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Neocosmocercella fisherae n. sp. (Nematoda: Cosmocercidae), a parasite of the large intestine of *Phyllomedusa bicolor* (Boddaert) (Anura: Phyllomedusidae) from the Brazilian Amazon

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Abstract *Neocosmocercella fisherae* n. sp. is the first nematode species found parasitising *Phyllomedusa bicolor* from the Brazilian Amazon Region. The new species has a triangular oral opening, with bilobed lips, and is distinguished from *N. bakeri* (triangular oral opening with simple lips), and from *N. paraguayensis* (hexagonal oral opening with bilobed lips). Additionally, the new species has ciliated cephalic papillae, which are absent in the other species of the genus. The reduced uterine sac and the presence of a single egg in the uterus in females are the main morphological characters that differentiate the new species from its congeners *N. bakeri* (8–10 eggs) and *N. paraguayensis* (10 eggs, based on the allotype). Additionally, the new species differs from the other two species of the genus by morphometric characters such as the size of spicules and gubernaculum in males and the vagina in females. Until now, phyllomedusid anurans are the only known hosts for the nematodes of this genus. The present work describes the third species of the genus and the first species of nematode parasitising *P. bicolor*.

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Introduction

The nematode family Cosmocercidae Railliet, 1916 comprises 23 genera of small and slender nematodes, with three lips, whose cuticle has fine transverse striations, and possessing an oesophagus with a small anterior pharynx, a posterior bulb with chitinous valves, and an excretory pore anterior to bulb. Females have a didelphic or monodelphic genital system and males have sub-equal spicules; gubernaculum may be present or absent (Fahel, 1952; Hodda, 2011).

Among the genera of the family Cosmocercidae, the genus *Neocosmocercella* Baker & Vaucher, 1983 was erected to accommodate *Neocosmocercella paraguayensis* Baker & Vaucher, 1983, a parasite of the large intestine of *Pithecopus hypochondrialis* (Daudin) from Paraguay. Recently, Santos et al.

(2017) described the second species of the genus based on material from *Phyllomedusa vaillantii* (Boulenger) in the Brazilian Amazon and expanded the diagnostic characters for the genus. Thus, until now, anurans of the family Phyllomedusidae (Günther) are the only known hosts for the nematodes of this genus.

According to Frost (2017), the family Phyllomedusidae contains eight genera and approximately 62 species, and among these genera, *Phyllomedusa* (Wagler) is distinctly known for being the most speciose. *Phyllomedusa bicolor* (Boddaert) is the largest species of the genus and is represented by arboreal and nocturnal amphibians commonly found in the Amazon Basin, Colombia, Peru, Venezuela, Bolivia and Guianas (Lima et al., 2005).

In spite of the increase of parasitological studies on helminths of amphibians in South America, only nine species of nematodes have been reported parasitising hosts of the family Phyllomedusidae, of which none have been reported from *Phyllomedusa bicolor* (see Campião et al., 2014).

The present study describes a new species of *Neocosmocercella* and reports the first species of nematode parasitising the large intestine of *P. bicolor*.

Materials and methods

Four specimens of *Phyllomedusa bicolor* (Boddaert) were collected in the "Reserva de Desenvolvimento Sustentável Mamirauá" in the Amazonas State, Brazil, during an expedition to collect helminthes parasites of amphibians and reptiles in March 2013. The hosts were euthanized by cardiac injection of lidocaine hydrochloride 2%, and their internal organs were examined under a dissecting microscope. Nematodes were collected, rinsed in saline solution and fixed in AFA (2% glacial acetic acid, 3% formaldehyde and 95% ethanol). For morphological and morphometric analysis, the nematodes were clarified in Aman's lactophenol and analysed using an Olympus BX41 microscope (Olympus, Tokyo, Japan) equipped with a drawing tube. All measurements are shown in micrometres unless otherwise indicated, and are presented as the range followed by the mean in parentheses.

