



A NEW SPECIES OF *COSMOCERCOIDES* (ASCARIDIDA: COSMOCERCIDAE) PARASITIC IN TREE FROGS FROM SOUTHERN AMAZONIA

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KEY WORDS ABSTRACT

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Cosmocercoides meridionalis n. sp. is described from the large and small intestines of 5 species of tree frogs belonging to the families Hylidae and Phyllomedusidae collected from 2 localities in Southern Amazonia. The new species is allocated to the genus *Cosmocercoides* Wilkie, 1930, due to the presence of papillae in rosettes, which are complex caudal papillae surrounded by punctuations. *Cosmocercoides meridionalis* n. sp. differs from its congeners by a combination of the following characters: the absence of the gubernaculum, the total length of the female (twice the size of the male), and the presence of 26 rosette papillae with a unique arrangement and distribution pattern: 11 pre-cloacal pairs, 1 ad-cloacal pair, and 1 post-cloacal pair. This is the first species of the genus described from the Amazonia region.

Parasites of the genus *Cosmocercoides* occur mainly in amphibians and reptiles (Burse et al., 2015), with some occasional findings in terrestrial snails and slugs (Anderson, 2000). The genus was proposed by Wilkie (1930) to accommodate 2 new species of nematodes in which male caudal papillae are surrounded by punctuations (or rosette papillae): *Cosmocercoides pulcher* Wilkie, 1930, was found in the rectum of a “Bull-frog” (*Rana japonica* Boulenger, 1879) and *Cosmocercoides tridens* Wilkie, 1930, from the large intestines of a salamander (*Echinotriton andersoni* Boulenger, 1892), both from Japan.

Among the 27 species of the genus, only 3 species have been described from the Neotropical region: *Cosmocercoides lilloi* Ramallo, Bursey, and Goldberg, 2007, parasitizing the toad *Rhinella arenarum* (Hensel, 1867); *Cosmocercoides latrans* Draghi, Drago, and Lunaschi, 2020, parasitizing the frog *Leptodactylus latrans* (Steffen, 1815) both from Argentina; and *Cosmocercoides sauria* Ávila, Strussmann, and Silva, 2010, from the lizard *Iphisa elegans* Gray, 1851, from Brazil (Ramallo et al., 2007; Ávila et al., 2010; Liu et al., 2019; Draghi et al., 2020).

During a parasitological survey of an amphibian community from Southern Amazonia, Mato Grosso State, Brazil, we found an intestinal nematode species, that corresponded to *Cosmocercoides*, parasitizing the intestines of *Boana geographica* (Spix, 1824); *Boana boans* (Linnaeus, 1758); *Dryaderces* cf. *inframaculata* (Boulenger, 1882); *Osteocephalus taurinus* Steindachner, 1862; and *Phyllomedusa camba* De la Riva, 1999. However, based on morphological comparisons of these nematodes, they differed from its congeners. Thus, the new species *Cosmocercoides meridionalis* is described herein.

MATERIALS AND METHODS

Host specimens were collected from 2 localities in the Southern Amazonia: São Nicolau farm (09°49'11”S, 58°15'31”W), municipality of Cotriguaçu, and Amazonia-Cerrado Transition Zone (11°46'26”S, 55°41'27”W) in the municipality of Sinop, both in Mato Grosso State, Brazil.

During the helminthological survey carried out from January 2017 to January 2020, we collected: 25 specimens of *O. taurinus*; 8 *B. geographica* Spix, 1824; 3 *B. boans* (Linnaeus, 1758); 9 *P. camba* De la Riva, 2000; and 14 *D. cf. inframaculata* (Boulenger, 1882) from Cotriguaçu, and 1 specimen of *O. taurinus* was collected in the municipality of Sinop. The frogs were captured in

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the shrubs near small water bodies (ponds and streams) inside of the primary forest by visual and auditory surveys.

