



Two new species of *Cosmocercidae* (Nematoda: *Cosmocercoidea*) of *Leptodactylus macrosternum* Miranda-Ribeiro (Anura: *Leptodactylidae*) from Caatinga Biome, Brazil

GABRIELA FELIX-NASCIMENTO^{1,2,7}, FABIANO MATOS VIEIRA^{3,4*},
LUÍS CLÁUDIO MUNIZ-PEREIRA^{3,4,8}, GERALDO JORGE BARBOSA DE MOURA^{1,5},
LEONARDO BARROS RIBEIRO⁶ & JAQUELINE BIANQUE DE OLIVEIRA^{1,2,9}

¹Programa de Pós-graduação em Ciência Animal Tropical (PPGCAT), Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manoel de Medeiros, Recife CEP 52051-360, Brazil.

²Laboratório de Parasitologia, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manoel de Medeiros s/n°, Recife CEP 52051-360, Brazil.

³Laboratório de Helminthos Parasitos de Vertebrados, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, Rio de Janeiro CEP 21040-900, Brazil.

⁴Programa de Pós-graduação em Biodiversidade e Saúde (PPGBS), Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, Rio de Janeiro CEP 21040-900, Brazil

⁵Laboratório de Estudos Herpetológicos e Paleoherpétológicos, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manoel de Medeiros s/n°, Recife CEP 52051-360, Brazil.

✉ geraldojbm@gmail.com; <https://orcid.org/0000-0001-6035-516X>

⁶Universidade Federal do Vale do São Francisco (UNIVASF), Rodovia BR-407, KM 12 Lote 543 S/n Projeto de Irrigação Nilo Coelho, Petrolina, 56300-000, Brazil. ✉ leonardo.ribeiro@univasf.edu.br; <https://orcid.org/0000-0003-4491-0236>

⁷✉ gabriela.veterinaria@yahoo.com.br; <https://orcid.org/0000-0001-8623-9546>

⁸✉ lmuniz@ioc.fiocruz.br; <https://orcid.org/0000-0002-7468-6274>

⁹✉ bianque01@yahoo.com.br; <https://orcid.org/0000-0002-6120-7895>

*Corresponding author. ✉ fmatosvieira@gmail.com; <https://orcid.org/0000-0002-5220-7252>

Abstract

In the current study, two new species of *Cosmocercidae* nematodes parasitizing the anuran *Leptodactylus macrosternum* (*Leptodactylidae*) from two localities in the municipality of Petrolina, sub-middle São Francisco river, state of Pernambuco, Brazil, were described. *Oxysomatium petrolinensis* **n. sp.** differs from all congeneric species for having the male caudal papillae distribution pattern unique in this genus, in which the last three pairs of pre-cloacal papillae are located lateroventrally on the anterior border of the cloacal aperture. Additionally, the new species has an unpaired medial precloacal papilla, seven pairs of postcloacal papillae, and a relatively unique spicule size. *Oxyascaris caatingae* **n. sp.** differs from all congeneric species mainly for having males with a medial unpaired precloacal papilla and a distribution pattern of caudal papillae unique to this species, being the only species of the genus with adcloacal papillae. The current study proposes the description of *Oxysomatium petrolinensis* **n. sp.**, fourth species and the first report of this genus in hosts from the Neotropical region. In addition, it describes *Oxyascaris caatingae* **n. sp.**, which is the fifth species of the genus.

Key words: Helminth, Neotropical anurans, nematode biodiversity, taxonomy, *Oxyascaris*, *Oxysomatium*

Introduction

The family *Cosmocercidae* (Ascaridida: *Cosmocercoidea*) is composed by gastrointestinal nematodes of amphibians and squamates (lizards, snakes and amphisbaenians), occurring in the Americas, Europe, Asia and Oceania (Chabaud 1978, Moravec 1998, Ávila & Silva 2010, Campião *et al.* 2014). Currently, this family consists of 11 genera: *Aplectana* Railliet & Henry; *Cosmocerca* Diesing; *Cosmocercella* Steiner; *Cosmocercoides* Wilkie; *Maxvachonia* Chabaud & Brygoo; *Neocosmocercella* Baker & Vaucher; *Oxyascaris* Travassos; *Oxysomatium* Railliet & Henry; *Paradollfusnema* Baker; *Paraplesiohedruris* Bursey, Goldberg & Kraus; and *Raillietnema* Travassos