For scanning electron microscopy, the nematodes were post-fixed in 1% osmium tetroxide, dehydrated in ethanol series, critical-point dried, coated with gold-palladium and examined using a JEOL JSM-5800LV of the Universidad Nacional del Nordeste (UNNE), Argentina. Some specimens were also examined using a Vega3 Microscope (TESCAN, Brno, Czech Republic) at the Laboratory of Embryology and Histology of the Federal University of Amazonia (Universidade Federal da Amazônia, UFRA).

The holotype, allotype and paratypes of the new species are deposited in the invertebrate collection of the Emilio Goeldi Museum (Museu Paraense Emílio Goeldi), Belém, Pará State, Brazil.

Family Cosmocercidae Railliet, 1916
Genus *Neocosmocercella* Baker & Vaucher, 1983

Neocosmocercella fisherae n. sp.

Type-host: *Phyllomedusa bicolor* (Boddaert) (Anura: Phyllomedusidae).
Type-locality: Reserva de Desenvolvimento Sustentável Mamirauá Tefé (03°02′22″S, 64°51′41″W), Amazonas State, Brazil.
Type-material: The holotype (MPEG 0121), allotype (MPEG 0122) and paratypes (MPEG 0123–0139) were deposited in the Invertebrate collection of the Emilio Goeldi Museum, Belém, Pará State, Brazil.
Infection parameters: Prevalence: 100% (4 out of 4 host infected); mean intensity: 477.25 per infected host (range 145–902); mean abundance 477.25 per host.
Site in host: Large intestine.
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Neocosmocercella fisherae* n. sp. is urn:lsid:zoobank.org:act:B0F545C5-D599-4B08-AF0B-AC8E407E1414
Etymology: The specific epithet is a patronymic in honor of the actress Carrie Fisher.

Description (Figs. 1–3)

General. Small, slender nematodes (Fig. 1A, F). Sexual dimorphism evident, females larger than males, males with coiled caudal regions (Figs. 1A, 3D). Conspicuous transverse striations and lateral alae present in both sexes, lateral alae extending from