The hosts were transported to the Herpetological Collection of the “Acervo Biológico da Amazônia Meridional (ABAM)” from Universidade Federal de Mato Grosso, Campus Universitário de Sinop, Mato Grosso state, where they were anesthetized through the application of 2% lidocaine hydrochloride, euthanized, and necropsied. All internal organs were placed in Petri dishes with saline solution (NaCl 0.9%) and examined for helminths using a stereomicroscope. Helminths found were washed in saline solution, killed using pre-heated 70% ethanol, and preserved in the same solution at room temperature. Some specimens were heat-killed and fixed with 2.5% glutaraldehyde in pH 7.4 phosphate buffer solution, for scanning electron microscopy study.

For light microscopy observations, the nematodes were cleared using Aman's lactophenol and examined under an Olympus BX41 microscope (Olympus, Tokyo, Japan) equipped with a drawing tube; drawings were made for morphometric comparisons.

Some specimens were also observed by scanning electron microscopy (SEM). Nematodes fixed using glutaraldehyde were post-fixed in 1% osmium tetroxide, dehydrated in an increasing ethanolic series, dried to the CO₂ critical point, coated with gold-palladium, and examined using a Vega3 microscope (TESCAN, Brno, Czech Republic) at the “Laboratório de Histologia e Embriologia Animal” from Universidade Federal Rural da Amazônia, Belém, Pará state, Brazil.

All measurements are in micrometers (µm), unless otherwise indicated, and are presented as those for the holotype followed by mean and the range of all paratypes in parentheses. Prevalence and mean intensity are presented following Bush et al. (1997).

The holotype and paratypes of the new nematode species described herein were deposited in the Other Invertebrate collection of the Museu Paraense Emílio Goeldi (MPEG), municipality of Belém, Pará State, Brazil. Hosts were deposited at Acervo Biológico da Amazônia, Meridional (ABAM) from Universidade Federal do Mato Grosso, Brazil.

DESCRIPTION

Cosmocercoides meridionalis n. sp. Anjos, Rodrigues, & Melo, 2020 (Figs. 1, 2)

General: Small nematodes. Cuticle with fine transverse striations. Somatic papillae present only in males. Sexual dimorphism evident, males approximately one-third length of females (Fig. 1a, b). Lateral alae present only in males (Fig. 2c). Oral opening triangular, surrounded by 3 lips: dorsal lip bearing 2 papillae, 2 sub-ventral lips with 1 ventral papilla and lateral amphidial pores (Fig. 1e); each lip with 1 pharyngeal tooth. Oxyuroid esophagus divided in short pharynx, corpus cylindrical, small isthmus, and a well-developed bulb with sclerotized valves. Nerve ring at middle part of esophageal corpus. Excretory pore anterior to esophageal bulb, close to isthmus-bulb junction. Tail conical, sharply pointed in both sexes (Fig. 2b, d).

Male (based on the holotype and 14 paratypes): Total length 4.0 (4.8, 3.6–5.7) mm. Lateral alae beginning at 284 (278, 192–395) from anterior end, 5.7% of the body length, extending until 789 (801, 657–973) from posterior end, 16% of the body length.

Esophagus 604 (579, 520–650) in length including bulb; pharyngeal portion 52 (51, 39–60) long, corpus 378 (366, 326–410) long; isthmus 56 (51, 31–68) long; bulb 118 (111, 89–126) × 124 (124, 100–150). Nerve ring 236 (274, 210–344; corresponding to 5.6% of the body length) and excretory pore 485 (499, 426–571; corresponding to 10% of the body length) from anterior end. Body width at esophago-intestinal junction 265 (260, 181–342). Thirteen pairs of rosette papillae located at posterior end distributed as follows: 11 pairs pre-cloacal, 1 pair ad-cloacal, and 1 pair post-cloacal, all in ventral position (Figs. 1d, 2c). Rosettes composed of 2 complete rings of about 15 punctations around central papillae (Fig. 2c). One unpaired papilla present on anterior cloacal lip. Two post-cloacal pairs of simple papillae ventral in position (Fig. 2b). Somatic papillae distributed in 2 longitudinal ventrolateral lines along posterior end. Testis single, tubular, flexing posteriorly at last third of body length (Fig. 1b). Spicules slightly sclerotized, curved ventrally, proximal ends slightly expanded, distal ends sharply pointed, equal in length 131 (166, 130–200) long, representing 3.43% of body length (Fig. 1f). Gubernaculum absent. Tail conical, ventrally curved, terminating in slender filament 324 (372, 290–446; corresponding to 7.7% of the body length) long.

