstract Two new species of parasitic copepods of the genus Colobomatus Hesse, 1873 (Cyclopoida) are proposed based on specimens collected from the squirrelfishes (Holocentridae) off the coast of Rio de Janeiro, Brazil. Colobomatus luquei n. sp. from the interorbital canals of Holocentrus adscensionis (Osbeck) can be distinguished from its closest congeners by the following combination of characters in the adult female: first to fourth thoracic somites fused, two pairs of thoracic processes, fifth pedigerous somite without processes, and process in the third abdominal somite not transpassing the margins of the caudal rami; and in the adult male: antenna with two elements in the second endopodal segment, leg 1 with three spines and three setae in the second endopodal segment, and leg 2 with three spines and three setae in the second exopodal segment. Colobomatus freirei n. sp. from the interorbital canals of Holocentrus rufus (Walbaum) can be distinguished from its closest congeners through the possession of an anterior pair of thoracic processes four times longer than the posterior pair. The two new species described herein are the first representatives of the family Philichthyidae found to parasitize fish of the order Holocentriformes.

Introduction

Copepods of the family Philichthyidae Vogt, 1877 are highly modified internal parasitic copepods found in the subcutaneous spaces associated with the sensory canals of the lateral line and skull bones of marine actinopterygians and rarely elasmobranchs (Boxshall & Halsey, 2004; Madinabeitia & Iwasaki, 2013; Pombo et al., 2015). Presently this family comprises about 92 species of the following nine genera: Colobomatoides Essafi & Raibaut, 1980; Colobomatus Hesse, 1873; Ichthyotaces Shiino, 1932; Leposphilus Hesse, 1866; Lernaeascus Claus, 1886; Philichthys Steenstrup, 1862; Procolobomatus Castro-Romero, 1994; Sarcotaces Olsson, 1872; and Sphaerifer Richardi, 1876 (Boxshall & Halsey, 2004). Philichthyids have global distribution, but most species can be found in the Mediterranean Sea and Australian waters (Boxshall & Halsey, 2004; Madinabeitia & Iwasaki, 2013). However, some authors are convinced that the current diversity of this family may represent only the "tip of the iceberg" because philichthyids are usually overlooked during fish dissections and their uneven biogeographical distribution may be explained by the sampling effort of researchers (Kabata, 1979; Madinabeitia et al., 2013; Paschoal et al., 2016a).

Records of the family in the western South Atlantic are scarce, with only six species of three genera recorded so far: four belonging to *Colobomatus*,

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i.e. C. belizensis Cressey & Schotte, 1983 from *Haemulon steindachneri* (Jordan & Gilbert) and *Orthopristis ruber* (Cuvier); *C. kimi* Paschoal, Pereira & Luque, 2016 from *Upeneus parvus* Poey; *C. stelliferi* Pombo, Turra, Paschoal & Luque, 2015 from *Stellifer brasiliensis* (Schultz), *S. rastrifer* (Jordan) and *S. stellifer* (Bloch); and *C. sudatlanticus* Pereira, Timi, Lanfranchi & Luque, 2012 from *Mullus argentinae* (Hubbs & Marini); one species of *Sarcotaces, i.e. S. verrucosus* Olsson, 1872 from *Pseudopercis semifasciata* (Cuvier); and one of *Leposphilus, i.e. L. vogti* Paschoal, Nagasawa & Luque, 2016 from *Micropogonias furnieri* (Desmarest) (Luque & Tavares, 2007; Paschoal et al., 2016a).

During a parasitological survey of specimens of *Holocentrus* spp. (Holocentriformes, Holocentridae) from the Brazilian coastal zone some internal parasitic copepods were recovered. A detailed morphological study of these specimens revealed two unknown species of *Colobomatus*, which are described in the present study.

Materials and methods

The philichthyid specimens studied were collected from the interorbital canals of the squirrelfish, H. adscensionis and the longspine squirrelfish H. rufus, caught in the littoral zone of the State of Rio de Janeiro (22°55'S, 43°12'W). The copepods were collected using the procedures described by Madinabeitia & Nagasawa (2012) and subsequently fixed and preserved in 70% ethanol. 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 & Gooding (1964). Drawings were made with the aid of a Zeiss Standard 20 microscope (Carl Zeiss Foundation, Germany) equipped with a drawing tube. Measurements, all in micrometers unless otherwise stated, were made using an ocular micrometer and are given by average values followed by the range in parentheses. The descriptive terminology and classification follows Boxshall & Halsey (2004). The terms prevalence and intensity are used according to Bush et al. (1997). Host identification was based on the key of Figueiredo & Menezes (1980); the nomenclature and classification were updated according to FishBase (Froese & Pauly, 2021). Type-specimens were deposited in the Museum of Zoology of the University of São Paulo (MZUSP), Brazil.