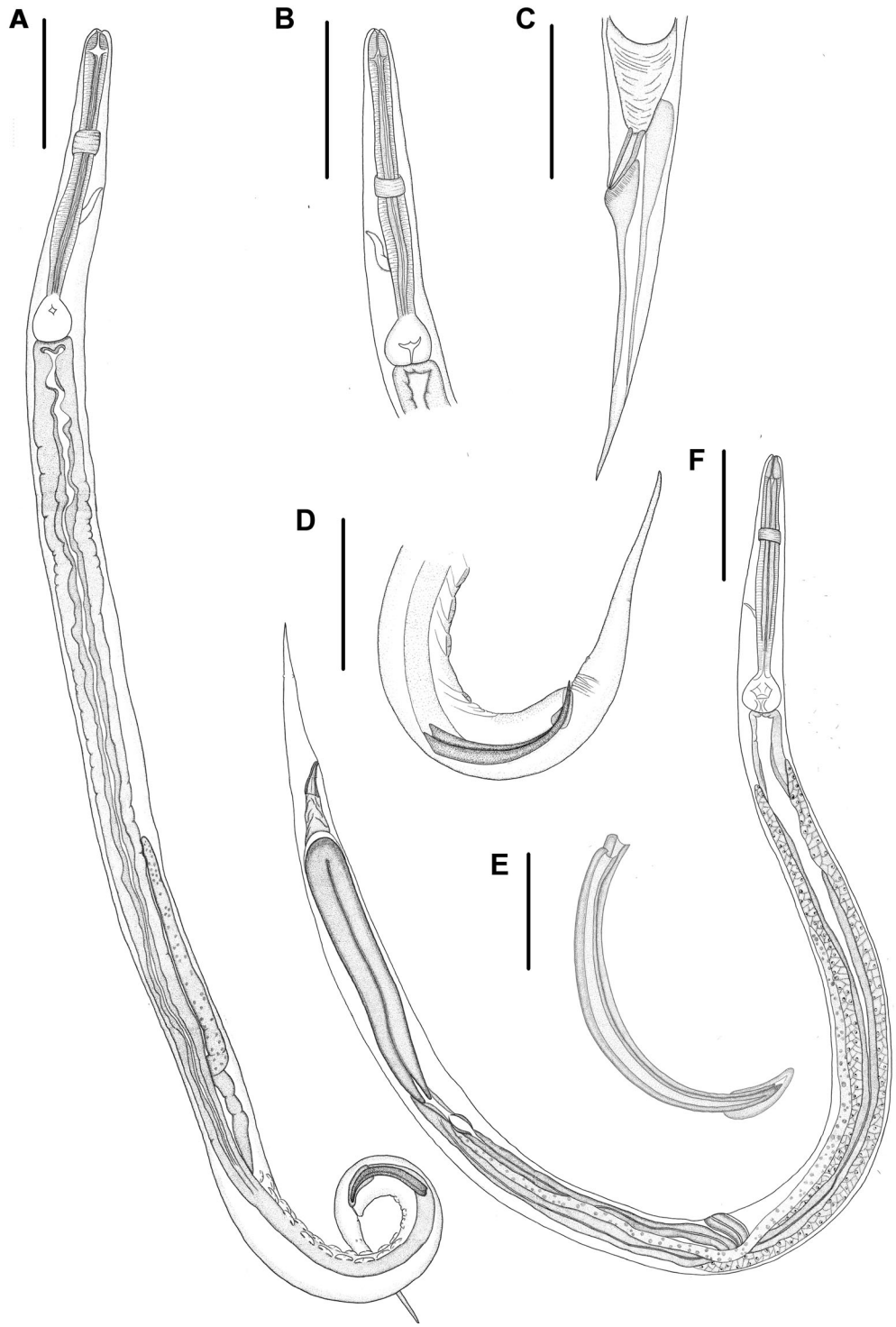

Fig. 1 Line drawings of *Neocosmocercella fisherae* n. sp. ex *Phyllomedusa bicolor*. A, Male, total view; B, Male anterior extremity, lateral view; C, Female, posterior extremity, lateral view; D, Male posterior extremity, lateral view; E, Spicules and gubernaculum, lateral view; F, Female, total view. *Scale-bars*: A–D, F, 200 μm; E, 50 μm

pharynx region to cloaca in males and posterior to anus in females. Oral opening triangular, with 3 bi-lobed lips; each lip with ciliated papillae, amphids located on ventrolateral lips. Cephalic extremity with 8 large papillae (Figs. 2A, 3A). Oesophagus comprises pharyngeal region, corpus, isthmus and large bulb with chitinous valves (Fig. 1B). Nerve-ring situated at anterior third of oesophagus; excretory pore at mid-region of oesophagus (Fig.1B). Female didelphic, opisthodelphic. Somatic papillae absent. Tail slender, sharply-pointed in both sexes (Figs. 1A, C, D, F, 3C, D).

Male [Based on the holotype and 9 paratypes.] Total length 1.94–2.68 (2.36) mm (Fig. 1A). Oesophagus 369–588 (469) long including bulb; pharynx 32–43 × 21–37 (37 × 30); corpus 235–397 (316) long; isthmus 40–69 (49) long; bulb 59–85 × 51–75 (67 × 63) (Fig. 1A, B). Nerve-ring and excretory pore at 141–209 (179) and 248–397 (301) from anterior extremity, respectively (Fig. 1A, B). Body width at oesophago-intestinal junction 61–115 (82). Posterior extremity ventrally coiled. Precloacal region with 2 ventral rows of 19–23 vesiculated papillae becoming gradually smaller, varying between 9–11 on left side and 10–12 on the right side (Figs. 1A, 3D). Precloacal papillae 2 pairs, sessile. Anterior lip of cloaca with 2 pairs of paired papillae and large unpaired superior papilla. Adcloacal papillae 2 pairs; post cloacal papillae 3 pairs (2 subventral and 1 lateral) (Figs. 2B, 3E). Spicules subequal 129–181 (150) long (Fig. 1D, E), gubernaculum triangular, concave, 25–42 (34) (Fig. 1D, E). Tail slender, sharply-pointed, 132–245 (190) long (Figs. 1D, 3D).