Female (based on 13 paratypes): Total length (10.5, 9.9–12.0) mm. Esophagus (734, 620–806) in length including bulb; pharyngeal portion (63, 56–71); corpus (487, 448–548) long; isthmus (49, 41–56) long; bulb (144, 139–156) × (152, 146–162). Nerve ring (291, 259–352; corresponding to 2.9% of the body length) and excretory pore (608, 540–633; corresponding to 6% of the body length) from anterior end. Body width at esophago-intestinal junction (275, 255–306), width at vulva (425, 306–468). Vulva slightly pre-equatorial (5.5, 4.9–6.4) mm from anterior end, 43% of the body length; vagina muscular directed anteriorly in proximal half and then flexed posteriorly in distal half; divided in 1 anterior and a posterior uterus (Fig. 1c). Amphidelphic. Ovary directed anteriorly to vagina not extending beyond bulb (Fig. 1a). Uteri, containing eggs in different stages of development, larvated eggs, and free larvae present near the vagina. Eggs thin-shelled (87 × 56, 79–94 × 47–67) (based on eggs with larvae, n = 13). Tail (577, 517–687; corresponding to 5.4% of the body length) long, conical terminating in slender filament.

Taxonomic Summary

Type host: *Osteocephalus taurinus* (host deposit numbers: ABAM-H 3955; ABAM-H 3875; ABAM-H 3914; ABAM-H 3872 (hosts of type material)).

Additional hosts: *Boana geographica* (deposit number: ABAM-H 3914; ABAM-H 3916); *B. boans* (host deposit number: ABAM-H 4143); *P. camba* (host deposit number: ABAM-H 3373; ABAM-H 3787); *D. cf. inframaculata* (host deposit number: ABAM-H 3120).

Type locality: São Nicolau farm/municipality of Cotriguaçu (09°49'11"S, 58°15'31"W), Mato Grosso State, Brazil.

Additional locality: Municipality of Sinop (11°26'26.36"S, 55°27'16.48"W), Mato Grosso State, Brazil.

Site of infections: Small and large intestines.

Infection parameters: *Osteocephalus taurinus*, prevalence 15% (4 out of 26 analyzed), mean intensity, 11 (2–15); *B. geographica*, prevalence 25% (2 out of 8 analyzed), mean intensity, 1.5; *B. boans*, prevalence 33% (1 out of 3 analyzed), mean intensity, 2; *P.*