Order Cyclopoida Burmeister, 1834

Philichthyidae Vogt, 1877 Colobomatus Hesse, 1873

Type-species: *Colobomatus lamnae* Hesse, 1873 by original designation.

Colobomatus luquei n. sp.

Type-host: The Squirrelfish *Holocentrus adscensionis* (Osbeck) (Holocentriformes: Holocentridae).

Type-locality: Coastal zone of the State of Rio de Janeiro (22°55′S, 43°12′W), Brazil.

Site in host: Interorbital canals.

Prevalence and intensity: 5.1% (five infected out of 98 fish examined); mean of 1.4 copepods per infected fish (range 1–2).

Type-material: Holotype female (MZUSP-42214); allotype male (MZUSP-42215); paratypes: three females (MZUSP-42217) and three males (MZUSP-42216).

ZooBank registration: urn:lsid:zoobank.org:act:EBBC10EF-DB46-4D92-8584-A621F0C47691

Etymology: The new species is named in honour of Prof. Jose Luis Fernando Luque Alejos (Federal Rural University of Rio de Janeiro, Brazil), for his contribution to the Neotropical ichthyoparasitology.

Description (Figs. 1–3)

Adult female [Based on 4 specimens; Fig. 1] Body elongate and transformed (Figs. 1A, B), 2.14 mm (1.63–2.46 mm) long. Pre-oral area of cephalosome with two anterior cephalic processes (Figs. 1A, B); paired processes with sharp tips and setae ornamentation (Fig. 1C), 146 (90–190) long. Cephalossome globular (Figs. 1A, B), longer than wide, 382 (289–430) \times 288 (221–350). First to fourth pedigerous somites fused, forming octagonal to ovoid thoracic region, 525 (351–676) long, representing about 25.2% (21.5%–30.4%) of total body length, 260 (150–350) wide, excluding thoracic processes. Prosomal region with two pairs of thoracic processes arising from



Fig. 1 *Colobomatus luquei* **n. sp.** (adult female). A, habitus, ventral, p1 = leg 1, p2 = leg 2, p3 = leg 3, p6 = leg 6; B, habitus, lateral, p1 = leg 1, p2 = leg 2, p3 = leg 3, p6 = leg 6; C, detail of cephalic and thoracic process, ventral; D, detail of distal part of the abdomen, ventrolateral; E, antennule, ventral; F, buccal area, showing position of antenna (a), maxillule (mx), maxilla (Mx) and labium (la); G, leg 1, ventrolateral; H, leg 2, ventrolateral; I, leg 3, ventrolateral; J, leg 6, ventrolateral. Scale bars: A–B = 200µm; C = 15µm; D = 100µm; E–F= 25µm; G–H = 10µm; I–J = 5µm

dorsolateral surfaces (Figs. 1A, B); both pairs with sharp tips and setae ornamentation (Fig. 1C), similar in size, with anterior pair measuring 252 (200-290) long and posterior pair 296 (223-370) long. First to fourth pairs of legs located on ventro-lateral surface of fused somites. Fifth pedigerous somite longer than wide and separated from preceding fused somites by slight constriction, 344 (255–438) × 226 (152–273). Genital somite square, 223 $(175-287) \times 252$ (210-297), with pair of lateral naked processes with rounded tips (Figs. 1A, B), 341 (300-390) long. Abdomen 4-segmented, first two abdominal somites longer than wide, measuring 296 (215–376) \times 184 (131–204), 263 $(170-315) \times 181$ (161-193), respectively. Third abdominal somite elliptic, 182 (137–219) \times 190 (172-200), with one ventral process armed with one digitate tip (Fig. 1D), 191 (139-250) long. Last abdominal somite shorter, 102 (79-119) long. Caudal rami fused to last abdominal somite, naked and with rounded distal margin (Fig. 1D).