(Chabaud 1978, Baker 1980, Baker & Vaucher 1985, Bursey *et al.* 2012). Only eight of these genera are reported in the South America (Ávila & Silva 2010, Campião *et al.* 2014). In Brazil, three species of Cosmocercidae were recorded in lizards and six species in amphisbaenians (Ávila & Silva 2010, Amorim *et al.* 2017). However, the highest species richness of this family occurs in anurans, which until the present study consist of 22 nematode species (Camião *et al.* 2014, Santos *et al.* 2017).

Leptodactylus macrosternum Miranda-Ribeiro (Anura: Leptodactylidae) is an abundant anuran species in the Caatinga biome, and although its biology is relatively well known, studies on its relationship with its helminths can still be considered scarce (Teles *et al.* 2018). In Brazil, *Cosmocerca podicipinus* Baker & Vaucher (Camião *et al.* 2014); *C. brasiliense* Travassos; *C. parva* Travassos; *Brevimulticaecum* Mozgovoy, Skrzjabin, Shikobalova & Mozgovoy (Goldberg *et al.* 2002); *Rhabdias breviensis* Nascimento, Gonçalves, Melo, Giese, Furtado & Santos (Nascimento *et al.* 2013); *Oxyascaris oxyascaris* Travassos (Camião *et al.* 2014); *Rhabdias* sp. (Teles *et al.* 2018); and *Foleyella convoluta* Molin (Teles *et al.* 2017) were nematodes previously reported in this host species.

In the current study, two new species of Cosmocercidae nematodes were described. This included a new record of a species of the genus *Oxysomatium* in a Neotropical host.

Material and methods

Specimens of *L. macrosternum* were collected in two localities in the municipality of Petrolina, sub-middle São Francisco river, state of Pernambuco, Brazil. This municipality, which is inserted in the northeastern semi-arid, presents typical *sensu stricto* Caatinga vegetation, with the climate characterized by high temperatures, and irregular and scarce periods of rain (Prado 2003). The first collection site is an agricultural area (9°0'17.85"S, 40°17'30.82"W), where a total of 51 anurans were collected in May 2018, and February, September and October 2019. The second collection site is an area of Caatinga *sensu stricto* (9°7'35.58"S, 40°21'30.79"W), where a total of 46 hosts were collected in May, September and October 2018, and February 2019. All hosts were collected manually by active search and sent alive to the Laboratório de Morfofisiologia do Centro de Conservação e Manejo de Fauna da Caatinga (CEMAFAUNA-CAATINGA), until necropsy. Hosts were identified according to Frost (2020) and representative specimens were deposited in the Coleção Herpetológica do Museu de Fauna da Caatinga (MFCH—5346, 5347, 5349, 5350, 5352, 5354–5357, 5363, 5365, 5381, 5382, 5388, 5402–5404, 5406, 5408, 5411, 5418, 5420, 5423, 5424, 5431, 5432, 5434, 5442), Universidade Federal do Vale do São Francisco (UNIVASF), state of Pernambuco, Brazil.

Hosts were killed with an overdose of lidocaine hydrochloride applied topically to the animal's dorsal region, according to the recommendations of the Conselho Nacional de Controle de Experimentação Animal (2018). Necropsies were performed under a stereomicroscope. Once collected, the live nematodes were placed in Petri dishes with 0.85% saline. Live parasites were fixed in hot 4% formaldehyde solution, remaining for 15 days at room temperature, and posteriorly preserved in 70° GL ethanol for morphological studies.

For identification, the nematodes were cleared in Amann's lactophenol and mounted on temporary slides, identified under light microscopy. Drawings were made using a drawing tube attached to an Olympus BX 51 microscope in the Laboratório de Helminthos Parasitos de Vertebrados, in the Oswaldo Cruz Institute (IOC), FIOCRUZ, Rio de Janeiro, Brazil. Measurements are given in micrometers.