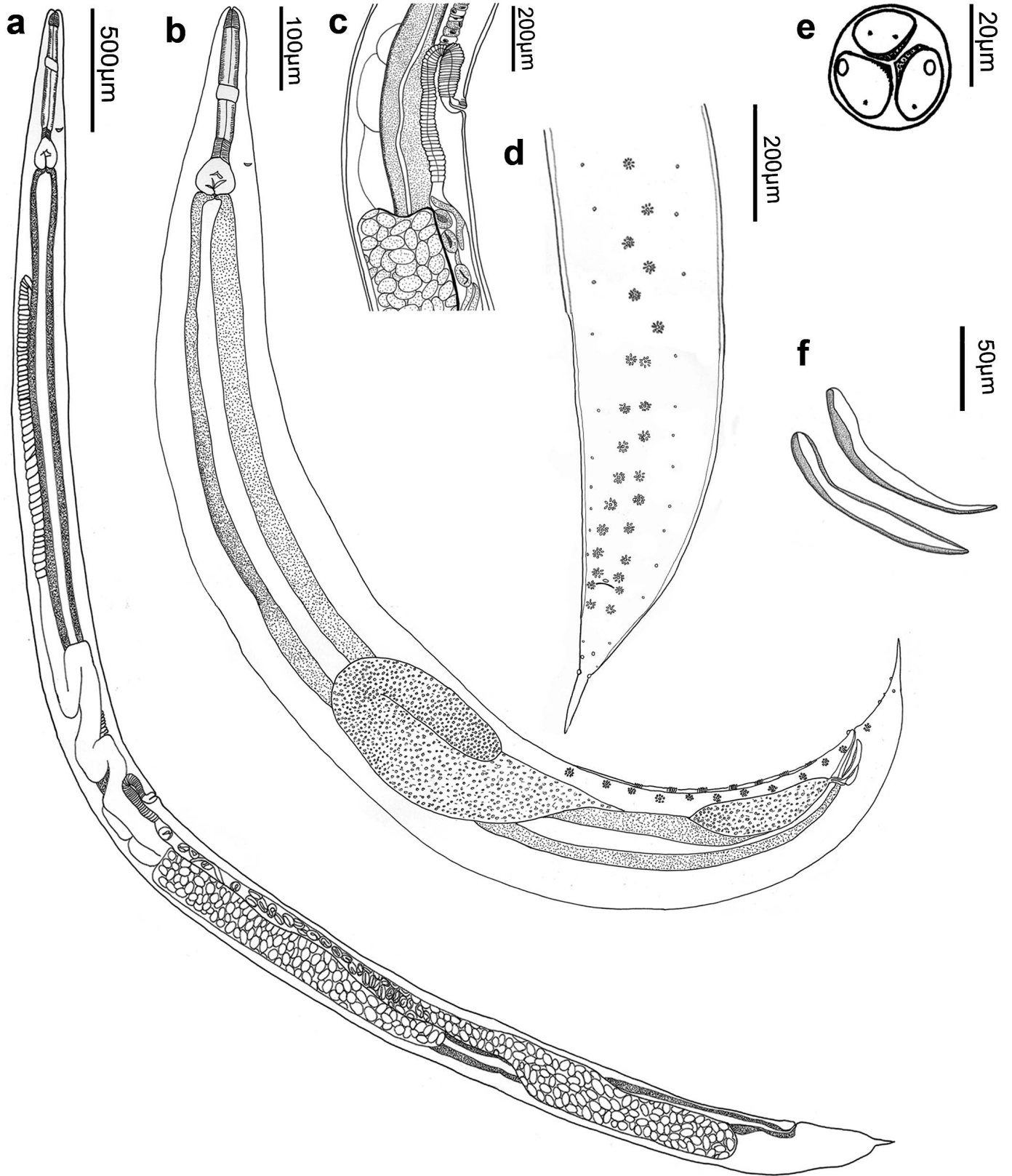


Figure 1. Line drawings of *Cosmocercoides meridionalis* n. sp., a parasite of *Osteocephalus taurinus* from Southern Amazonia. **(a)** Female, entire worm, lateral view. **(b)** Male, entire worm, lateral view. **(c)** Female, lateral view of vulva region. **(d)** Male, posterior end, ventral view. **(e)** Male, Anterior end, apical view. **(f)** Spicules.

