



ANIMAL SCIENCE

Morphological study of the rare nematode *Paradollfusnema amphisbaenia* (Baker, 1981) (Cosmocercidae) and its first report in an amphibian host

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Abstract: The present study aimed to perform a morphological analysis of *Paradollfusnema amphisbaenia*, a parasite of *Leptodactylus podicipinus* (Leptodactylidae) in the Caatinga of Pernambuco, Brazil, using light and scanning electron microscopy (SEM), and to report for the first time the genus infecting an amphibian. *Paradollfusnema* species differs from other Cosmocercidae by having interlabia in the cephalic end. Main morphological characters that distinguish species in this genus are the number and distribution of caudal papillae, size of spicules and gubernaculum, and size of eggs. Nematodes analysed had lateral alae in both males and females, arrangement of caudal papillae: 14 pairs, distributed in 8 pairs + 1 unpaired precloacal, 1 adcloacal, and 5 postcloacal, non-alate spicules with 100–170 µm long, and eggs 87–125 long x 68–110 µm wide. SEM analyses revealed the arrangement of labial papillae with greater accuracy, diverging from what was described in a previous study. This also enabled a more detailed analysis of the distribution of the caudal papillae and the presence of interlabia. There are no records of this genus parasitizing amphibians worldwide. This is the first morphological study of *P. amphisbaenia* using SEM, and the first report of this species infecting an anuran host.

Key words: Cosmocercidae, Anura, Nematoda, taxonomy, biodiversity, Caatinga Biome.

INTRODUCTION

The family Cosmocercidae is composed mainly of intestinal parasitic nematodes of amphibians and reptiles (Anderson 2000). Females can be oviparous or viviparous, having a vulva posterior to the oesophageal bulb and didelphic uterus, with amphidelphic, prodelphic or opistodelphic branches (Anderson et al. 2009). Additionally, the morphology of female reproductive system has great taxonomic importance in identifying the genera since the position of uterus and ovaries and the number and size of eggs are determinant characters for differentiating most genera (Baker 1981, Anderson et al. 2009). Males exhibit

a posterior region without precloacal sucker, with two spicules and gubernaculum. Distinctive diagnostic features include the shape and length of the spicules and gubernaculum, as well as the number and arrangement of caudal papillae (Baker 1981, Anderson et al. 2009). Regardless of the characteristics listed above, species of the little-known genus *Paradollfusnema* Baker, 1982 differ from all other genera by the presence of a cuticular thickening located between the lips, called interlabia (see Baker 1982).

Paradollfusnema allocates two species, *P. amphisbaenia* (Baker, 1981) and *P. telfordi* Bursey, 2002, parasites of amphisbaenians in the USA

and Brazil (Baker 1981, Bursey 2002, Filogônio et al. 2013). The previous studies on these species are restricted to their original descriptions (Baker 1981, 1982, Bursey 2002) and two other non-taxonomic studies in Brazil (Filogônio et al. 2013, Amorim et al. 2014). Furthermore, the morphological data on these two species have been based only on light microscopy (Baker 1981, Bursey 2002). Therefore, the present study aims to describe the morphology of *P. amphisbaenia* using light and scanning electron microscopy and record this species for the first time in an amphibian host.

MATERIALS AND METHODS

The nematodes were collected alive from a recently euthanized individual of *Leptodactylus podicipinus* (Cope, 1862) (Anura: Leptodactylidae), on February 2019, by active search, near intermittent and perennial water bodies at an area of Caatinga morphoclimatic domain, municipality of Petrolina (9°20'4.68" S; 40°35'11.25" W), State of Pernambuco, Brazil. Host were euthanized with an overdose of lidocaine hydrochloride applied topically on the dorsal region, according to the recommendations of the CONCEA (2018), necropsied under a stereomicroscope, and deposited in the Coleção Herpetológica do Museu de Fauna da Caatinga (MFCH) (MFCH 5299), State of Pernambuco, Brazil. The frog was identified according to Vaz-Silva et al. (2020).

Nematodes were fixed in 4% hot formalin and stored in 70% ethanol for morphological studies. For species identification, the specimens were cleared in Amann's lactophenol, mounted in temporary slides, and analysed using an Olympus BX-41 microscope equipped with a drawing tube, at the Laboratório de Helmintos Parasitos de Vertebrados do Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil. Unless

otherwise indicated, all measurements are given in micrometers and are presented as the range, with mean and standard deviation in parentheses.