For scanning electron microscopy (SEM) studies, some specimens were dehydrated through a graded ethanol series, dried in 1,1,1,3,3,3-Hexamethyldisilazane 97% (HMDS) (Sigma-Aldrich), coated with gold and observed in a JEOL JSM 6390LV microscope (operating at 15 kV), in the Plataforma de Microscopia Eletrônica Rudolf Barth, of the IOC, FIOCRUZ, Rio de Janeiro, Brazil.

Nematodes were identified at generic level according to Chabaud (1978) and Baker & Vaucher (1985). The prevalence, mean intensity, and mean abundance of helminths were calculated according to Bush *et al.* (1997). Holotypes, allotypes and paratypes were deposited in the Coleção Helminológica do Instituto Oswaldo Cruz (CH-IOC), IOC, FIOCRUZ.

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 N° 62680-1), and license of the Comitê de Ética no Uso de Animais (CEUA) of the UNIVASF (UNIVASF N° 0001/221018).

Results

We recorded a total of 171 nematodes: 157 specimens of *Oxysomatium petrolinensis* n. sp. in the large intestine of 14 hosts from the organic agriculture area (Fig. 1), and 50 specimens of *Oxyascaris caatingae* n. sp. in the small intestine of 14 hosts from the Caatinga *stricto sensu* area (Fig. 1). Each of these parasite species has been observed to parasitize hosts from respective areas. No co-infections were observed between these two new nematode species.