Antennule (Fig. 1E), indistinctly 4-segmented, with armature formula 1, 3, 2, 4 + aesthetasc; all setae naked. Antenna modified, forming buccal capsule with mouth parts, and bordered posteriorly by simple and undivided labium (Fig. 1F). Labrum not observed. Maxillule (Fig. 1F) minute, 1-segmented, located mid-laterally in bucal area and bearing two apical setae. Maxilla (Figure 1F) robust, apparently 1-segmented and bearing one apical seta. Maxilliped absent. Posterior rim of bucal capsule divided.

Legs 1–2 inserted in rugose area. Leg 1 (Fig. 1G) biramous, located immediately posterior to junction of cephalosome and fused somites, with numerous fine spinules distributed on the anterior surface of protopod and exopod; protopod with naked lateral seta; exopod indistinctly 1-segmented, armed with one lateral seta and five distal setae, all setae naked; endopod vestigial, unsegmented and unarmed. Leg 2 (Fig. 1H) biramous, located posterior to leg 1, in the second part of the fused somites; protopod with one naked lateral seta; exopod indistinctly 1-segmented, armed with one lateral seta and three distal setae, all setae naked; endopod indistinctly 2-segmented, armed with two distal naked setae. Leg 3 (Fig. 11), located in the third part of the fused somites (third pedigerous somite) and reduced to two setae of unequal sizes, largest seta annulated. Leg 4 not observed. Leg 5 absent. Leg 6 (Fig. 1J) located near genital apertures, represented by two naked setae of unequal sizes.

Adult male [Based on 4 specimens; Figs. 2–3]. Body cylindrical and not transformed (Figs. 2A, B), 1.05 mm (0.91–1.23 mm) long. Cephalosome elongated and with rounded posterolateral corners (Figs. 2A, B), 201 (169-235) × 190 (160-230). First pedigerous somite wider than long, $85(71-100) \times 194(170-233)$. Second pedigerous somite wider than long 77(70–90) \times 181 (149–212), with paired dorsolateral processes directed backwards, distal part recurved dorsally, hook-like (Figs. 2A, B). Third to fifth pedigerous somite, each wider than long, measuring 70 (54–85) \times 127 (107–162), 80 (60–100) \times 130 (110–151), 72 $(52-94) \times 124$ (102–149), respectively. Genital somites not expanded, with 2 setae on posterolateral corner of genital operculum, 85 (70–99) \times 112 (93-139). Abdomen 4-segmented, first abdominal somite wider than long 91 (79–110) \times 108 (92–130); second abdominal somite square, 99 $(89-115) \times 98$ (80-120); third and fourth abdominal somites longer than wide, measuring 95 (85–112) \times 83 (69–106), 99 (89–109) \times 71 (60–81), respectively. Caudal rami, 89 (72-100) long, each ramus armed with six setae (Fig. 2C), one lateral on outer margin, one ventrolateral on inner margin, and four terminal setae of unequal size, two medial in opposite corners and two long apical, longest seta measuring 64 (50-85) long.

Rostrum absent. Antennule (Fig. 2D), 6-segmented, with armature formula 1, 5, 3, 3, 2, 6 + 1 aesthetasc; all setae naked. Antenna (Fig. 2E) 4-segmented and consisting of coxobasis and 3-segmented endopod; coxobasis armed with one seta; first endopodal segment elongated with one ventrolateral seta; second endopodal segment with two medial setae; third endopodal segment with two medial setae of unequal size. Labrum (Fig. 2F) reduced, ventral surface armed with two anterior teeth and four posterior stout teeth of similar size; lateral regions of labrum sclerotized, with one blunt hump and one small tooth. Mandible (Fig. 2G) large, comprising broad based coxa and distal claw-like blade. Maxillule (Figure 2G) 1-segmented, located near the margin of mandible, with two setae distally. Maxilla (Figure 2H) 2-segmented; basal segment unarmed; distal segment ending in one seta and one spinulose spine. Maxilliped absent.