For scanning electron microscopy (SEM), three males and three females were dehydrated through a graded ethanol series, dried in pure Hexamethyldisilazane (HMDS) (Sigma-Aldrich), coated with gold, and observed in a JEOL JSM 6390LV (operating 15 kV) microscope.

Voucher specimens were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Fundação Oswaldo Cruz (FIOCRUZ), Brazil.

Ethics approval

This study was conducted under the authorizations of the Sistema de Autorização e Informação em Biodiversidade (SISBIO) of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio no. 29558-1), license of the Comitê de Ética no Uso de Animais (CEUA) of the UNIVASF (UNIVASF no. 0001/221018), and National System for the Management of Genetic Heritage and Associated Traditional Knowledge, Brazil (SISGEN #AA68CDE).

RESULTS

Description

Paradollfusnema amphisbaenia (Baker, 1981)

(Figs. 1 and 2)

General. Sexual dimorphism evident, females larger than males. Cuticle with small transverse striations throughout the body (Figs 2a-f). Narrow lateral alae present in males and females (Figs. 1d, g, 2b, f), extending up to the level of the third pair of pre-cloacal papillae in males and anterior to the anus in females. Somatic papillae distributed irregularly from nerve ring to caudal region in both sexes. Cephalic end with three lips, separated by cuticular triangular