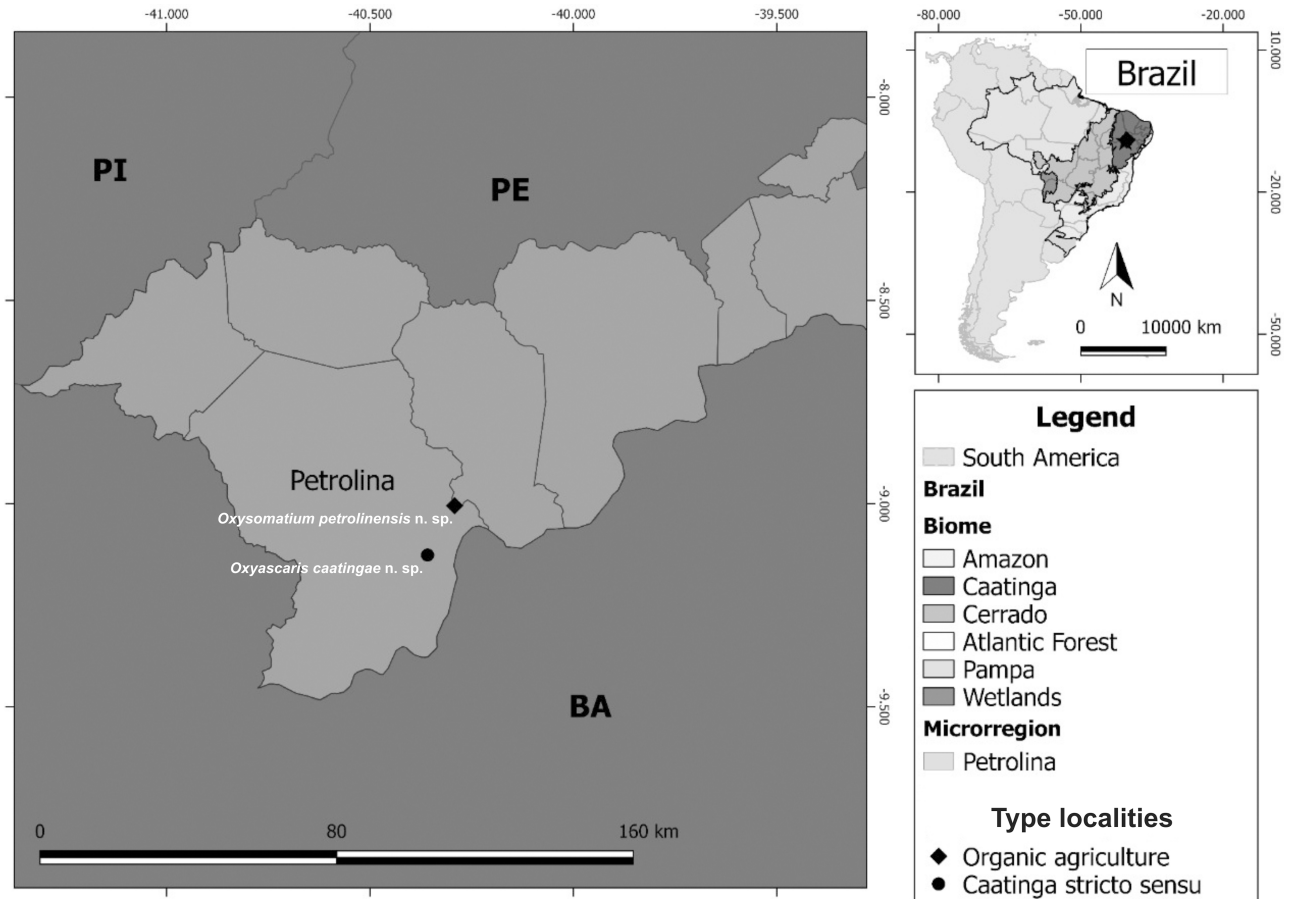


FIGURE 1. Type localities of *Oxysomatium petrolinensis* n. sp. and *Oxyascaris caatingae* n. sp. in municipality of Petrolina, state Pernambuco, Brazil. Abbreviations: BA – state of Bahia; PE – state of Pernambuco; PI – state of Piauí.

Descriptions

Oxysomatium petrolinensis n. sp.

(Fig. 2 and 3)

General. Sexual dimorphism not evident. Presence of narrow lateral body alae and numerous somatic papillae in both sexes (Fig. 2C, D, E, F, I, 3A, C, F). Cephalic end with three lips and triangular buccal aperture (Fig. 2B, 3B). Dorsal lip with two large papillae on the edge; a small posterior papilla, on the same longitudinal line as each large papilla, totalling four papillae. (Fig. 2B, 3B). Lateroventral lips with two papillae on the edge of each lip, with the ventral papilla larger than the medial which is close to the buccal aperture; lateral to the medial papilla has one small papilla, totalling three papillae in each lateroventral lip (Fig. 2B, 3B). Lateral amphids (Fig. 2B, 3B). Oesophagus oxyuriform, divided into four parts: pharynx, corpus, isthmus and bulb (Fig. 2C, D). Nerve ring anterior to excretory pore, located between the anterior third and middle third of the oesophagus (Fig. 2C, D). Excretory pore located at the level of oesophageal isthmus, between the middle third and the posterior third of the oesophagus (Fig. 2C, D).

Male (based on 10 specimens): Total body length 1.8–2.8 (2.6 ± 0.3) mm, body width at level of oesophagus-intestinal junction 82–135 (111 ± 14). Total oesophagus length 404–526 (482 ± 34); pharynx 29–40 (34 ± 3) long, corpus

280–375 (328±27) long, isthmus 20–44 (35±7) long, bulb 74–90 (84±4) long. Nerve ring 173–298 (215±33) and excretory pore 330–368 (346±23) from anterior end. Lateral body alae that appear at the level of the initial third of the oesophagus, anterior to nerve ring (Fig. 2C), and ends up before the cloaca, between the second and third pairs of precloacal papillae (Fig. 2F, 3C). Caudal papillae slightly larger in size than somatic papillae (Fig. 3A–F). Cloacal aperture has protuberances within the posterior border that form a row of cuticular fringes (Fig. 3C, D). Posterior region with a total of 16 pairs of papillae and one unpaired medial precloacal papilla (8+1:1:7) (Fig. 2E–G, 3C–E). Eight pairs of precloacal papillae, five lateroventral pairs, with the first located at level of proximal end of spicules and the fifth pair at level of the gubernaculum, the other three pairs lateroventral on the anterior border of cloaca (Fig. 2E, G, 3C, D). One unpaired medial precloacal papilla in the centre of a protuberance on the anterior border of cloacal aperture (Fig. 2E, G, 3C, D). One pair of lateral adcloacal papillae (Fig. 2E, G, 3C, D). Seven pairs of postcloacal papillae, of which the first two pairs are lateroventral, close together, with the second pair posterior and diagonal to the first pair; the third is laterodorsal and distant from the first two; the fourth pair is lateroventral close to the third pair; the fifth pair is dorsal, between the fourth and sixth pair. Sixth pair lateral and close to the tip of tail; the seventh pair is lateroventral and slightly later than the sixth pair (Fig. 2E–G, 3C, E). Phasmids not found. Spicules equal and slender, with rounded proximal end and sharply pointed distal end, not alate (Fig. 2E, F), 187–283 (207±28) long. Gubernaculum present, oval in ventral view, with lateral and distal margins more sclerotized in relation to the proximal region (Fig. 2G, H), 56–80 (68±8) long. Tail conical with short filamented distal end (Fig. 2E–G, 3C, E), 161–218 (182±15) long.