Swimming Legs 1 (Fig. 3A) and 2 (Fig. 3B) biramous, each with 2-segmented protopod comprising coxa and basis; interpodal plates lacking spinules; coxa with inner plumose seta and smooth margins;

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Fig. 2 *Colobomatus luquei* **n. sp.** (adult male). A, habitus, dorsal; B, habitus, lateral, p1 = leg 1, p2 = leg 2, p3 = leg 3, p6 = leg 6; C, caudal ramus, ventral; D, antenulle, ventral; E, antenna, ventral; F, labrum, ventral; G, mandible (ma) and maxillule (mx), ventrolateral; H, maxilla, ventral. Scale bars: A–B = 500µm; C = 50µm; D–E = 50µm; F = 10µm; G = 20µm; H = 10µm

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Fig. 3 *Colobomatus luquei* **n. sp.** (adult male). A, leg 1, ventral; B, leg 2, ventral; C, leg 3, ventral; D, leg 6, ventrolateral. Scale bars: $A-B = 50\mu m$; $C = 10\mu m$; $D = 20\mu m$

basis with an outer naked seta present on posterior surface. Rami 2-segmented with spines on endopods and exopods with denticulate and pilose setae; first exopodal segment of leg 2 with one small process near denticulate spine.

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

	Coxa	Basis	Exopod	Endopod
Leg1	0, 1	1, 0	I, 0; III, 4	0, 1; III, 3
Leg 2	0, 1	1, 0	I, 0; III, 3	0, 1; III, 2

Leg 3 (Fig. 3C) lobate, wider than long, armed with 3 naked distal setae. Leg 4 not observed. Leg 5 absent.

Leg 6 (Fig. 3D) represented by 2 unequal setae on genital operculum of genital somite.

Remarks

The presence of a process at the posterior end of the third abdominal somite in the female of *C. luquei* **n. sp.** is also shared by six other species of *Colobomatus*: *C. absens* Madinabeitia, Tang & Nagasawa, 2013 from *Pterocaesio digramma* (Bleeker) (Caesionidae) off the Ryukyu Islands, Japan (Madinabeitia et al., 2013); *C. collettei* Cressey, 1977 from *Hemiramphus robustus* Günther and *H. far* (Forsskål) (Belonidae) in the Gulf of New Guinea and off the Ryukyu Islands, Japan, respectively (Cressey, 1977; Madinabeitia et al., 2013); *C. creeveyae* West, 1992 from *Pseudocaranx dentex* (Bloch & Schneider) (Carangidae) in the Deception Bay, Australia (West, 1992); *C. exilis*

Izawa, 1974 from *Caprodon schlegelii* (Günther) (Serranidae) in the Tanabe Bay, Japan (Izawa, 1974); *C. haeckeli* (Richiardi, 1877) from *Brama brama* (Bonnaterre) (Bramidae) in the Mediterranean Sea (Delamare-Deboutteville, 1962); and *C. ornatus* West, 1992 from *Pentapodus setosus* (Valenciennes) (Nemipteridae) off the Moreton Island, Australia (West, 1992). All these species can be easily differentiated from *C. luquei* **n. sp.** by the possession of the second to fourth thoracic somites fused, while the new species possesses the first to fourth thoracic somites fused (Delamare-Deboutteville, 1962; Izawa, 1974; West, 1992; Madinabeitia et al., 2013).

In addition, the female of C. luquei n. sp. differs from C. collettei, C. haeckeli and C. exilis by the process at the posterior end of the third abdominal somite not transpassing the margins of the caudal rami (process transpassing the margins of the caudal rami in the last three species) (Delamare-Deboutteville, 1962; Izawa, 1974; Madinabeitia et al., 2013), from C. ornatus by the abscense of processes at the fifth pedigerous somite (fifth pedigerous somite with processes in the latter species) (West, 1992), and from C. creeveyae by the genital somite with a pair of lateral processes (three pairs of lateral processes in the latter species) (West, 1992). Finally, C. luquei n. sp. differs from C. absens in having two pairs of thoracic processes, against only one such pair in the latter species (Madinabeitia et al., 2013).

Another species of *Colobomatus* that displays a process at the posterior end of the third abdominal somite in the female is C. goodingi Cressey & Collette, 1970. This species was established based on 190 females from seven species of needlefish (Belonidae) distributed across the Atlantic, Pacific, and Indian Oceans (see Cressey & Collete, 1970). However, in the original description of this species, Cressey & Collete (1970) indicated the presence of a cephalosome, three thoracic somites (after the cephalosome), and a greatly expanded somite (perhaps the fourth). In most species of the genus Colobomatus, the somites in the thoracic region are fused, more often from the first (or second) to the fourth thoracic somite (West, 1992; Hayward, 1996; Madinabeitia et al., 2013; Pombo et al., 2015; Paschoal et al., 2016b). Considering these data, there is not enough information to establish the prossomal region in this species, but C. luquei n. sp.

can be separated from *C. goodingi* by having two cephalic processes (only one cephalic process in the latter species) and by the thoracic processes with sharp tips (thoracic processes with round tips in the latter species) (Cressey & Collete, 1970).

