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ECOSYSTEMS

Cucullanus lithodorasi n. sp. (Nematoda: Cucullanidae), a parasite of *Lithodoras dorsalis* (Siluriformes: Doradidae) on the north coast of Brazil

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Abstract: *Cucullanus lithodorasi* n. sp. (Nematoda: Cucullanidae), collected from the intestine of *Lithodoras dorsalis* (Siluriformes) and waters of the north coast of Brazil is described based on light and scanning electron microscopic observations. The new species differs from its congeners in the number and arrangement of cloacal papillae: five precloacal pairs and five postcloacal pairs and presence of unpaired ventral papillae located slightly anterior to the cloaca. This is the third nominal species of the genus infecting fishes from brackish water from Brazil.

Key words: Parasite, Nematoda, morphology, Amazon.

INTRODUCTION

Nematodes of the superfamily Seuratoidea Hall, 1916, represent a phylogenetically non-natural group (Choudhury & Nadler 2018), and are included in four families: Cucullanidae Cobbold, 1864; Quimperiidae Gendre, 1928; Schneidernematidae Freitas, 1956 and Chitwoodchabaudiidae Puylaert, 1970 (Nemys 2023). *Cucullanus* Müller, 1777 and *Dichelyne* Jägerskiöld, 1902 are the most representative genera within Cucullanidae considering the number of valid species, and both are taxonomically problematic (Pereira & Luque 2016).

Cucullanus comprises more than 100 species parasitizing several fishes from various orders and families around the world (Xu et al. 2014, Bouderbala et al. 2022). Although the genus contains numerous species, the high number of poor descriptions and the rather uniform morphology of cucullanids make detailed comparisons among all of them impossible and represent a real challenge for taxonomists who deal with these parasites (Moravec et al. 1993, Vieira et al. 2015).

An attempt should be made to organize and group the different species of *Cucullanus*, using taxonomic comparisons according to the taxonomic group of the host and the zoogeographic region in which the host was found (Yooyen et al. 2011, Pereira et al. 2014, Lacerda et al. 2015). In the freshwater, estuarine and marine waters of Brazil, *Cucullanus* species parasitize ten orders of fish: Siluriformes (*C. pinnai pinnai* Travassos, Artigas & Pereira, 1928; *C. pauliceae* Vaz & Pereira, 1934; *C. zungaro* Vaz & Pereira, 1934; *C. bagre* Petter, 1974; *C. oswaldocruzi* Santo, Vicente & Jardim, 1979; *C. brevispiculus* Moravec, Kohn & Fernandes, 1993; *C. pimelodellae* Moravec, Kohn & Fernandes, 1993; *C. pinnai pterodorasi* Moravec, Kohn & Fernandes, 1997; *C. pseudoplatystomae* Moravec, Kohn & Fernandes, 1997; *C. heliomartinsi* Moreira, Rocha & Costa, 2000 and *C. ageneiosus* Giese, Furtado, Lanfredi & Santos, 2010), Perciformes

(*C. cirratus* Müller, 1777; *C. carioca* Vicente & Fernandes, 1973; *C. protrudens* Pereira, Vieira & Luque, 2015 and *C. pseudopercis* Pereira, Vieira & Luque, 2015), Tetraodontiformes (*C. dodsworthi* Barreto, 1922; *C. brevicaudatus* Pereira, Vieira & Luque, 2014 and *C. marajoara* Pinheiro, Santana, Monks, Santos & Giese, 2018), Cichliformes (*C. tucunarensis* Lacerda, Takemoto, Marchiori, Martins & Pavanelli, 2013 and *C. opisthoporus* Pereira & Luque, 2016), Characiformes (*C. mogi* Travassos, 1947 and *C. colossomi* Díaz-Ungría, 1968), Anguilliformes (*C. pedroi* Timi & Lanfranchi, 2006), Gymnotiformes (*C. rhamphichthydis* Moravec, Kohn & Fernandes, 1997), Ophidiiformes (*C. genypteri* Sardella, Navone & Timi, 1997), Lophiiformes (*C. gastrophysi* Pereira, Vieira & Luque, 2015) and Gadiformes (*C. cirratus*) (Pinheiro et al. 2018).

As part of an ongoing study of the helminths of vertebrates of eastern Brazil, specimens of *Lithodoras dorsalis* (Valenciennes, 1840) were collected and necropsied. Cucullanid nematodes were found as parasites of these fish, but could not be assigned to a known species; therefore, the new species is described herein.