Female (based on 10 specimens): Total body length 3.2–3.7 (3.4±0.6) mm, body width at level of oesophagus-intestinal junction 137–180 (160±16). Total oesophagus length 435–580 (516±48); pharynx 30–45 (37±4) long, corpus 236–405 (336±52) long, isthmus 31–45 (38±4) long, bulb 90–128 (104±11) long. Nerve ring 204–350 (236±45) and excretory pore 345–420 (388±24) from anterior end. Lateral body alae originate at the level of the initial third of the oesophagus, anterior to nerve ring, and terminate posterior to anus. Didelphic and amphidelphic, with the ovary of the anterior uterus located posterior to the vulvar opening and the ovary of the posterior uterus located anterior to the vulva (Fig. 2A). Vulva post equatorial, slightly protruded, between 2nd and 3rd thirds of the body, 2–2.4 (2.1±0.1) mm from anterior end. Ovipositor thin and muscular along its entire length, anteriorly directed (Fig. 2J). Oval larvated eggs in ovipositor (Fig. 2J), 80–115 (98±9) long and 40–83 (57±9) width. Tail conical, with filamented distal end (Fig. 2I, 3F), 201–247 (224±13).

Taxonomic summary

Type host: *Leptodactylus macrosternum* Miranda-Ribeiro (Anura, Leptodactylidae) (Miranda's White-lipped Frog, Caçote)

Type locality: Organic agriculture area (9°0'17.85"S, 40°17'30.82"W), in the municipality of Petrolina, state of Pernambuco, Brazil

Site of infection: Large intestine

Prevalence: 27.5% (14 infected hosts)

Mean intensity: 6.2±3.2 parasites per infected hosts

Mean abundance: 3.1±1.3 parasites per analyzed hosts

Range of infections: 1–50

Type material: Holotype male: CHIOC 38970a, allotype female: CHIOC 38970b, paratypes: CHIOC 38970c.

Etymology: The specific name refers to the first species of this genus described in a host in the municipality of Petrolina, state of Pernambuco, Brazil.

Remarks. Currently, three species of the genus *Oxysomatium* Railliet & Henry are known. They differ from each other by the number and position of caudal papillae in males, presence/absence and size of gubernaculum, size of spicules, position of vulva, and egg size (Sharpilo 1974, Baker 1980) (Table 1).

One of the differences between *Oxysomatium* species is the presence/absence of an unpaired papilla on the anterior border of the cloacal aperture. Thus, *Oxysomatium petrolinensis* **n. sp.**, *O. brevicaudatum* (Zeder) and *O. dolfusi* Baker differ from *O. caucasicum* Sharpilo due to the presence of an unpaired precloacal papilla, while in the latter this papilla is absent (Sharpilo 1974, Baker 1980) (Table 1).