Among the males, the new species is most similar to C. gymnoscopeli Grabda & Lindowski, 1978 from Gymnoscopelus nicholsi (Gilbert) (Myctophidae) off South Georgia Island, Artic Waters (Grabda & Linkowski, 1978), C. nanus West, 1992 from Pelates quadrilineatus (Bloch) (Terapontidae) in the Deception Bay, Australia (West, 1992) and C. pupa Izawa, 1974 from Parupeneus spilurus (Bleeker) (Mullidae) in the Tanabe Bay, Japan (Izawa, 1974). These four species share the absence of maxilliped and leg 3 with three setae. However, the new species is easily distinguishable from these congeners by the combination of the following features: (1) antenna with two elements in the second endopodal segment (one in C. nanus; three in C. gymnoscopeli and C. pupa) (Izawa, 1974; West, 1992); (2) Leg 1 with three spines and three setae in the second endopodal segment (two spines and four setae in C. gymnoscopeli; two spines and two setae in C. nanus) (Grabda & Linkowski, 1978; West, 1992); (3) Leg 2 with three spines and three setae in the second exopodal segment (two spines and one seta in C. pupa) (Izawa, 1974).

Colobomatus freirei n. sp.

Type-host: The Longspine squirrelfish *Holocentrus rufus* (Walbaum) (Holocentriformes: Holocentridae). *Type-locality*: Coastal zone of the State of Rio de Janeiro (22°55'S, 43°12'W), Brazil.

Site in host: Interorbital canals.

Prevalence and intensity: 36.3% (four infected out of 11 fish examined); 1 copepod per infected fish.

Type-material: Holotype female (MZUSP-42218) and three paratypes females (MZUSP-42219).

ZooBank registration: urn:lsid:zoobank.org:act: DCF5DACA-7878-4C85-913D-58E9D4A6014E

Etymology: The new species is named in honour of Prof. Paulo Reglus Neves Freire (1921–1997) from Brazil, for his contribution to philosophy and the Brazilian education.

Description (Fig. 4)

Adult female [Based on 4 specimens; Fig. 4]. Body elongate and transformed (Fig. 4A), 2.13 mm (1.76-2.48 mm) long. Pre-oral area of cephalosome with two anterior cephalic processes laterally directed, naked and with rounded distal margin (Fig. 4A), 84 (60-115) long. Cephalosome slightly extended anteriorly, longer than wide, 306 (249–400) \times 180 (152-209). First pedigerous somite subcylindrical and forming short neck, 160 (127–192) \times 201 (179–217). Second to fourth pedigerous somites fused, forming octagonal to ovoid thoracic region 425 (349-462) long, representing about 20.1% (18.1%-21.4%) of total body length, 258 (180-310) wide excluding thoracic processes; Prosomal region with two pairs of thoracic processes arising from dorsolateral surfaces (Fig. 4A); anterior pair of processes elongated, directed anteriorly around four times as long as the posterior pair, with distal part laterally curved, 311 (248-342) long; posterior pair of processes short, 78 (50-95) long; both pairs of processes naked and with rounded tips. First to fourth pairs of legs located on ventro-lateral surface of fused somites. Fifth pedigerous somite swollen and separated from preceding fused somites by slight constriction, 324 $(270-410) \times 333$ (248-390). Genital somite square, $213 (153-271) \times 300 (194-388)$, with pair of lateral tapering processes with denticulate tips (Fig. 4B), 212 (201-232) long. Abdomen 4-segmented, first three abdominal somites wider than long, measuring 208 $(180-249) \times 253 (176-317), 216 (165-262) \times 241$ (170-283), 185 $(147-210) \times 237$ (176-272), respectively. Last abdominal somite shorter, 131 (100-170) long. Caudal rami fused to last abdominal somite, armed with 4 rudimentary setae: one ventrolateral on inner margin, two medial in opposite corner and one apical; and with rounded distal margin (Fig. 4C).