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MATERIALS AND METHODS

Ten specimens of *L. dorsalis* were captured by artisanal fishermen from the island of Caratateua (Marajó Bay 0°47'54.7"S 48°24'10.7"W), State of Pará, Brazil. Fish were collected from March to July 2022 and transported dead on ice to the Laboratório de Histologia e Embriologia Animal, Instituto da Saúde e Produção, Universidade Federal Rural da Amazônia, City of Belém, state of Pará. All applicable institutional, national, and international guidelines for animal care and use were followed (permission number SISBIO N 68028-4). In the laboratory, the digestive tract of each specimen was separated and placed in Petri dishes with saline and examined using a stereomicroscope. Live nematodes were recovered from the intestine of *L. dorsalis*, washed in saline solution and then fixed in a hot ethanol–formaldehyde–acetic acid solution and preserved in 70% ethanol. They were then examined using light microscopy and scanning electron microscopy following procedures described by Pinheiro et al. (2018). All measurements are presented in millimeters, unless otherwise indicated. The system adopted for describing male caudal papillae is according to Moravec & Justine (2011), Yooyen et al. (2011), Pereira et al. (2014) and Vieira et al. (2015). The fish nomenclature adopted was according to FishBase (Froese & Pauly 2023). The type material was deposited in the Coleção de Invertebrados não Arthropoda of the Museu Paraense Emílio Goeldi (MPEG), municipality of Belém, State of Pará, Brazil: Holotype male (MPEG 000403-1), allotype female (MPEG 000404-1) and 16 paratypes (8 males: MPEG 000405-8; 8 females: MPEG 000406-8).

RESULTS

Family Cucullanidae Cobbold, 1864
Genus *Cucullanus* Müller, 1777
***Cucullanus lithodorasi* n. sp.** (Figs. 1, 2)
ZooBank Life Science Identifier (LSID) - urn:lsid:zoobank.org:act:C31C94AA-49A2-413E-9DF5-3264862896CD

Etymology: The specific name refers to the generic name of the type host.

Type host: *Lithodoras dorsalis* (Valenciennes, 1840) (Siluriformes: Doradidae).

Site of infection: Intestine.

Biome: Amazon - **Environment:** Estuarine.