The number and distribution of caudal papillae in males of *Oxysomatium* can also be considered a specific differentiation. *Oxysomatium petrolinensis* **n. sp.** and *O. brevicaudatum* present seven pairs of postcloacal

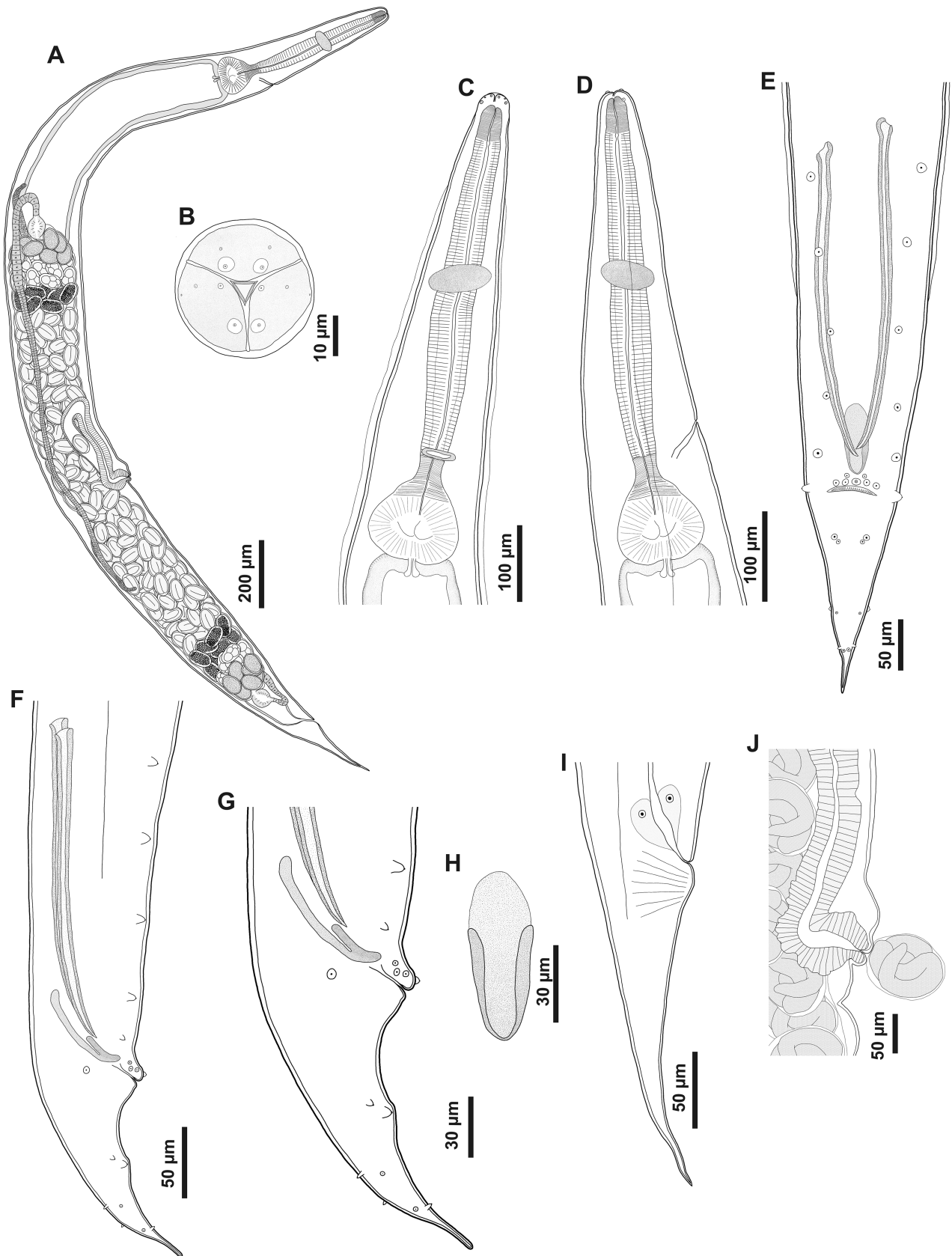


FIGURE 2. *Oxysomatium petrolinensis* n. sp. (A) total female length. (B) anterior end, male, apical view. (C) anterior region, male, ventral view. (D) anterior region, male, lateral view. (E) posterior region, male, ventral view. (F) posterior region, male, lateral view. (G) tail, male, lateral view. (H) gubernaculum, ventral view. (I) tail, female, lateral view. (J) vulva, ovjector and eggs, lateral view.