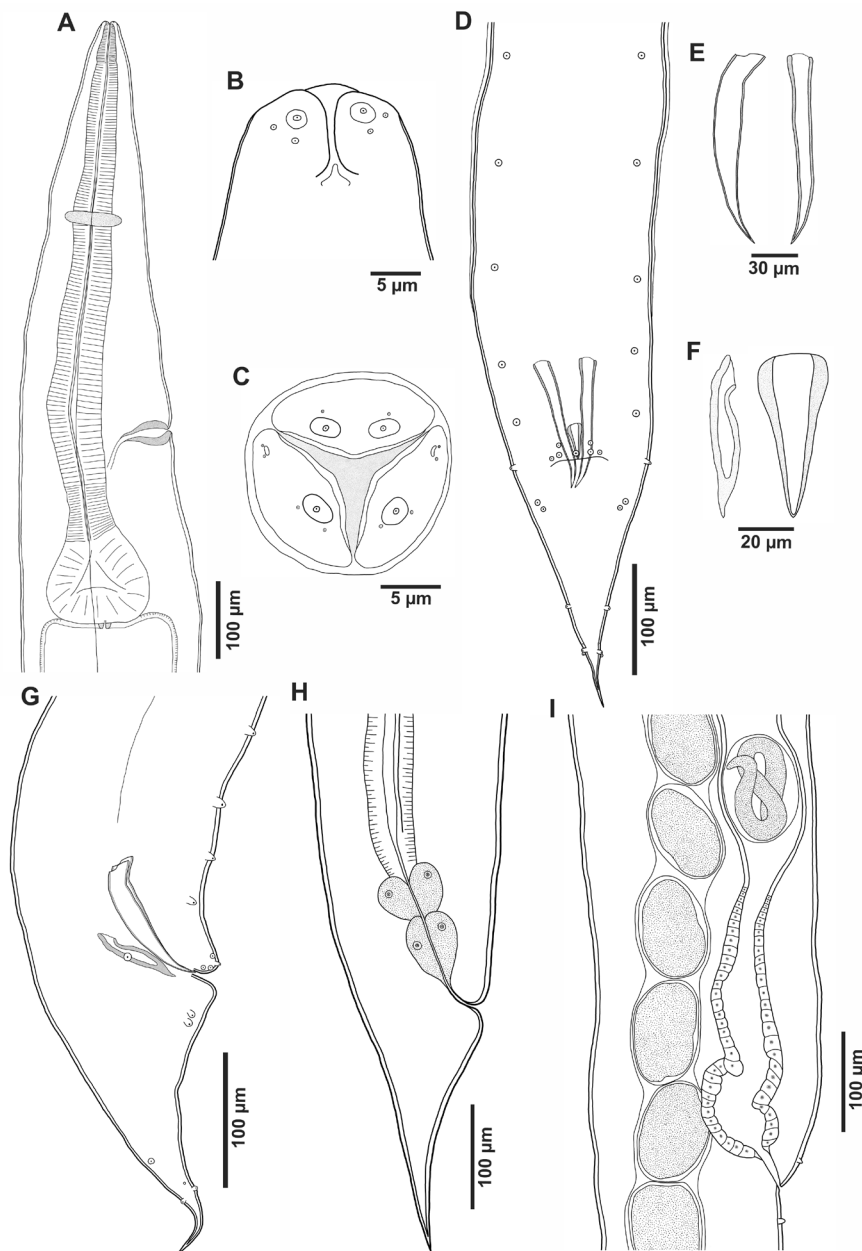


Figure 1. *Paradollfusnema amphisbaenia*. a) Female, anterior end, lateral view. b) Female, cephalic region, ventral view. c) Female, cephalic region, *en face* view. d) Male, posterior region, ventral view. e) Spicules, lateral and ventral view. f) Gubernaculum, lateral and ventral. g) Male, posterior region, lateral view. h) Female, tail, lateral view. i) Female, genital system, lateral view.

interlabia (Figs. 1b, 2a). Dorsal lip with two large papillae adjacent to smaller papilla each (Figs. 1c, 2a); latero-ventral lips with one large papilla adjacent to a smaller papilla, and amphidial pore adjacent to two small papillae (Figs. 1b, c, 2a). Oesophagus oxyuroid, divided into four parts: pharynx, corpus, isthmus, and bulb (Fig. 1a). Nerve ring located at first 1/3 of oesophagus

(Fig. 1a). Excretory pore slightly anterior to oesophageal isthmus (Fig. 1a).

Male (based on 10 specimens): Body length 2.7–3.6 (3.17 ± 0.31) mm, body width at level of oesophagus-intestinal junction 200–290 (238 ± 28.63). Oesophagus total length 618–785 (703 ± 51.03); pharynx 58–65 (61 ± 4.48) long, corpus 450–525 (491 ± 31.12) long, isthmus 70–100 (85 ± 12.39) long, bulb 100–145 (122 ± 16.17) long. Nerve