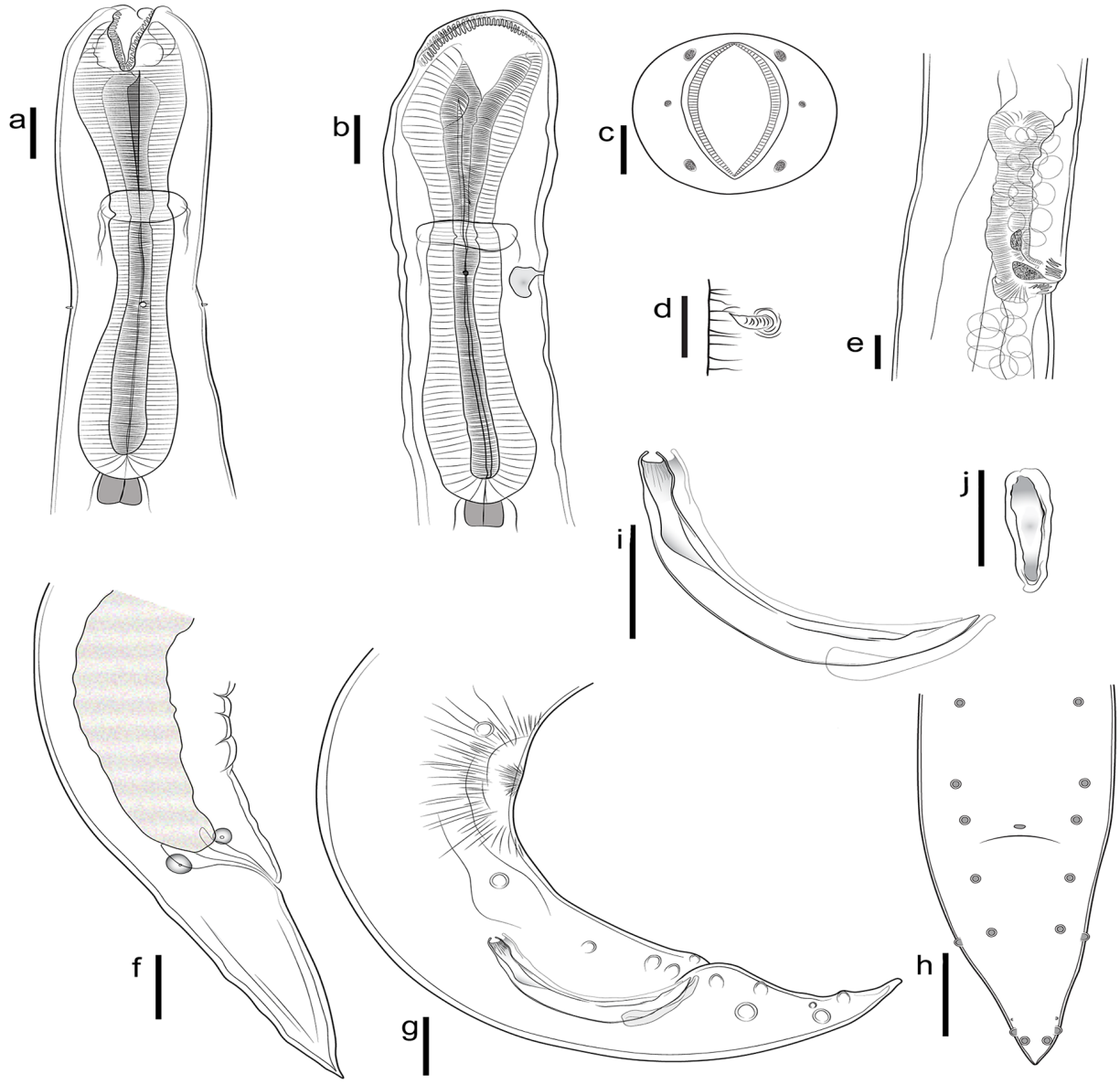

Figure 1. ***Cucullanus lithodorasi*** **n. sp., from** ***Lithodoras dorsalis*****, eastern Amazon: (a) Anterior end of female, ventral view; (b) Anterior end of male, lateral view; (c) Cephalic end of male, apical view; (d) Deirid, lateral view; (e) Region of vulva, lateral view; (f) Posterior end of female, lateral view; (g, h) Posterior end of male, lateral and ventral views, respectively; (i) Spicule, gubernaculum outlined, lateral view; (j) Gubernaculum, ventral view. Scale bars: 100 µm (a-c, e- i); 20 µm (d).**

Type locality: Marajó Bay, state of Pará, Amazon, Brazil.

Prevalence: 50% (5 infected fish/10 examined).

Mean intensity of infection: 30 nematodes per infected host (range 2–10).

Description

General. Medium-sized nematode, opaque white when alive. Cuticle finely striated transversally. Cephalic region slightly asymmetrical in lateral view; without alae. Cephalic end rounded, oral aperture in form of a dorsoventral cleft surrounded by a membranous ala (collarette)