Female [Based on the allotype and 9 paratypes.] Total length 2.36–3.31 (2.8) mm (Fig. 1F), width at vulva 91–155 (109). Oesophagus 386–614 (479) in length including bulb; pharynx 29–48 × 24–40 (36 × 31); corpus 251–451 (326) long; isthmus 40–53 (47) long; bulb 61–83 × 56–83 (70 × 66). Nerve-ring and excretory pore at 131–242 (172) and 245–387 (296) from anterior extremity, respectively. Body width at oesophago-intestinal junction 75–133 (95). Vulva prominent, situated at median region of the body at 1.33–1.86 (1.54) mm from anterior extremity

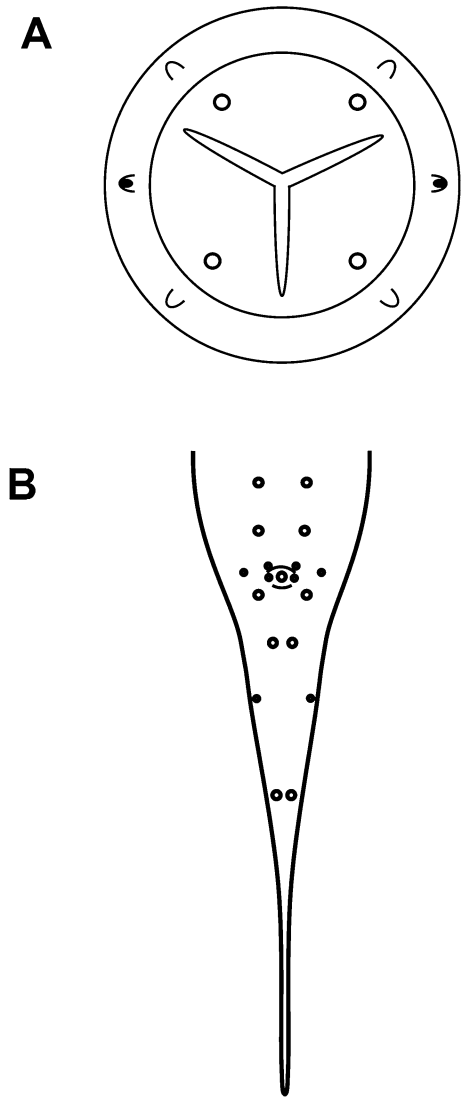

Fig. 2 Schematic drawings of *Neocosmocercella fisherae* n. sp. ex *Phyllomedusa bicolor*. A, Anterior extremity, apical view; B, Male posterior extremity, ventral view

(Figs. 1F, 2B). Vagina well developed, 0.57–0.87 mm long, directed anteriorly [*vagina vera* 63–107 (85) long] and folding back posteriorly [*vagina uterina* 504–760 (632)] ending with muscular uterine sac. Ovoviviparous. Uteri with one egg, some presenting well-developed larvae out of the egg (Fig. 1F); egg in morula 224–392 × 85–141 (290 × 113) (n = 7). Anus well sclerotised. Tail slender, 232–411 (288) long, with long, sharp point (Figs. 1C, 3C).