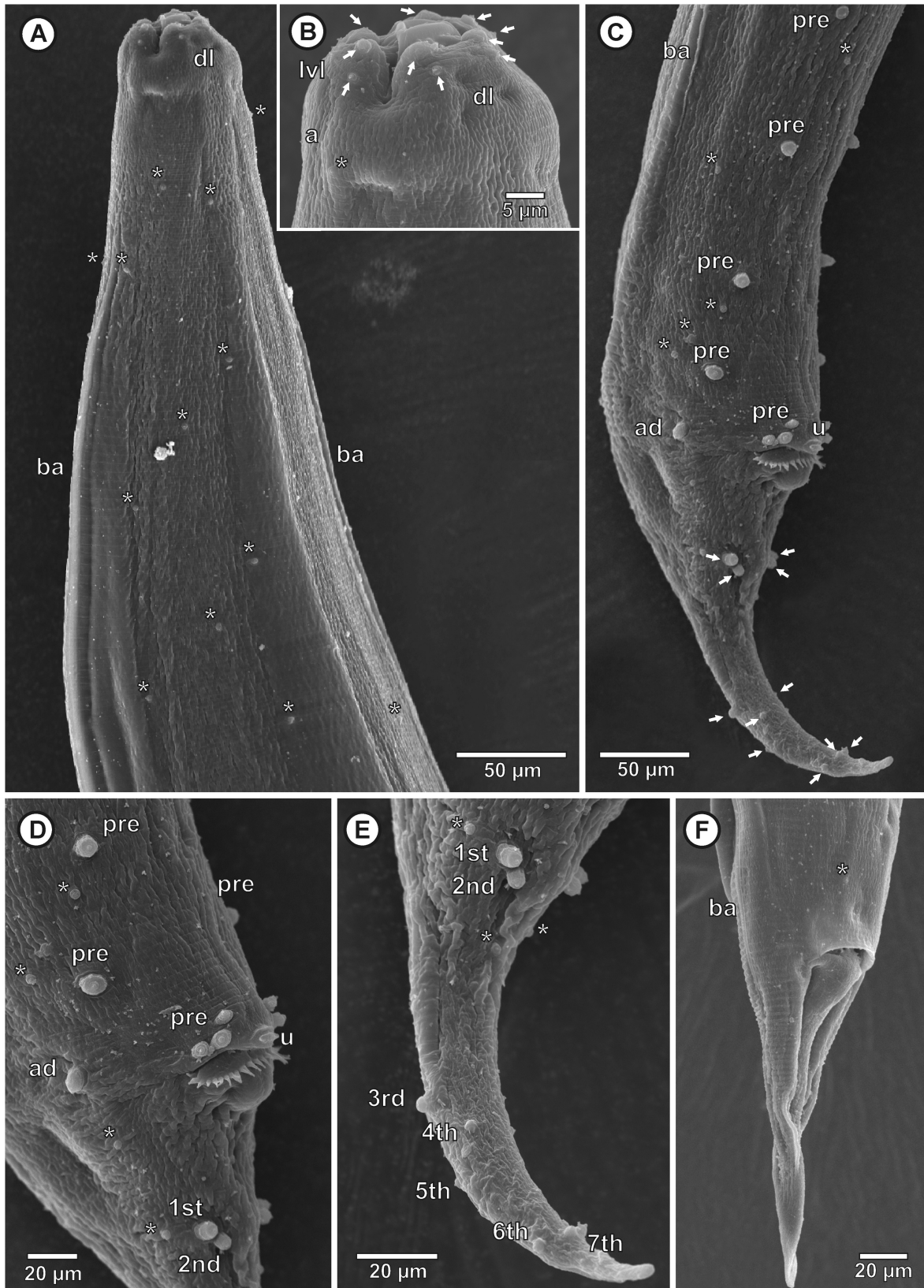


FIGURE 3. *Oxyssomatium petrolinensis* n. sp. (A) anterior region, male, dorsal view. (B) anterior end, male, dorsal view (white arrows indicate the cephalic papillae). (C) posterior region, male, latero ventral view (white arrows indicate post cloacal papillae). (D) cloacal region, male, latero ventral view. (E) tail filament, male, latero ventral view. (F) tail, female, latero ventral view. (*—somatic papillae; 1st–7th—first to seventh post cloacal papillae; a—amphid; ad—ad cloacal papillae; ba—body alae; dl—dorsal lip; lvi—latero ventral lip; pre—pre cloacal papillae; u—unpaired pre cloacal papillae).