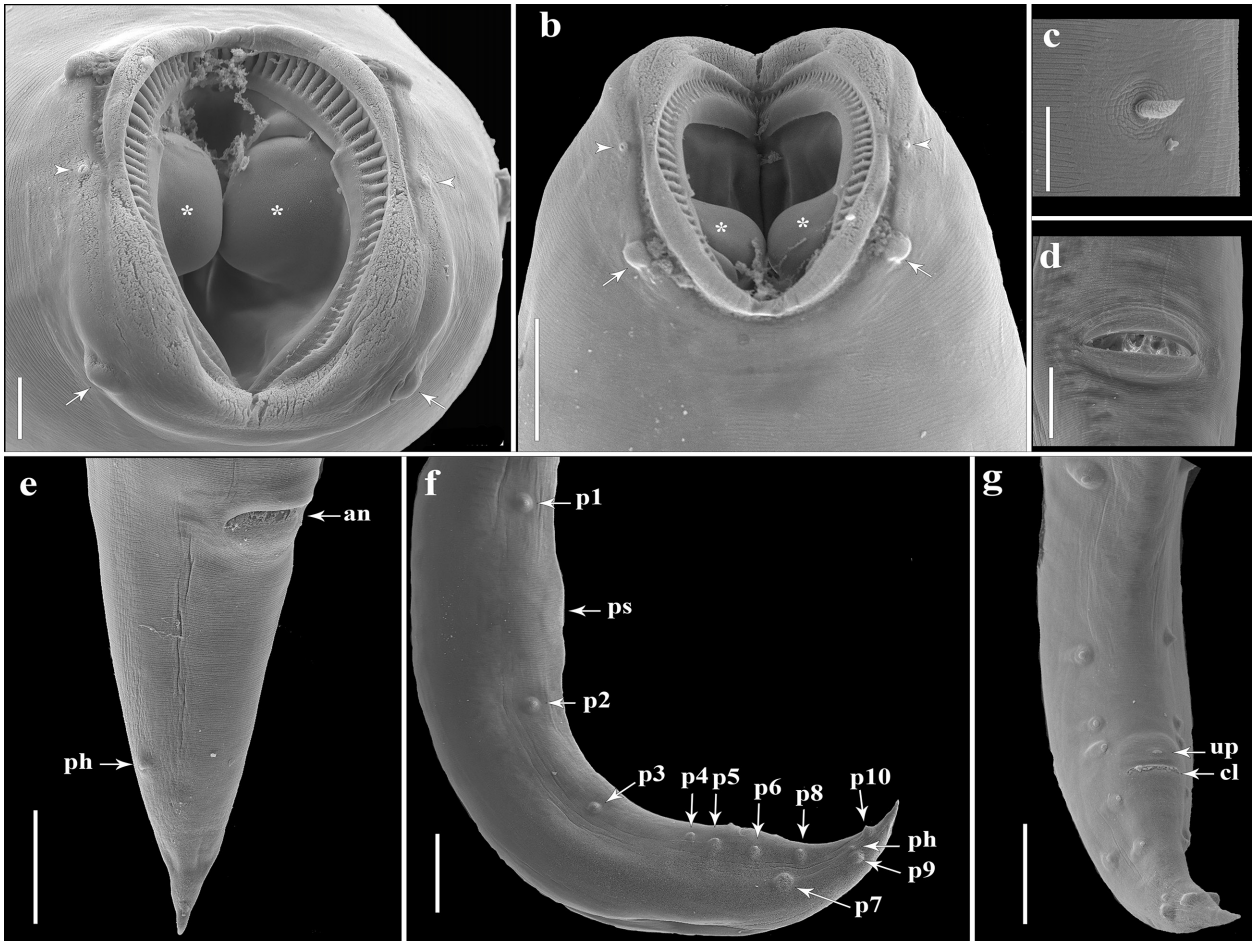

Figure 2. ***Cucullanus lithodorasi*** **n. sp., from** ***Lithodoras dorsalis*****, eastern Amazon, scanning electron micrographs: (a, b) Cephalic region, apical and lateral views, respectively (arrows indicate papillae, arrowheads indicate amphids, asterisks indicate conspicuous sclerotized plates present); (c) Deirid, lateral view; (d) Vulva, ventral view; (e) Tail of female, sublateral view; (f) Posterior extremity of male, lateral view; (g) Posterior extremity of male, subventral view. Scale bars: 20 µm (a, c); 50 µm (b, d, e); 200 µm (f); 100 µm (g). Abbreviations: anus (an), cloaca (cl), papillae (p1-p10), phasmid (ph), pseudosucker (ps), and unpaired papilla (up).**

armed with row of *c.* 50 conical basal teeth (Figures 1a, b; 2a). Cephalic end with four submedian papillae and pair of lateral amphids (Figures 1c; 2b). Esophagus muscular, expanded at anterior end forming well-developed pseudobuccal capsule (esophastome) (Figure 1a). Conspicuous sclerotized plates present in esophastome: 1 pair of lateral elongate structures (Figure 2a, b). Posterior end of esophagus expanded; esophageal valve well-developed, nonsclerotized, opening into intestine. Nerve ring surrounds esophagus in final portion of esophastome (Figure 1b). Small pointed deirids (Figures 1d, 2c). Excretory pore approximately at level of deirids, both located anterior to esophagus-intestine junction.

Male (based on 10 specimens, holotype measurements in parentheses): Body length 8.00–9.00 (8.00), maximum width at junction between esophagus and intestine 0.230–0.317 (0.250). Nerve ring, deirids, and excretory pore 0.310–0.397 (0.317), 0.516–0.600 (0.550), and 0.516–0.650 (0.590), respectively, from anterior end of body. Esophastome 0.300–0.403 (0.300) long and 0.136–0.173 (0.147) wide. Esophagus 0.730–0.870 (0.743) long and 0.100–0.153 (0.100)