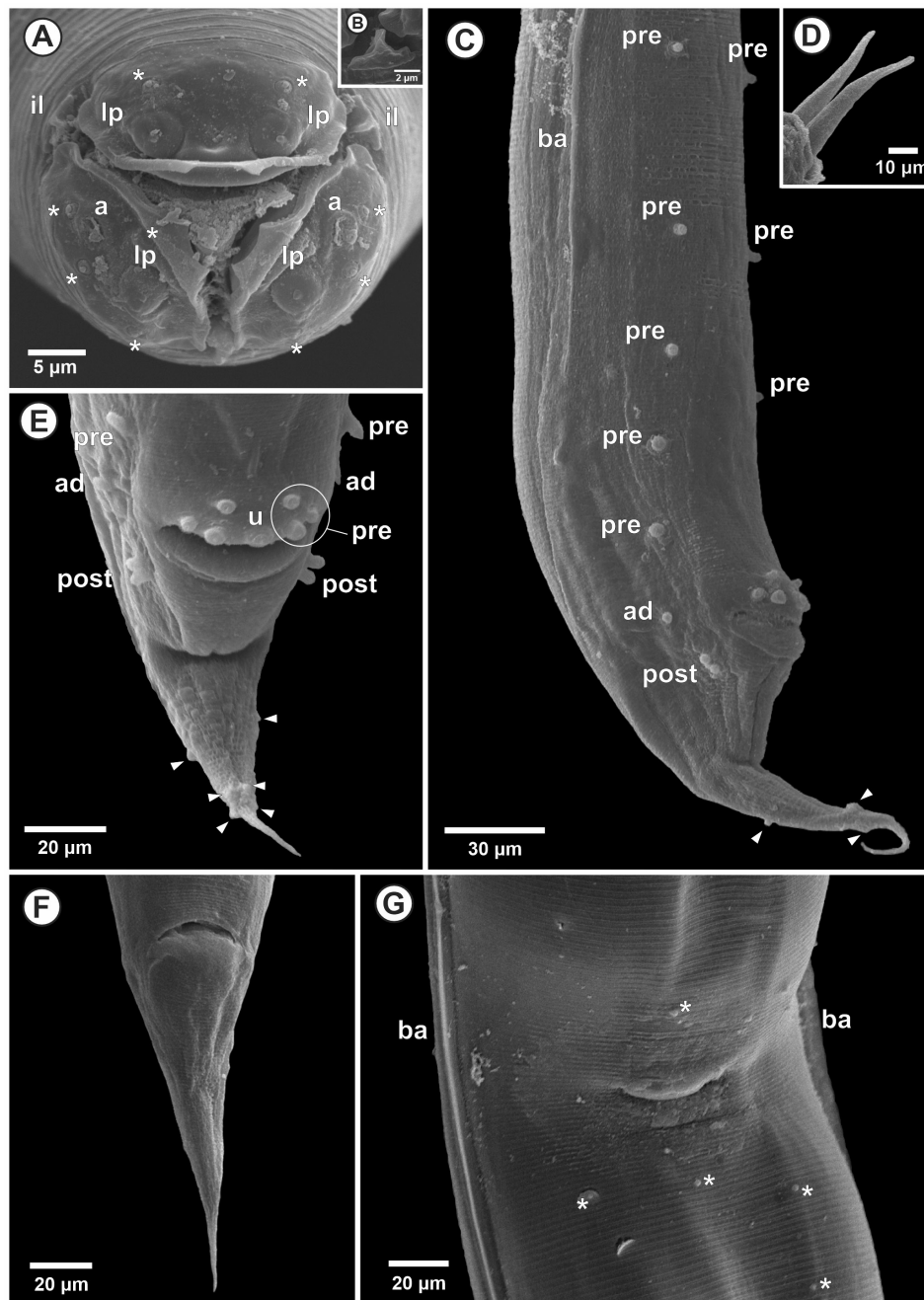


Figure 2. *Paradollfusnema amphisbaenia*. a) Female, anterior region, apical view (asterisks indicate the small labial papillae). b) Interlabia, lateral view. c) Male, posterior end, lateral view (white arrowhead indicate small postcloacal papillae). d) Detail of spicules. e) Male, cloacal region, ventral view (white arrowhead indicate small postcloacal papillae). f) Female, tail, ventral view. g) Female, detail of vulvar opening, ventral view (asterisks indicate the somatic papillae). **Abbreviations:** a, amphid; ad, adcloacal papilla; pre, precloacal papillae; u, unpaired papilla; lp, labial papillae; il, cuticular interlabia; ba, lateral alae; ae; post, postcloacal; Triangles, small postcloacal.

ring 225–335 (261 ± 30.3) and excretory pore 415–576 (540 ± 47.1) from anterior end, respectively. Caudal papillae markedly larger than somatic. Fourteen pairs of papillae and one unpaired median precloacal papilla (Figs. 1d, 2b). Eight pairs of precloacal papillae (Figs. 1d, 2b), first four pairs ventral, aligned longitudinally (Figs. 1d, 2b), equidistant from each other; fifth pair

laterally displaced (Figs. 1d, g, 2b, d), three pairs on anterior cloacal lip, sixth pair anterior to the seventh, and eighth pair lateral to the seventh. (Figs. 1d, g, 2d). Unpaired medial papilla on the anterior border of cloaca, with a sensilla pointed with one projection, located between last three pairs of precloacal papillae (Fig. 1d, g, 2b, d). One pair of adcloacal papillae (Figs. 1d, g,

2b, d), lateral to cloaca. Five pairs of postcloacal papillae, first and second pairs latero-ventral, in tandem, third pair latero-dorsal far from the first two pairs, fourth and fifth pairs near the tip of the tail, fourth pair ventral and fifth pair dorsal (Figs. 1d, g, 2b, d). Papillae arrangement 8+1:1:5 (precloacal+unpaired:adcloacal:postcloacal). Posterior cloacal lip smooth, without cuticular fringe (Figs. 1g, 2d). Phasmids not observed. Spicules equal in length and shape, not alate (Fig. 2c), with prominent curved proximal extremity and pointed distal extremity (Figs. 1d, f, g), 100–170 (124 ± 21.1) long. Gubernaculum triangular in ventral view, well sclerotized (Fig. f), 48–75 (60 ± 9.9) long. Tail conical, 200–250 (224 ± 19.5) long.