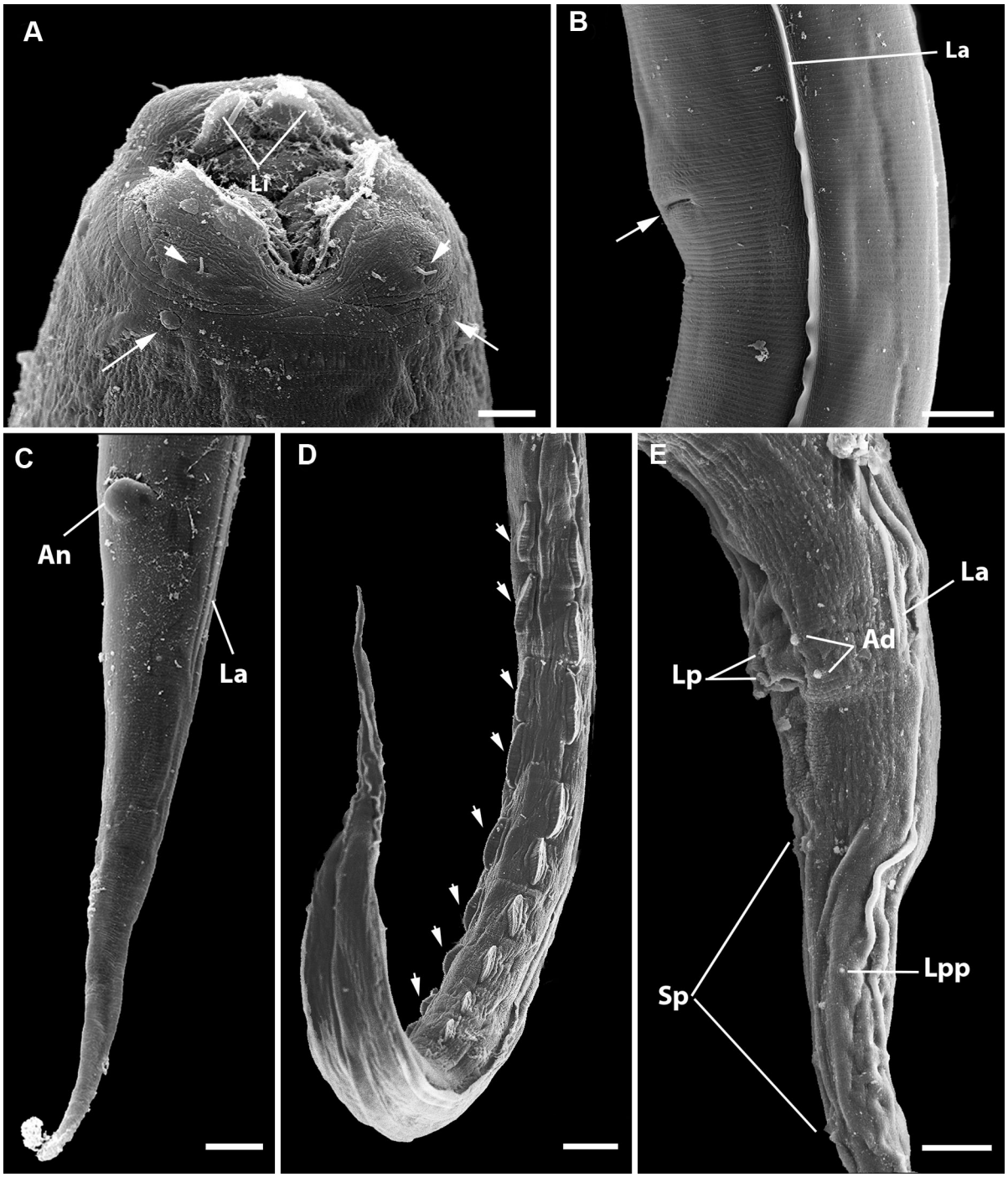

Fig. 3 Scanning electron micrographs of *Neocosmocercella fisherae* n. sp. ex *Phyllomedusa bicolor*. A, Triangular oral opening with three bi-lobed lips, oral ciliated papillae (*arrowheads*) and two pairs of cephalic papillae (*arrows*). B, Vulvar region showing vulva (*arrow*) and lateral alae. C, Female posterior extremity, anus and lateral alae. D, Male posterior extremity, distribution of vesiculated papillae (*arrowheads*). E, Detail of cloacal region and distribution of papillae. *Abbreviations*: Ad, adcloacal papillae; An, anus; Li, lips; La, lateral alae; Lp: labial papillae; Lpp, Lateral post-cloacal papillae; Sp, ventral post cloacal papillae. *Scale-bars*: A, 4 μm; B, C, 20 μm; D, 25 μm; E, 10 μm

Discussion

The nematodes found in the large intestine of *Phyllomedusa bicolor* from the Amazonas, Brazil, possess a triangular oral opening, with three bi-lobed lips, females have a well-developed vagina ending with a muscular uterine sac, and males have a coiled posterior region with vesiculated papillae. Therefore, morphological characteristics of the nematodes described herein corroborate with those proposed by Baker & Vaucher (1983) that allocate them in the subfamily Cosmocercinae of the family Cosmocercidae Railliet, 1916.

