

A new species of *Neocosmocercella* Baker & Vaucher, 1983 (Nematoda: Cosmocercidae), a parasite of *Phyllomedusa vaillantii* Boulenger (Anura: Phyllomedusidae) in the Caxiuanã National Forest, eastern Amazon, Brazil

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Abstract *Neocosmocercella bakeri* n. sp. is described from the large intestine of *Phyllomedusa vaillantii* Boulenger collected in the Caxiuanã National Forest in the Eastern Brazilian Amazon. The new species is easily distinguished from the type-species of the genus, *Neocosmocercella paraguayensis* Baker & Vaucher, 1983 in possessing a triangular mouth opening with three simple lips (*vs* three bilobed lips and hexagonal mouth opening) and somatic papillae, which are absent in *N. paraguayensis*. The

males of the new species are distinguished by the distribution of the sessile cloacal papillae and the dimensions of the gubernaculum, whereas the females are distinguished by their smaller size and opisthodelphic uterus. This study expands the diagnostic characters of *Neocosmocercella* Baker & Vaucher, 1983, reports the first species parasitising anurans of the Brazilian Amazon, a new host record for the genus, and the description of the second species of the genus.

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Introduction

Phyllomedusa Wagler (Anura: Phyllomedusidae) contains 16 species occurring from Central to South America, including Trinidad, to northern Argentina and Uruguay (Frost, 2016). *Phyllomedusa vaillantii* Boulenger is nocturnal and usually found in leaf litter and shrubs near streams or permanent bodies of water in tropical forests (Duellman, 1978). In South America, this species occurs from the Guianas and Amazon Basin of Guyana, Suriname, French Guiana, and northeastern Brazil to Colombia, Peru, northern Bolivia, State of Amazonas, and Venezuela (Frost, 2016).

To date, species of four nematode genera have been found parasitising *Phyllomedusa* spp.: *Cosmocercella* Steiner, 1924; *Physalopteroides* Wu & Liu, 1940; *Oswaldocruzia* Travassos, 1917; and *Cosmocerca* Diesing, 1861 (see Campião et al., 2014). The nematode genera *Cosmocercella* and *Cosmocerca*

belong to the family Cosmocercidae Railliet, 1916 and are commonly found parasitising the intestines of amphibians (Vicente et al., 1990). Among the genera of the family Cosmocercidae, *Neocosmocercella* Baker & Vaucher, 1983 is prominent because it is a monotypic genus; the type-species *Neocosmocercella paraguayensis* Baker & Vaucher, 1983 was described from *Pithecopus hypochondryalis* (Daudin) in Paraguay (Baker & Vaucher, 1983).

Only species of three nematode genera have been recorded in *Phyllomedusa vaillantii*: *Cosmocercella*, *Physalopteroides*, and *Oswaldocruzia* (see Campião et al., 2014). This study describes a new species of *Neocosmocercella* based on material from *P. vaillantii* in the eastern Brazilian Amazon.

Materials and methods

Ten specimens of *Phyllomedusa vaillantii* were collected in the Ferreira Pena Scientific Station (host specimens were collected under permits 0004/06 NUC SUPES PA and SISBIO 32660-1), located in the Caxiuanã National Forest, in the municipality of Melgaço, state of Pará, Brazil, during an expedition conducted in March 2015 for collection of helminths that parasitise amphibians and reptiles. The hosts were euthanised by injection of lidocaine hydrochloride 2%, and their internal organs were examined under a dissecting microscope. Helminths were collected, transferred to saline and fixed in 70% ethanol. For morphological and morphometric analysis, the nematodes were cleared in Amman's lactophenol and analyzed using an Olympus BX41 microscope (Olympus, Tokyo, Japan) equipped with a drawing tube. All measurements are given in micrometres unless otherwise indicated and are presented as the range followed by the mean in parentheses.

For scanning electron microscopy, samples were post-fixed in 1% OsO₄, subjected to dehydration in an ethanol series, critical point-dried using CO₂, coated with gold and examined using a Vega3 microscope (TESCAN, Brno, Czech Republic) at the Laboratory of Embryology and Histology of the Federal Rural University of Amazonia (Universidade Federal Rural da Amazônia, UFRA).

The holotypes, allotype and paratypes of the new species were deposited in the Invertebrate Collection of the Emílio Goeldi Museum (Museu Paraense

Emílio Goeldi, MPEG), and the hosts were deposited in the herpetological collection of MPEG.