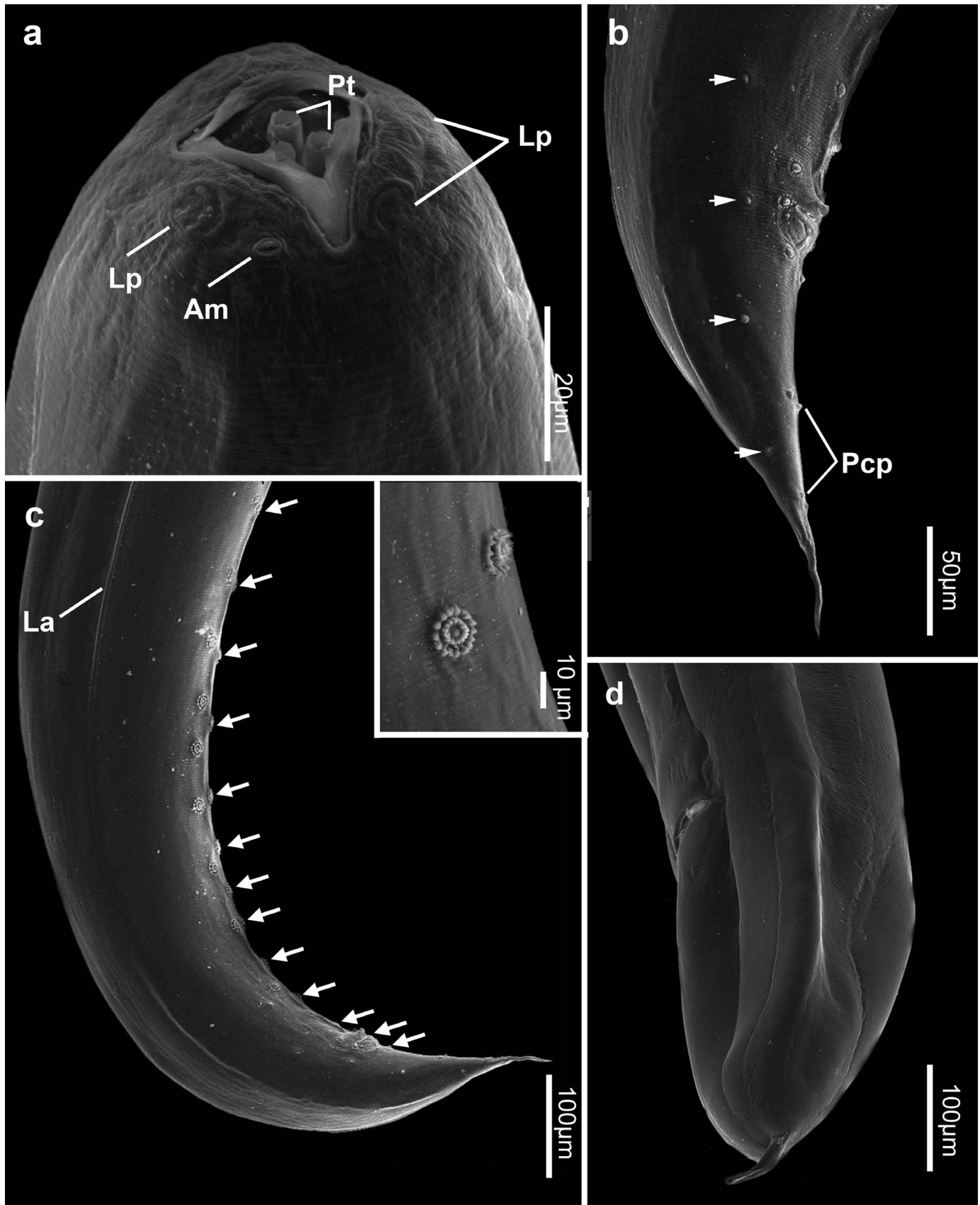


Figure 2. *Cosmocercoides meridionalis* n. sp., a parasite of *Osteocephalus taurinus* from Southern Amazonia. Scanning electron micrographs. (a) Male apical view, showing triangular oral opening surrounded by 3 lips, pharyngeal teeth (b) Male posterior end, single papillae on cloacal lip and somatic papillae (arrows). (c) Male posterior end, papillae in rosettes (arrows). Inset: Detail of rosette. (d) Female posterior region. Abbreviations: Am = amphidial pore; La = lateral alae; Lp = labial papillae; Pcp = post-cloacal papillae; Pt = pharyngeal teeth.

camba, prevalence 22% (2 out of 9 analyzed), mean intensity, 6 (4 out of 8 analyzed); *D. cf. inframaculata*, prevalence 14% (2 out of 14 analyzed), mean intensity, 2 (1–3).