The main morphological character used to distinguish the genera of this subfamily is the presence of different kinds of ornate papillae on the ventral surface of the posterior extremity of the male body (Chabaud, 1978). The genera in this subfamily that resemble each other are *Cosmocerca* Diesing, 1861, *Cosmocercoides* Wilkie, 1930, *Cosmocercella* Steiner, 1924 and *Neocosmocercella* Baker & Vaucher, 1983. The presence of vesiculated papillae and the absence of other ornate papillae in the ventral region of males is the main character to distinguish the later genus (Anderson et al., 2009; Gibbons, 2010).

Additionally, according to Baker & Vaucher (1983) and Santos et al. (2017), the presence of hexagonal or triangular oral opening, simple or bi-lobed lips, and a well-developed vagina and elongated uterine sac in females are other morphological characters also present in species of *Neocosmocercella*, allowing us to assign the new species to this genus.

To date, only two species of the genus *Neocosmocercella* have been described: *Neocosmocercella paraguayensis* Baker & Vaucher, 1983 and *Neocosmocercella bakeri* Santos, Rodrigues, Santos, González & Melo, 2017, both described from hosts of the family Phyllomedusidae, *Pithecopus hypochondrialis* (Daudin) from Paraguay and *Phyllomedusa vaillantii* Boulenger from the Brazilian Amazon, respectively. Furthermore, *Pithecopus azureus* (Cope) has also been found parasitised by *N. paraguayensis* in Formosa, Argentina (Draghi et al., 2015).

Neocosmocercella fisherae n. sp. is similar to *N. paraguayensis* in having bi-lobed lips and females presenting a uniform body shape. However, the new species can be differentiated from *N. paraguayensis* by the presence of a triangular oral opening (*vs* hexagonal oral opening), ciliated cephalic papillae (*vs* four large papillae), the presence of a single egg in the uterus of females (*vs* 10 eggs) and 19–23 vesiculated papillae in males (9–11 on the left side and 10–12 on the right side *vs* 12 on the left side and 13 on the right side). According to Baker & Vaucher (1983), there is no evidence that the larvae undergo development outside of the egg in the uterine sac, in contrast to the viviparous biology of the related family Atractidae (Railliet, 1917). Some larvae were observed free in the uterine sac of females, together with larvated eggs, a feature observed in *N. paraguayensis* and *N. bakeri* indicating the presence of ovoviviparity in *Neocosmocercella.*

Additionally, differences of morphometric parameters were also observed between the new species and *N. paraguayensis* regarding the position of the excretory pore (at the mid-region of oesophagus *vs* third portion of oesophagus) and nerve-ring (anterior third of oesophagus *vs* mid-region of oesophagus), tail length (132–245 *vs* 114–162 μm in males and 232–411 *vs* 46–267 μm in females), gubernaculum (25–42 *vs* 34–43 μm) and eggs (224–392 × 85–141 *vs* 190–225 × 110–130 μm). The two species differ especially in the length of the vagina (including *vagina vera* and *vagina uterina*) (0.57–0.87 *vs* 1.9 mm).

The new species shares the morphology of the oral opening (triangular) and of the female genital system (opisthodelphic) with *N. bakeri*, but differs in the presence of bi-lobed lips (*vs* simple lips) and by the lateral alae located between close to pharynx region and the cloacal region in males (posterior to the anus in females) (*vs* from level of oesophageal bulb and cloacal region in males and posterior to anal region in females for *N. bakeri*). Additionally, females of *N. fisherae* n. sp. have a uniform body shape, an uterus containing only one egg, a reduced uterine sac whereas females of *N. bakeri* described by Santos et al. (2017) bear 8–10 eggs *in utero*, the female body is dilated posteriorly and the vagina and uterine sac are more elongated. Regarding the male morphology, *N. fisherae* n. sp. has fewer vesiculated papillae of the caudal region [19–23 (9–11 on the left side and 10–12 on the right side) *vs* 25–29 (12–14 on the left side and 13–15 on the right side) in *N. bakeri*] and a different number of sessile preclocal papillae (two pairs *vs* one pair in *N. bakeri*). A summary of the main morphometric differences between the new taxon and congeneric species is provided in Table 1.