wide, representing 9–10% (9%) of total body length. Ventral pseudosucker present, situated at 0.606–0.910 (0.817) from cloacal aperture. Cloacal opening, without protruding lips (Figure 2g). Postdeirids not observed. Ten pairs of caudal papillae: five precloacal pairs (first pair located in anterior rim of pseudosucker, pairs 2nd, 3rd, 4th, and 5th subventral and located posteriorly to cloacal sucker) and five postcloacal pairs (pairs 6, 8, and 10 subventral, pairs 7 and 9 lateral); a pair of lateral phasmids located between pairs 8 and 10 (Figures 1g; 2f, g). Unpaired ventral papilla located slightly anterior to cloaca (Figure 1j). Spicules small, filiform, equal, and weakly sclerotized, 0.242–0.340 (0.263) long, representing 3–4% (3%) of body length (Figure 1h). Gubernaculum weakly sclerotized and spoon-shaped in lateral view, 0.108–0.151 (0.128) long (Figure 1i). Tail 0.141–0.330 (0.293) long. Caudal alae and cuticular projection absent.

Female (based on 11 gravid females with immature eggs, allotype measurements in parentheses): Body length 8.00–14.00 (9.29), maximum width at junction between esophagus and intestine 0.190–0.410 (0.247). Nerve ring, deirids, and excretory pore 0.367–0.550 (0.367), 0.447–0.843 (0.553), and 0.480–0.860 (0.557), respectively, from anterior end of body. Esophastome 0.360–0.547 (0.370) long and 0.150–0.233 (0.150) wide. Esophagus 0.794–1.087 (0.797) long and 0.100–0.187 (0.100) wide, representing 8–11% (9%) of total body length. Vulva postequatorial, 5.00–9.00 (5.70) from anterior extremity, representing 61–66% (61%) of body length (Figures 1e; 2d). Vagina directed anteriorly from vulva. Uterus filled with numerous oval eggs with uncleaved contents 0.047–0.073 long and 0.037–0.053 wide. Rectum a short hyaline tube, 0.083–0.200 (0.113) long. Papilla-like phasmids laterally located in second half of tail (Figure 2e). Tail conical, 0.227–0.387 (0.270) long (Figure 1f).

DISCUSSION

The most effective strategies for grouping and comparing *Cucullanus* spp. are to consider the spectrum of hosts that an individual species parasitizes (mainly at family level) together with its geographic distribution (Moravec et al. 2005, López-Caballero et al. 2009, Yooyen et al. 2011, Pereira et al. 2014, Vieira et al. 2015, Pinheiro et al. 2018). However, there will obviously be exceptions for some species. For example, *C. cirratus* is parasitic in fishes of the order Perciformes (*Micropogonias undulatus* (Linnaeus) and Gadiformes (*Urophycis brasiliensis*) (see Vicente & Santos 1973, Vieira et al. 2015). Thus, in the present work, to avoid describing a species that might be synonymized in the future, we will compare the new taxon with all species of the genus in Brazil.

Cucullanus lithodorasi n. sp. can be easily distinguished from other *Cucullanus* spp. parasitizing marine fishes from the Atlantic coast off Brazil in having spicules shorter than 0.500 mm; while *C. brevicaudatus*, *C. carioca, C. cirratus*, *C. dodsworthi*, *C. gastrophysi*, *C. genypteri*, *C. pedroi* and *C. pseudopercis* have spicules > 0.500 mm. The gubernaculum of *Cucullanus lithodorasi* n. sp. is spoon-shaped, with a different morphology than those of *C. cirratus* and *C. pedroi*, which are Y-shaped, *C. gastrophysi* (V-shaped), *C. dodsworthi* (rod-shaped). *Cucullanus brevicaudatus* presents a distally thin gubernaculum that is enlarged and has a small ornament proximally and differs from new species in the smaller size of the gubernaculum (0.080–0.087 mm vs. 0.108–0.151 mm). Furthermore, deirids in the new species are situated anterior to the esophagus-intestine junction (vs. far posterior to it in *C. pseudopercis*). *Cucullanus carioca* is much smaller than the new species (4.47–7.71 mm males and 5.88 mm females vs. 8.00–9.00 mm males and 8.00–14.00

mm females), with a smaller gubernaculum (0.06 mm vs. 0.108–0.152 in the new species) (Vicente & Fernandes 1973, Vicente et al. 1985). *Cucullanus genypteri* also differs from *C. lithodorasi* n. sp. in the arrangement of caudal papillae (3 precloacal, 4 adcloacal and 4 postcloacal vs. 5 precloacal and 5 postcloacal pairs). *Cucullanus pulcherrimus* and *C. rougetae* (considered *species inquirenda*) differ from the new species in the spicule/body length ratio (4.7% and 25.9% vs. 3–4%, respectively). Additionally, *C. rougetae* has a three-pointed structure on the tip of the tale in males, which is absent in the new species and males of *C. pulcherrimus* are much larger than those of the new species (13.3 mm vs. 8.00–9.00 mm) (see Vieira et al. 2015).