Family Cosmocercidae Railliet, 1916

Genus *Neocosmocercella* Baker & Vaucher, 1983

Neocosmocercella bakeri n. sp.

Type-host: *Phyllomedusa vaillantii* Boulenger, 1882.

Type-locality: Caxiuanã National Forest (1°47'00"N, 51°26'02.5"W), Pará, Brazil.

Type-material: The holotype (MPEG 0068), allotype (MPEG 0069) and 18 paratypes (MPEG 0070) were deposited in Invertebrate Collection of the Emílio Goeldi Museum.

Site in host: Large intestine.

Infection parameters: Prevalence: 30% (3 out of 10 hosts infected); mean intensity: 409 nematodes per infected host (range 5–1,227); mean abundance: 129.3 nematodes per host.

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 bakeri* n. sp. is urn:lsid:zoobank.org:act:89C5632F214F41D39E6CB7C383BF572C.

Etymology: The new species is named after Michael R. Baker in recognition of his many contributions on the systematics and biology of parasites of amphibians and reptiles.

Description (Figs. 1, 2)

General. Small, slender nematodes. Sexual dimorphism evident, females larger than males, males with curved caudal region (Fig. 1A, D). Cuticle with delicate transverse striations; lateral alae present in both sexes, from level of oesophageal bulb and cloacal region in males and posterior to anal region in females. Mouth triangular, with 3 lips; dorsal lip with 2 cephalic papillae; each ventrolateral lip with 1 cephalic papilla and 1 large amphid (Fig. 1C, 2A). Oesophagus divided into anterior pharyngeal portion, corpus, isthmus and large bulb with chitinous valves (Fig. 1B). Nerve-ring located at mid-region of oesophagus. Excretory pore close to oesophageal bulb