The scanning electron microscopy (SEM) analysis revealed details of the morphological characters of the species especially the morphology of the lips, anterior extremity, lateral alae, and the number and distribution of the ornate and sessile papillae. The SEM images of the present study provided evidence for the bi-lobed structure of the lips as described by Baker & Vaucher (1983); however the oral opening is distinguishably triangular and thus different from hexagonal oral opening in their generic diagnosis. A SEM examination of *N. paraguayensis* may help confirm the morphology of the lips and oral opening and add new information to the diagnosis of *Neocosmocercella.*

According to Santos et al. (2008) and Feitosa et al. (2015) the SEM analysis is helpful to add new morphological characters to the descriptions of helminth species and to visualize details not easily distinguishable under light microscopy. For small and slender nematodes such as species of the Cosmocercidae, this technique also allows a better characterisation of the ornate and sessile caudal papillae in males as well as the shape and structure of the oral opening and lips, which are important for species differentiation.

Cosmocercid nematodes are commonly found parasitising amphibians (Vicente et al., 1990) and according to Campião et al. (2014), in South America, the genera *Raillietnema* Travassos, 1927, *Aplectana* Railliet & Henry, 1916, *Cosmocerca* Diesing, 1861 and *Cosmocercella* Steiner, 1924 have been reported in different families of anurans whereas species of the genus *Neocosmocercella* were found only parasitising

Table 1 Morphometric data for *Neocosmocercella* spp.

Species	*N. fisherae* n. sp		*N. paraguayensis* Baker & Vaucher, 1983		*N. bakeri* Santos, Rodrigues, González, Santos & Melo, 2017	
Host	*Phyllomedusa bicolor*		*Pithecopus hypochondrialis*		*Phyllomedusa vaillantii*	
Locality	Brazil		Paraguay		Brazil	
Source	Present study		Baker & Vaucher (1983)		Santos et al. (2017)	
Character	Female	Male	Female	Male	Female	Male
Body total length (mm)	2.3–3.3	1.9–2.6	3.2–4.1 (4.3)	1.8–3.2	2.44–2.68	1.47–2.06
Body width	75–133	61–115	–	–	91–115	45–66
Oesophagus total length	386–614	369–588	416–460	388–444	403–451	303–355
Pharynx length	29–48	32–43	40	35 (holotype)	29–37	23–30
Corpus length	251–451	235–397	328	271	267–304	204–253
Isthmus length	40–53	40–69	37	30	35–45	26–43
Bulb length	61–83	59–85	68	62 (holotype)	59–77	47–52
Bulb width	24–40	53–75	–	–	56–67	38–48
Nerve-ring from anterior end	131–242	141–209	170–206 (207)	163–194	139–186	127–148
Excretory pore from anterior end	245–387	213–397	302–348 (361)	252–333	209–288	200–221
Tail length	232–411	132–245	246–267 (297)	114–162	229–277	126–148
Spicule length		129–181		123–153		104–139
Gubernaculum length		28–40		34–43		25–32
No. of vesiculated papillae		19–23		19–27		25–29
Egg length	224–392		190–225		165–197	
Egg width	85–141		110–130		90–120	
Vulva from anterior end (mm)	1.33–1.86		1.5–1.9 (1.9)		1.02–1.14	
Width at vulva	91–155		–		128–173	
Vagina vera	63–107		90		–	
Vagina total length (mm)	0.57–0.87		1.9		–	

anurans of the family Phyllomedusidae. These findings suggest that *Neocosmocercella* spp. may be specific to this anuran family; however morphological, molecular and phylogenetic studies are necessary to confirm this hypothesis.

The morphological and morphometric data revealed that the nematode specimens from the large intestine of *P. bicolor* represent a new species of *Neocosmocercella*, the first parasite species reported for this host, the third species of the genus and the second species in the Brazilian Amazon.

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Compliance with ethical standards

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. Host specimens were collected under permits 0004/06 NUC SUPES PA, project "Biodiversity survey of the herpetofauna according to the Research Program on PPBIO eastern Amazonia" and SISBIO 32660-1, project "Amphibian and reptile diversity and associated helminth parasites in the Amazon region".

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