Specimens deposited: The holotype (MPEG 00251), and paratypes (MPEG 00252 males; MPEG 00253 females).

ZooBank registration: urn:lsid:zoobank.org:act:8B673BE9-3F4A-4860-AEE7-E685BA90F3D1.

Etymology: The new species name refers to the Southern Amazonia region known as the “Meridional Amazonia Region.”

DISCUSSION

Here we describe a new intestinal parasite species found in 5 species of tree frogs from the Southern Brazilian Amazonia. The new nematode species is assigned to the genus *Cosmocercoides* Wilkie, 1930, based on the presence of rosette papillae on the male caudal region. According to Rizvi (2009) and Chen et al. (2018), the main morphological characteristics to distinguish species of this genus are the number and position of papillae in rosettes, size and shape of the gubernaculum (if present), size and shape of spicules, and presence or absence of lateral alae and somatic papillae. Considering the number of papillae in rosettes, 17 of the 27 described species of *Cosmocercoides* have more than 24 papillae: *Cosmocercoides bufonis* Karve, 1944; *Cosmocercoides himalayanus* Rizvi and Bursey, 2014; *Cosmocercoides karnatacaensis* Rizvi, 2009; *Cosmocercoides kiliwai* Martínez-Salazar, Falcón-Ordaz, González-Bernal, Parra-Olea, and Pérez-Ponce de León 2013; *Cosmocercoides kumaoni* Arya, 1991; *C. lilloi* Ramallo, Bursey, and Goldberg, 2007; *Cosmocercoides malayensis* Bursey, Goldberg, and Grismer, 2015; *Cosmocercoides multipapillata* Khera, 1958; *Cosmocercoides nainitalensis* Arya, 1979; *C. pulcher* Wilkie, 1930; *Cosmocercoides qingtianensis* Chen, Zhang, Nakao, and Li, 2018; *Cosmocercoides rickae* Ogden, 1966; *Cosmocercoides tibetanum* (Baylis, 1927); *Cosmocercoides tonkinensis* Tran, Sato, and Luc 2015; *C. tridens* Wilkie, 1930; *Cosmocercoides variabilis* (Harwood, 1930); and *Cosmocercoides wuyiensis* Liu, Yu, Shu, Zhao, Fang, and Wu, 2019 (Baylis, 1927; Harwood, 1930; Wilkie, 1930; Karve, 1944; Khera, 1958; Ogden, 1966; Arya, 1979, 1991; Hasegawa, 1989; Ramallo et al., 2007; Rizvi, 2009; Martínez-Salazar et al., 2013; Rizvi and Bursey 2014; Bursey et al., 2015; Tran et al., 2015; Chen et al., 2018, Liu et al., 2019).

Of the 17 species mentioned above, *C. meridionalis* differs from 15 species by the absence of a gubernaculum. Thus, the only 2 species with a combination of more than 22 rosette papillae and absence of gubernaculum are *C. lilloi* and *C. kumaoni*. The new species can also be easily distinguished from *C. lilloi* by the number and distribution of rosette papillae; *C. meridionalis* has 11 pairs of pre-cloacal rosettes, 1 pair of ad-cloacal rosettes, and a post-cloacal pair, while *C. lilloi* has 9 pairs of pre-cloacal rosettes, 5 post-cloacal pairs, and the absence of ad-cloacal rosettes. Moreover, *C. lilloi* also differs from the new species by the absence of lateral alae and somatic papillae (present in the male of the new species). *Cosmocercoides meridionalis* differs from *C. kumaoni* by the number of post-cloacal rosettes (1 pair in the new species and 5 pairs in *C. kumaoni*), by the presence of single papillae on post-cloacal region (absent in *C. kumaoni*), and the latter species also have a singular hook shape structure near pre-cloacal region. In addition, they differ by male body size (3.6 to 5.7 mm in *C. meridionalis* and 2.0 to 3.0 mm in *C. kumaoni*).