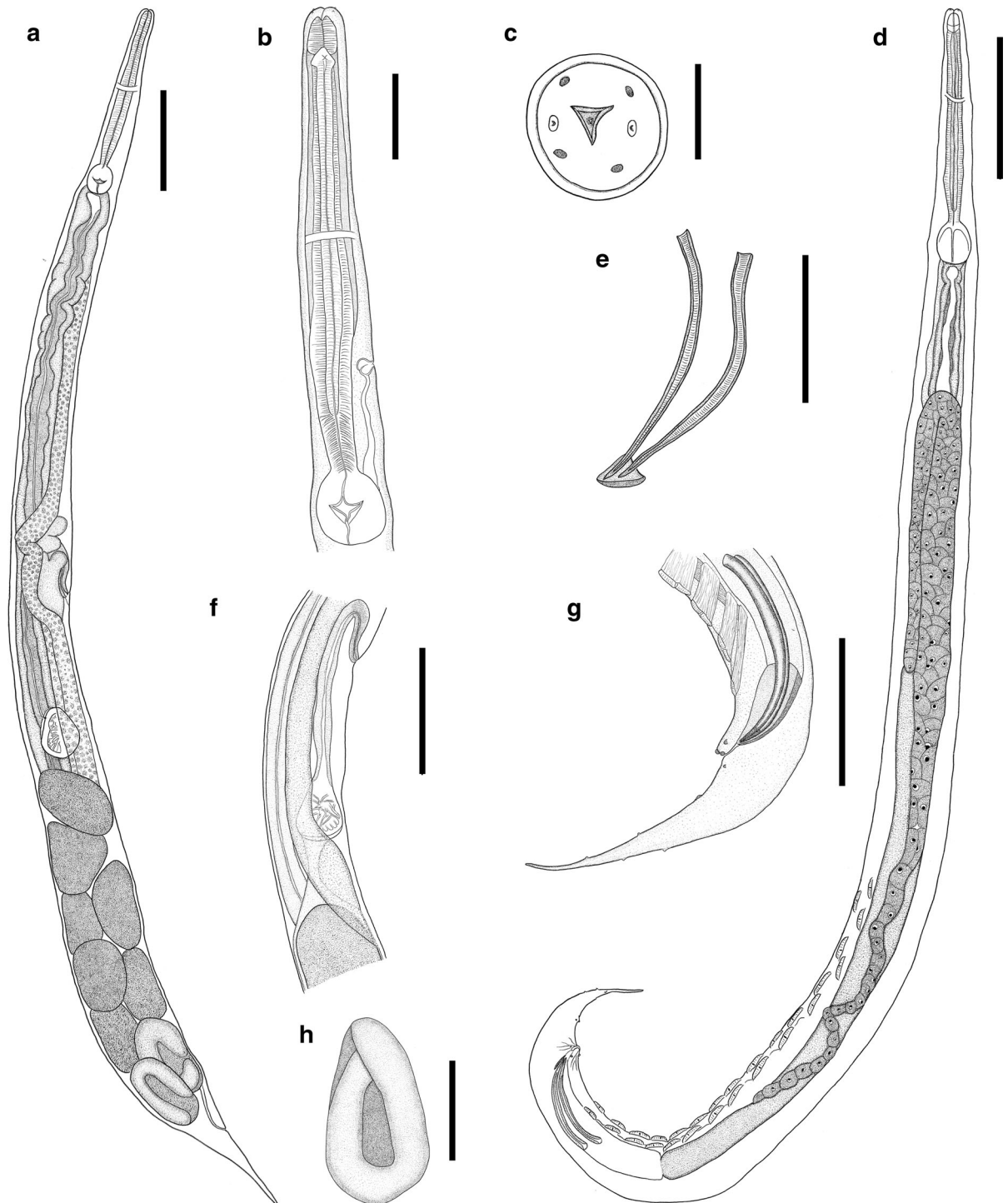
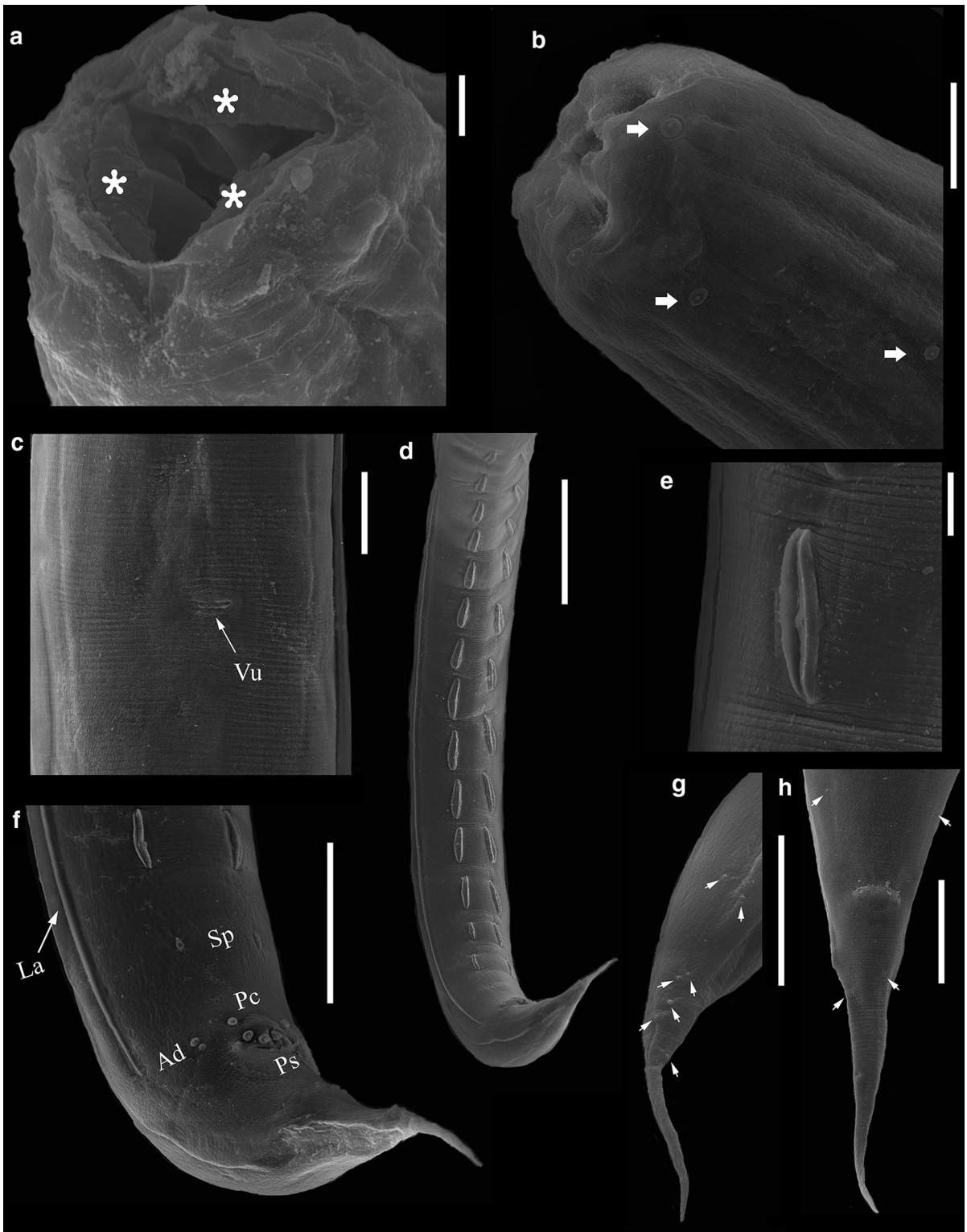