Antennule (Fig. 4D), apparently 3-segmented, with armature formula 4, 4, 3 + aesthetasc; all setae naked. Antenna modified, forming buccal capsule with mouth parts, and bordered posteriorly by simple and undivided labium (Fig. 4E). Labrum not observed. Maxillule (Fig. 4E) minute, 1-segmented, located midlaterally in bucal area and bearing one apical seta. Maxilla (Fig. 4E) large, aparentlly 1-segmented, with one ventrolateral seta and apical seta. Maxilliped (Fig. 4E) with basal segment and one distal spine. Posterior rim of bucal capsule undivided.

Legs 1–2 inserted in rugose area. Leg 1 (Fig. 4F) biramous, located immediately posterior to junction of cephalosome and first pedigerous somite; protopod with one irregularly annulated lateral seta; exopod indistinctly 1-segmented, armed with three distal annulated setae; endopod 1-segmented and armed with one apical annulated seta. Leg 2 (Fig. 4G) biramous, located posterior to leg 1, located immediately posterior to junction of first pedigerous somite and fused somites; protopod with one irregularly annulated lateral seta; exopod 1-segmented, armed with one distal annulated seta; endopod vestigial, unsegmented and unarmed. Leg 3 (Fig. 4H), located in the second part of the fused somites (third pedigerous somite), and reduced to two setae of unequal sizes. Leg 4 (Fig. 4I), located in the last part of the fused somites (fourth pedigerous somite), and represented by a single seta. Leg 5 absent. Leg 6 (Fig. 4J) located near genital apertures, represented by two naked setae of unequal sizes.

Adult Male Unknown

Remarks

The new species has the anterior pair of thoracic processes longer than the posterior pair, this feature can be observed in another five species of Colobomatus: C. acanthuri Madinabeitia, Tang & Nagasawa, 2013 from Acanthurus olivaceus Bloch & Schneider (Acanthuridae) and C. gymnocranii Madinabeitia, Tang & Nagasawa, 2013 from Gymnocranius griseus (Temminck & Schlegel) (Lethrinidae) off Okinawajima Island, Japan (Madinabeitia et al., 2013); C. similis Kim, 1995 from Ditrema temminckii Bleeker (Embiotocidae) off Chungmu Strait, South Korea (Kim, 1995); C. sparsi Essafi, 1982 from Diplodus annularis (Linnaeus) (Sparidae) in the littoral of Tunisia (Essafi, 1982); and C. steenstrupi (Richardi, 1876) from two species of Mullus (M. barbatus Linnaeus and M. surmuletus Linnaeus) in the western Mediterranean Sea (Essafi et al., 1983). Colobomatus freirei n. sp. can be readily distinguished from the five congeners mentioned above by possessing the anterior pair of thoracic processes four times as long as the posterior pair. Each of the other five species has the anterior pair of thoracic processes up to twice as long



Fig. 4 *Colobomatus freirei* **n. sp.** (adult female). A, habitus, ventral, p1 = leg 1, p2 = leg 2, p3 = leg 3, p4 = leg 4, p6 = leg 6; B, detail of genital process, ventral; C, caudal ramus, ventral; D, antennule, ventral; E, buccal area showing position of antenna (a), maxillule (mx), maxilla (Mx), maxilliped (mp) and labium (la); F, leg 1, ventrolateral; G, leg 2, ventrolateral; H, leg 3, ventrolateral; I, leg 4, ventrolateral; J, leg 6, ventrolateral. Scale bars: A = 250μ m; B = 50μ m; C = 100μ m; D = 25μ m; E–I = 10μ m; J = 20μ m

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as the posterior pair (Essafi, 1982; Essafi et al., 1983; Kim, 1995; Madinabeitia et al., 2013).

In addition, *C. freirei* **n. sp.** differs from *C. acanthuri* and *C. steenstrupi* by possessing the second to fourth thoracic somites fused (first to fourth thoracic somites fused in the last two species) (Essafi et al., 1983; Madinabeitia et al., 2013), from *C. gymnocranii* and *C. sparsi* by having two cephalic processes (four cephalic processes in the last two species) (Essafi, 1982; Madinabeitia et al., 2013) and from *C. similis* by the absence of a midventral cephalic process (present in the latter species) (Kim, 1995).