Cucullanus lithodorasi n. sp. parasitizes *Lithodoras dorsalis* (Siluriformes), unlike of *C. mogi* (Characiformes), *C. opisthoporus* (Cichliformes), *C. rhamphichthydis* (Gymnotiformes) and *C. tucunarensis* (Cichliformes), in terms of hosts and all are parasites of freshwater fish. *Cucullanus mogi* lacks a gubernaculum, whereas it is present in the new species. *Cucullanus tucunarensis* and *C. opisthoporus*, which are both parasites of *Cichla* Bloch & Schneider, 1801 (Cichliformes) in northern Brazil, differ morphologically from the new species in the presence of sclerotized spines at the tail in the ventral region. The new species can be differentiated from *C. rhamphichthydis* and *C. heliomartinsi* (Siluriformes) by the presence of deirids and excretory pore far posterior below to the end of the esophagus. This feature, associated with the morphology of the anterior end easily differentiates *C. rahmphichthydis* from *Cucullanus lithodorasi* n. sp.

Cucullanus lithodorasi n. sp. has smaller spicules (0.241–0.340 mm) than *C. oswaldocruzi* (Siluriformes) (1.14 mm), *C. pauliceae* (Siluriformes) (1.2 mm), *C. pinnai pinnai* (Siluriformes) (0.345–0.681 mm), *C. pinnai pterodorasi* (Siluriformes) (0.544–0.558 mm), *C. pseudoplatystomae* (Siluriformes) (0.486–0.534 mm), and *C. zungaro* (Siluriformes) (0.640–0.780 mm). Additionally, *C. pinnai pinnai* and *C. zungaro* have the excretory pore situated anterior to the deirids, while in the new species the deirids and excretory pore are located in the same region of the esophagus. *Cucullanus oswaldocruzi* has 7 pairs of postcloacal papillae vs. 5 postcloacal pairs in the new taxon. The males of C. *pimelodellae* do not have a precloacal pseudosucker, a structure present in the new species. *Cucullanus pauliceae* has a spicule length that overlaps that of *C. lithodorasi* n. sp. but differs by having deirids close to the posterior end of esophagus, and a different arrangement of caudal papillae. *C. pinnai pterodorasi* differs from the new species by having a larger gubernaculum (0.069 mm, 0.075–0.084 vs. 0.108–0.151 mm).

The new species resembles *C. brevispiculus* (Siluriformes) in the number of male caudal papillae. However, can be easily distinguished by the position of the vulva (3.47–4.54 mm vs. 5–9 mm), length of spicules (0.210 mm). *Cucullanus lithodorasi* n. sp. has the phasmids between pairs 8th and 10th of caudal papillae, thus it differs from *C. ageneiosus* (Siluriformes), in which the phasmids are located immediately posterior to the 10th pair of caudal papillae. In addition, the new species has larger spicules (0.241–0.340 mm) than *C. ageneiosus* (0.140–0.180 mm). *Cucullanus bagre* (Siluriformes) has an excretory pore far posterior to the esophago-intestinal junction, gubernaculum (Y-shaped), and clearly protruding cloacal lips, which differs completely from the new species.

Cucullanus marajoara is a parasite of the estuarine fish *Colomesus psittacus* (Bloch & Schneider 1801) (Tetraodontiformes), in Pará, Brazil, which differs from the new species in

its host order (Tetradontiformes), size of the spicules (0.56–1.00 mm) and distribution of the 5 postcloacal papillae (3 pairs subventral, 1 pair lateral and 1 pair dorsolateral). *Cucullanus lithodorasi* n. sp. is the third species of the genus (after *C. ageneiosus* and *C. marajoara*) proposed for fishes of estuarine environments in Brazil, and the fourth described for the State of Pará (namely, *C. oswaldocruzi*, *C. ageneiosus*, *C. marajoara* and *C. lithodorasi* n. sp.).

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R. H. S. Pinheiro wrote the main draft and prepared images. R. H. S. Pinheiro and L. A. A. S. Ruffeil helped with specimen observations and SEM analysis, reviewing, and writing the manuscript. E. G. Giese wrote the manuscript, revised and prepared the line drawings. All authors reviewed the manuscript.