Fig. 1 Line drawings of *Neoscomocercella bakeri* n. sp. ex *Phyllomedusa vaillantii*. A, Female, total view; B, Female, anterior extremity, lateral view; C, Female, anterior extremity, apical view; D, Male, total view; E, Spicules and gubernaculum; F, Vulva, vagina and uterine sac, lateral view; G, Male, posterior extremity, lateral view; H, Larvated egg. Scale-bars: A, D, F, G, 200 µm; B, E, 50 µm; C, 25 µm; H, 100 µm



◀ **Fig. 2** Scanning electron micrographs of *Neocosmocercella bakeri* n. sp. ex *Phyllomedusa vaillantii*. A, Triangular mouth opening with three simple lips (*asterisks*); B, Anterior extremity with somatic papillae (*arrows*); C, Region of vulva; D, Distribution of vesiculated papillae in the posterior region in males; E, Vesiculated papillae; F, Distribution of cloacal papillae; G, Posterior extremity of female, lateral view (*arrows* indicate somatic papillae); H, Posterior extremity of female, ventral view (*arrows* indicate somatic papillae). *Abbreviations*: Ad, adcloacal papillae; Pc, precloacal papillae; Ps, postcloacal papillae. Sp, sessile precloacal papillae; Vu, vulva. *Scale-bars*: A, 2 µm; B, E, 10 µm; C, 20 µm; D, 100 µm; F–H, 50 µm

(Fig. 1B). Female didelphic, opisthodelphic. Somatic papillae present (Fig. 2B, G, H). Tail slender, sharply pointed in both sexes (Fig. 1G, 2G, H).

Male. [Based on the holotype and 9 paratypes.] Total length 1.4–2.06 (1.6) mm, body width at esophago-intestinal junction 45–66 (50). Oesophagus length including bulb 303–355 (341) long. Pharynx 23–30 (27) long, corpus 204–253 (228) long, isthmus 26–43 (33) long, bulb 47–52 × 38–48 (50 × 44). Nerve-ring at 127–148 (139) and excretory pore at 200–221 (207) from anterior extremity. Posterior extremity ventrally curved. Precloacal region with 2 ventral rows of 25–29 vesiculated papillae (Fig. 2E) becoming gradually smaller, varying between 11–14 on left side and 12–15 on right side (Fig. 1D, G, 2D). One pair of sessile precloacal papillae present. Anterior lip of cloaca with 2 pairs of paired papillae and large unpaired superior papilla. Adcloacal papillae 2 pairs; postcloacal papillae 3 pairs: 2 subventral and 1 lateral (Fig. 2F). Spicules subequal 104–139 (113) long (Fig. 1E); gubernaculum triangular, concave, 25–32 (31) long. Tail slender, sharply-pointed, 126–148 (137) long.

Female. [Based on the allotype and 9 paratypes.] Ovoviviparous (Fig. 1A). Body slender anteriorly, dilating posteriorly, 2.4–2.6 (2.5) mm long, width at esophago-intestinal junction 91–115 (99); width at vulva 128–184 (154). Oesophagus length including bulb 403–451 (423). Pharynx 29–37 (33) long, corpus 267–304 (285) long, isthmus 35–45 (39) long, bulb 59–77 × 56–67 (65 × 60). Nerve-ring at 139–186 (166) and excretory pore at 210–288 (265) from anterior extremity. Vulva simple, pre-equatorial (Fig. 1F, 2C), located at 1.02–1.14 (1.08) mm from anterior end. Vagina well developed, directed anteriorly (*vagina vera*) and folding back posteriorly (*vagina uterina*) ending at muscular uterine sac

(Fig. 1F). Uteri filled with 8–10 eggs, eggs in terminal portion with larvae (Figs. 1A, H), eggs in morula 152–185 × 96–135 (168 × 114). Tail slender, 224–277 (241) long, sharply-pointed.