Discussion

Colobomatus currently comprises 75 species, including the two new species described in the present study. Copepods belonging to this genus are highly modified and can be characterized by an elongated body, comprising cephalosome, fused thoracic somites, abdomen and caudal rami; presence of at least two pairs of divergent lateral processes in the thoracic region, arranged in the shape of the letter 'X'; and leg 4 being reduced to a single seta or completely absent (West, 1992; Boxshall & Halsey, 2004; Pereira et al., 2012; Uyeno & Nagasawa, 2021). According to Paschoal et al. (2016b), most species of this genus use a wide range of perciform teleosts as hosts; however, some species are also associated with other Actinopterygii fish, i.e., Anguilliformes, Beloniformes, Myctophiformes, Mugiliformes, Scorpaeniformes members and one species have been described from lamniform elasmobranchs. So far, only three families of copepods have been recorded to parasitize fish of the order Holocentriformes: Bomolochidae Claus, 1875, Caligidae Burmeister, 1835 and Hatschekiidae Kabata, 1979 (Jones, 1985; Boxshall & Halsey, 2004; Boxshall & El-Rashidy, 2009). Thus, the first record of Colobomatus in Holocentriformes made in this study also represents the first record of representatives of the family Philichthyidae in this order.

Different hypotheses about the parasitic specificity of the genus *Colobomatus* have been discussed over decades of study. According to Grabda (1991), species of *Colobomatus* exhibit a strict host specificity, usually having a single host species or rarely two. However, some authors disagree with this Syst Parasitol (2021) 98:753-764

generalization and suggest that most species can be specific to host families or genera (Hayward, 1996) or even suborders (Muñoz & Romero, 2011). For Paschoal et al. (2016b), characteristics in the biology of hosts, such as the spatial overlap of close species exhibiting similar anatomy of the fixation site (subcutaneous spaces) may be related with the presence of *Colobomatus* in different hosts groups. In the present study, two species of *Colobomatus* were described from different holocentrid hosts, which may corroborate the hypothesis of Grabda; however, 90 species of fish are known in this order (see Froese & Pauly, 2021) and little is known about the parasitism of Philichthyidae in this group. Therefore, further studies are needed to define a pattern in these hosts.

Two species of Colobomatus were described from the interorbital canals of the holocentrid fish H. adscensionis and H. rufus in this paper. Species of Colobomatus have a narrow specificity with their hosts (see the previous paragraph) and their biogeographical distribution seems to be according to their host's distribution. Colobomatus pagri (Richiardi, 1877), for example, was originally described from the cephalic canals of Pagrus pagrus (Linnaeus) (Sparidae) in the Mediterranean Sea and was recently reported in the littoral of Brazil and Argentina in the same host, all previously known areas for the distribution of P. pagrus (Delamare-Deboutteville, 1962; Soares et al., 2018; Froese & Pauly, 2021). In the present study both host species are widely distributed in the Atlantic Ocean and can be found off the coast of the United States (Florida and North Carolina), Gulf of Mexico, Caribbean shores, along the coast of Brazil and there are some reports in the Eastern Atlantic, more specifically from the Canary Islands to Angola (see Froese & Pauly, 2021). Therefore, the distribution area of C. luquei n. sp. and C. freirei n. sp. may be extensive, not only in the littoral of Brazil but also in the Atlantic Ocean.

At present, 42 species of cyclopoids have been recorded to parasitize marine fish in Brazil; seven of these (16%) belong to the family Philichthyidae, *i.e.*, six species of *Colobomatus*, including the two species described herein, and one species of *Leposphilus* (Luque et al., 2013; Paschoal et al., 2016a). In contrast, 78 species of cyclopoids have been recorded to parasitize freshwater fish in Brazil, where in the last 2 years, five new species and two new genera have been described (Taborda et al., 2016; Narciso et al.,

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2019; Narciso & Da Silva, 2020). The Brazilian coastline extends for more than 7000 km, encompassing approximately 60% of the Atlantic coast of South America, and harbors one of the richest marine diversities on the planet, with approximately 1,227 species of fish recorded (Angulo et al., 2006; Froese & Pauly, 2021). However, as noted by Paschoal et al. (2016a), further studies are needed regarding the parasitic copepods in marine fish from Brazil, especially in the philichthyids, which remains a poorly known group and might be more abundant and diverse than previously thought.

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Declarations

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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