Female (based on 10 specimens): Body length 3.2–4.4 (3.9 ± 0.37) mm, width at level of oesophagus-intestinal junction 200–475 (318 ± 72.2). Oesophagus total length 712–787 (745 ± 25.7); pharynx 50–75 (62 ± 7.8) long, corpus 450–535 (493 ± 31.8) long, isthmus 60–100 (80 ± 14.76) long, bulb 110–150 (129 ± 21.5) long. Nerve ring 240–315 (275 ± 31.03) and excretory pore 525–615 (574 ± 36.3) from anterior end, respectively. Uterus didelphic and prodelphic, both ovaries anterior to vulvar opening (Fig. 1i). Vulva post equatorial without protruded lips, surrounded by somatic papillae (Fig. 1i, 2f), 2.0–2.8 (2.5 ± 0.24) mm from anterior end, corresponds to approximately 68.4% of total body length. Ovijector muscular along its entire length, anteriorly directed (Fig. 1i). Ellipsoidal larvated eggs in ovijector (Figs. 1i), 87–125 long (109 ± 10.2) and 68–119 (82 ± 10.6) wide. Tail conical, 200–263 (218 ± 19.7) long (Figs. 1h, 2e).

Taxonomic Summary

Host: *Leptodactylus podicipinus* (Cope, 1862) (Anura: Leptodactylidae) (Pointedbelly Frog).

Site of infection: large intestine.

Intensity of infection: 30 specimens collected from a single host.

Locality: Municipality of Petrolina (9°20'4.68" S; 40°35'11.25" W), Caatinga morphoclimatic domain, State of Pernambuco, Brazil.

Voucher specimens: Voucher specimens (6 males and 6 females): CHIOC 39680.

DISCUSSION

The nematodes analyzed belong to the genus *Paradollfusnema* due to the presence of triangular interlabia, caudal papillae without ornamentations (plectanes and/or rosettes punctuations) in males, and didelphic and prodelphic uterus in females, with larvated eggs on the ovijector, according to Baker (1981).

In relation to the specific diagnosis, the present specimens have morphological and morphometric characteristics similar to *P. amphisbaenia* as described by Baker (1981). Such characteristics are the presence of interlabia and lateral alae in both males and females, the size and shape of spicules and gubernaculum, and the shape and size of eggs (see Table I). However, the morphology and arrangement of the lips papillae in the present specimens differed from that in the description by Baker (1981), in which the newly collected material had two large papillae on dorsal lip with a small papilla at the base of each, and a large papilla on each latero-ventral lip with one small papillae at the base, and other two small papillae at the base of each amphid. In contrast, that in the original description (Baker 1981), the type specimens presented the two large papillae on the dorsal lip, but with smaller papillae closer to mouth opening, and a large papilla on the each latero-ventral lip with two smaller papillae also closer to mouth opening and a small papilla laterally to the amphidial pore. In this sense, our SEM analysis is more accurate in demonstrating the position of these labial papillae, as it showed that the specimens

Table I. Morphometrics of *Paradollfusnema* Baker, 1982 species, and number and arrangement of male caudal papillae.