Discussion

The genus *Neocosmocercella* was erected by Baker & Vaucher (1983) to accommodate *Neocosmocercella paraguayensis* Baker & Vaucher, 1983 based on specimens from the large intestine of *Pithecopus hypochondrialis* (Daudin) in Paraguay. Baker & Vaucher (1983) defined the genus based on characters such as the presence of a uterine sac, elongate vagina and, in males, a coiled posterior body region containing vesiculated papillae. Moreover, aspects related to the mouth opening and position of the uterus were reported as important generic characters: hexagonal mouth opening, formed by three bi-lobed lips and prodelphic uterus (Baker & Vaucher, 1983); all of these characteristics were corroborated by Draghi et al. (2015) in specimens of *N. paraguayensis* described from *Pithecopus azureus* (Cope) in Formosa, Argentina.

According to Anderson et al. (2009), the distribution and shape of the ornate papillae are also characteristics used for differentiation of the genera within the subfamily Cosmocercinae Railliet, 1916. Therefore, the nematode species described in the present study can be classified in the genus *Neocosmocercella* because the male specimens only present vesiculated papillae in the posterior region. The genus *Cosmocercella* Steiner, 1924 is morphologically similar to *Neocosmocercella*, as species of the latter genus also possess this type of papillae; however, *Cosmocercella* is easily differentiated by having rosette papillae in the posterior region (Baker & Adamson, 1977). Additionally, *N. bakeri* n. sp. differs from species of *Cosmocercella* and from the other genera of the subfamily Cosmocercinae by possessing an elongated vagina and uterine sac in females (absent in the other genera).

Neocosmocercella bakeri n. sp. can be easily distinguished from *N. paraguayensis* primarily by the presence of a triangular mouth opening, with three simple lips (in *N. paraguayensis*, the mouth opening is hexagonal, with three bi-lobed lips) and the presence of somatic papillae (absent in *N. paraguayensis*). The distribution of sessile cloacal papillae in males of *N.*

bakeri n. sp. is markedly different from that in males of *N. paraguayensis*: one pair of pre-cloacal sessile papillae (vs two pairs); upper lip of the cloaca with two pairs of papillae and a large unpaired papilla (vs three pairs of papillae); two pairs of lateral adcloacal papillae (vs lack of adcloacal papillae); and three pairs of postcloacal papillae, two ventral pairs and one lateral (vs five pairs of papillae, three subventral and two lateral pairs). In the same way, the gubernaculum in *N. bakeri* n. sp. is smaller (25–32 vs 34–43 µm). The uterus in females of the new species is opisthodelphic, and the body is smaller and dilated posteriorly (width 2.4–2.6 mm) whereas the females of *N. paraguayensis* are prodelphic, and the body is larger and uniform throughout its length (3.2–4.2 mm).

The distribution of the sessile cloacal papillae is widely used to separate species of the Cosmocercidae (see Baker & Adamson, 1977); therefore, the variation in papillae observed between *N. bakeri* n. sp. and *N. paraguayensis* associated with other morphological characteristics are sufficient to justify the new species, eliminating the possibility of intraspecific variation in the papillae. Thus, morphological variations combined with morphometric variations indicate the presence of a new species of *Neocosmocercella*. This is the second species of the genus and the second species of the Cosmocercinae reported to parasitise *P. vaillantii*. Bursey et al. (2001) reported that *Cosmocercella phyllomedusae* Baker & Vaucher, 1983 was the first species found to parasitise *P. vaillantii*.

The scanning electron microscopy analysis carried out was important to confirm some morphological features, allowing a better characterisation of the new species such as the pattern of distribution of the sessile papillae, the presence of vesiculated papillae, the morphology of the oral opening and lips, as well as the presence of somatic papillae in males and females. *Neocosmocercella bakeri* n. sp. is the first species of the genus analysed by scanning electron microscopy, adding new morphological data for the genus and supporting the description of the new species. This study is also the first to record infection of anurans with a species of *Neocosmocercella* in the Brazilian Amazon and describes the second species of the genus.

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Compliance with ethical standards

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

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

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