Cosmocercoides meridionalis also differs from *C. variabilis*, *C. kiliwai*, *C. tibetanum*, *C. tridens*, *C. himalayanus*, and *C. qingtianensis* by having smaller spicules (130–200 µm in the new species and ranging from 242–580 µm in the other species); from *C. karnatacaensis*, *C. tibetanum*, and *C. pulcher* by the presence of lateral alae; and from *C. variabilis*, *C. malayensis*, *C. pulcher*, *C. tibetanum*, and *C. bufonis* by the presence of somatic papillae. The new species differ from *C. multipapillata*, *C. nainitalensis*, *C. rickae*, *C. tonkinensis*, and *C. wuyiensis* by the number of post-cloacal rosettes (1 pair in the new species and ranging from 4 to 6 pair in the 5 other species). *Cosmocercoides meridionalis* also differs from *C. tonkinensis* by spicule size (130 to 200 in *C. meridionalis* and 220 to 250 in the *C. tonkinensis*), from *C. wuyiensis* by male body size (3.6–5.7 mm in the new species vs. 1.94–3.22 mm in the *C. wuyiensis*). Additional morphological and morphometric differences between *C. meridionalis* and congeneric species can be observed in Table I.

In our study, the SEM was an important tool to observe and confirm some important morphological characters of the new species, namely, the structure and arrangement of papillae, lateral alae, and oral opening structures, and the presence of pharyngeal teeth. Other authors also highlighted the importance of this tool for a detailed study and taxonomy of nematodes, which contribute significantly to the differentiation of species (González et al., 2012; Santos et al., 2018). We provided a detailed description of the first species of *Cosmocercoides* parasitizing anurans in the Amazonia region. This is also the second species of the genus report in Brazil. Thus, considering the country has the greatest diversity of amphibians in the world, further studies are necessary to know the anuran parasite diversity, which currently faces severe risk due to environmental impacts.

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The authors assert all applicable international, national, and institutional guidelines for the care and use of animals were followed, and the project was approved by the Animal Ethics Committee from Universidade Federal do Mato Grosso, Brazil, process number 3108.014041/2020-32.

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Table I. Morphological and morphometric data of selected characters of *Cosmocercoides* species from different biogeographic regions.