	<i>Paradollfusnema amphisbaenia</i> (Baker, 1981)				<i>Paradollfusnema telfordi</i> Bursey, 2002	
Hosts	<i>Leptodactylus podicipinus</i>	<i>Leposternon microcephalum</i>			<i>Rhineura floridana</i>	
Localities	Petrolina, Pernambuco, Brazil	Recife, Pernambuco, Brazil			Florida, USA	
References	Current study		Baker (1981)		Bursey (2002)	
	Male	Female	Male	Female	Male	Female
Body length (mm)	2.7–3.6	3.2–4.4	2.4–2.8	2.4–2.8	2.1–2.82	2.54–3.07
Body width	200–290	200–475	-	-	82–104	98–110
Oesophagus length	618–795	712–787	626–716	631–742	451–528	461–548
Pharynx length	58–65	50–75	47–50	47–62	34–49	40–52
Oesophageal corpus length	450–525	450–535	425–512	428–503	305–366	305–372
Oesophageal bulb length	115–140	110–150	113–135	118–137	-	73–91
Nerve ring from anterior end	225–335	240–315	222–237	212–234	189–220	189–213
Excretory pore from anterior end	415–576	450–535	443–556	453–560	366–403	337–449
Spicule length	100–170	-	96–113	-	67–104	-
Relative size of spicule (%)	4.2%	-	4.5%	-	-	-
Gubernaculum length	48–75	-	55–67	-	46–61	-
Number and arrangement of pairs of caudal papillae (Total)	8+1:1:5* (14+1)	-	7-8+1:1:5* (13–14+1)	-	11+1:4:5* (20+1)	-
Vulva from anterior end (mm)	-	2.0–2.8	-	1.6–1.9	-	1.34–1.54
Egg length	-	87–125	-	115	-	64–76
Egg width	-	68–110	-	70	-	34–43
Tail	200–250	200–263	161–179	175–213	67–93	-

*Precloacal papilla+unpaired papilla:adcloacal papillae:postcloacal papillae.

studied have a distinct arrangement of the small labial papillae compared to the type specimens described by Baker (1981).

There were no variations observed in the number and arrangement of caudal papillae among the specimens analysed, contrary to

Baker's (1981) findings, which indicated that the precloacal papillae in males of *P. amphisbaenia* can vary between 7 and 8 pairs. *Paradollfusnema amphisbaenia* differs from *P. telfordi*, a parasite of *Rhineura floridana* (Baird, 1858) (Squamata: Amphisbaenidae) from the USA (Bursey 2002),

by having a smaller number of caudal papillae than the latter, as well as a distinct arrangement of these papillae (14 pairs plus an unpaired medial precloacal papilla, arrangement 8+1:1:5 vs. 20 pairs plus an unpaired medial precloacal papillae, arrangement 11+1:4:5), non-alate spicules (vs. alate spicules), and larger eggs (87-125 x 68-110 vs. 64-76 x 34-43) (see Bursey 2002) (Table I).

The interlabia are key structures for differentiating the genera in Cosmocercidae (Baker 1981, 1982). Due to their difficult visualization in light microscopy, species of *Paradollfusnema* can be easily attributed to a different genus where males also present unornamented caudal papillae and females have didelphic and prodelphic uterus, such as *Aplectana* Railliet & Henry, 1916 and *Raillietnema* Travassos, 1927. Therefore, SEM analyses are essential to avoid misidentification of genera in Cosmocercidae.

The rare records of *Paradollfusnema* have been exclusive to amphisbaenid until now. *Paradollfusnema amphisbaenia* was reported parasitizing *Leposternon microcephalum* Wagler, 1824 (Squamata: Amphisbaenidae) in Recife, State of Pernambuco, Brazil (Baker 1982), *Leposternon wolchereri* (Peters, 1879) (= *Amphisbaenia wolchereri*) in Minas Gerais, Brazil (Filogônio et al. 2013), and *L. polystegum* (Duméril, 1851) in Ceará, Brazil (Amorim et al. 2014). Additionally, *P. telfordi* was described parasitizing only *Rhineura floridana* in Florida, USA (Bursey 2002).

The occurrence of a parasite in a species of anurans that was originally described in amphisbaenians may be explained by the fact that *L. podicipinus* occupies the same type of habitat as amphisbaenids. It is believed that this species of frog exhibits semi-fossorial habits and behaviour of digging into the soil for protection (Ponssa et al. 2011), which may facilitate the

transmission of certain cosmocercids, as these are monoxenous parasites with infective stages in soil or water (Anderson 2000). In Brazil, for example, this low host specificity was previously observed in another Cosmocercidae, *Cosmocerca rara* Freitas & Vicente, 1966, which was also recorded parasitizing the toad *Rhinella crucifer* (Wied-Neuwied, 1821) (Bufonidae), and the amphisbaenian *Leposternon microcephalum* Wagler, 1824 (Amphisbaenidae) (Ávila & Silva 2010, Campião et al. 2014).

Therefore, in addition to being the first study to use SEM to describe the morphology of *Paradollfusnema*, this is also the first record of this genus parasitising an anuran species worldwide.

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