<i>Cosmocercoides</i> spp.	Biogeographic regions								Reference
	Length (mm)	Tail (μ m)	Spicule (μ m)	Gubernaculum (μ m)	Rosette papillae*	Simple papillae*	Lateral alae	Somatic papillae	
Nearctic									
<i>C. dukae</i>	2.55	240	Equal: 290	102	22:0:2	Not stated	Not stated	Absent	Holl (1928)
<i>C. variabilis</i>	2.2–5.0	125–220	Equal: 250–420	110–160	24–36:2:6	0:0:4+1	Present	Absent	Harwood (1930)
<i>C. kiliwai</i>	3.1–4.8	Not stated	Equal: 360–430	130–150	32–40:4:2	1: 0:11–15	Present	Absent	Martínez-Salazar et al. (2013)
Neotropical									
<i>C. lilloi</i>	2.0–2.4	160–210	Equal: 100–120	Absent	18–19:0:8+2	Not stated	Absent	Absent	Ramallo et al. (2007)
<i>C. sauria</i>	1.3	54	Equal: 104	97	8:0:0	0:1:4	Present	Not stated	Ávila et al. (2010)
<i>C. latrans</i>	1.48–2.68	78.5–64.14	Equal: 95.2–154.7	Absent	6–8:0:0	0:2:8	Present	Present	Draghi et al. (2020)
<i>C. meridionalis</i> n. sp.	3.6–5.7	290–446	Equal: 130–200	Absent	22:2:2	0:0:6	Present	Present	Present study
Oriental									
<i>C. himalayanus</i>	4.6–6.8	200–250	Equal: 242–260	142–158	24:2:4	0:0:6	Present	Present	Rizvi and Bursey (2014)
<i>C. barodensis</i>	2.1	200	Subequal: 240; 230	60	16:0:4	0:0:14	Present	Absent	Rao (1979)
<i>C. bufonis</i>	4.6–4.9	200–240	Equal: 190–260	120–145	18–26:2:6	0:0:20	Present	Absent	Karve (1944)
<i>C. karnatakaensis</i>	1.3–1.6	250–290	Equal: 245–265	31–42	16:10:24	6:0:0	Absent	Present	Rizvi (2009)
<i>C. kumaoni</i>	2.0–3.0	130–150	Subequal: 100–160; 90–125	Absent	24:2:10	Absent	Present	Present	Arya (1991)
<i>C. lanceolatus</i>	1.3	250	Equal: 210	75	10:2:2	0:0:6	Present	Absent	Rao (1979)
<i>C. malayensis</i>	3.1–3.8	134–183	Equal: 146–159	79–104	26:2:6	0	Present	Absent	Bursey et al. (2015)
<i>C. multipapillata</i>	5.8–6.4	250–280	Equal: 200–240	130–140	21–27:2:8	0:0:10	Present	Present	Khera (1958)
<i>C. nainitalensis</i>	4.9–5.5	230–250	130–151; 112–134	100–120	26–32:2:12	0:0:12	Present	Present	Arya (1979)
<i>C. rickae</i>	3.07	161	Equal: 168	121	28:2:6	0:0:8	Present	Present	Ogden (1966)
Palaearctic									
<i>C. microhylae</i>	2.24	157	Equal: 140	Absent	20:0:0	20:2:18	Present	Absent	Wang et al. (1978)
<i>C. oligodentis</i>	2.62	320	Equal: 112	45	16:0:0	0:0:6	Present	Absent	Wang et al. (1981)
<i>C. pulcher</i>	6.9	220	Equal: 247	143	28:4:2	Many:0:14	Absent	Absent	Wilkie (1930)
<i>C. ranae</i>	2.4	302	Equal: 192	105	20:0:0	8:0:8	Present	Absent	Wang et al. (1981)
<i>C. skrajabini</i>	Not stated	Not stated	Equal: 212	210	16:0:0	Not stated	Present	Present	Ivanitzky (1940)
<i>C. speleomantis</i>	1.8–2.3	115–119	Not stated	81–88	8:0:0	Not stated	Present	Not stated	Ricci (1987)
<i>C. tibetanum</i>	4.5–5.0	170	Equal: 580	188	44:0:2	0:0:6	Absent	Absent	Baylis (1927)
<i>C. tridens</i>	2.7–4.9	140–210	Equal: 270–530	96–146	22–30:0:6	0:0:16+1	Present	Present	Hasegawa (1989)
<i>C. tonkinensis</i>	4.2–5.1	210–230	220–250	113–122	24–26:2:6	0:0:8	Present	Present	Tran et al. (2015)
<i>C. qingtianensis</i>	6.30–8.00	233–330	272–340	126–175	24–28:4:2	0:0:8–10	Present	Present	Chen et al. (2018)
<i>C. wuyiensis</i>	1.94–3.22	61–220	Subequal: 151–163; 189–206	54–105	18–24:2:6	14:(many):4	Present	Present	Liu et al. (2019)

* Papillae distribution on male posterior region, Pre-cloacal:ad-cloacal:post-cloacal.

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