TABLE 1. Comparative morphometrics of *Oxysomatium* Railliet & Henry species.

Type hosts	<i>O. petrolinensis</i> sp. n.		<i>O. brevicaudatum</i> (Zeder)		<i>O. caucasicum</i> Sharpilo		<i>O. dollfusii</i> Baker	
	Petrolina, Pernambuco, Brazil		Unspecified type host		Caucasus		Casa Blanca, Morocco	
Type localities	Miranda-Ribeiro (Anura, Leptodactylidae)		Unspecified type host		Caucasus		Casa Blanca, Morocco	
References	Current study		Baker (1980)		Sharpilo (1974)		Baker (1980)	
Body length (mm)	Male	Female	Male	Female	Male	Female	Male	Female
Body width (µm)	1.8–2.8 (2.5±0.3)	3.2–3.7 (3.4±0.6)	5.0–6.9	6.3–8.2	4.2–4.6	6.0–6.5	4.8	5.0
Oesophagus length (µm)	82–135 (111±14)	137–180 (160±16)	-	-	180–190	310–330	-	-
Pharyngeal region length (µm)	404–526 (482±34)	435–580 (516±48)	750–878	882–1154	690–710	710–770	651	780
Isthmus length (µm)	29–40 (34±3)	30–45 (37±4)	53–69	59–72	55	55	51	58
Oesophagus corpus (µm)	20–44 (35±7)	31–45 (38±4)	31–41	34–41	-	-	39	55
Oesophagus bulb length (µm)	280–375 (328±27)	236–405 (336±52)	566–666	691–913	-	-	463	555
Oesophagus bulb width (µm)	74–90 (84±4)	90–128 (104±11)	97–109	109–128	-	-	98	112
Nerve ring (µm)	67–85 (76±6)	92–110 (102±7)	-	-	90–99	110–120	-	-
Excretory pore (µm)	173–298 (215±33)	204–350 (236±45)	356–422	400–484	330	350	275	320
Spicule length (µm)	330–368 (346±23)	345–420 (388±24)	447–563	513–669	400–480	450–500	402	453
Relative size of spicule (%)	187–283 (207±28)	-	1400–2000	-	380–400	-	192	-
Gubernaculum length (µm)	10.1–10.4	-	28–28.9	-	8.7–9.04	-	4	-
Total number of pairs of papillae	56–80 (68±8)	-	113–119	-	82	-	78	-
Number and position of pairs of caudal papillae (pre:ad:post)	15+1	-	14–16+1	-	17+0	-	12–13+1	-
Unpaired papillae	8:1:7	-	6–8:1:7	-	10:1:6	-	7–8:1:4	-
Vulva from anterior end (mm)	Yes	-	Yes	-	No	-	Yes	-
Vulva position	-	2.0–2.4 (2.1±0.1)	-	3.5–4.5	-	2.0–2.6	-	2.8
Egg length (µm)	-	Post equatorial	-	Equatorial	-	Pre equatorial	-	Post equatorial
Egg width (µm)	-	80–115 (98±9)	-	62–72	-	80–82	-	69–78
Tail (µm)	-	40–83 (57±9)	-	35–45	-	49–50	-	40–45
	161–218 (182±15)	201–247 (224±13)	153–172	209–241	110–113	170–180	137	208

papillae, while *O. dolfusi* present only four pairs (Baker 1980). The relative spicule size is also a distinguishing feature between these three species. In *O. petrolinensis* **n. sp.**, the spicules represent about 10.2% of the total body length of males, while in *O. dolfusi* it represents 4% of total body length and in *O. brevicaudatum* it represents 28.5% (Baker 1980) (Table 1).

Although *O. petrolinensis* **n. sp.** and *O. brevicaudatum* have the same number of caudal papillae, other features besides the relative size of the spicules differentiate these two species. The females' vulva (Table 1) is postequatorial in *O. petrolinensis* **n. sp.** and equatorial in *O. brevicaudatum* (Baker 1980). In addition, the caudal papillae distribution pattern in males of *O. petrolinensis* **n. sp.** is unique to this species, especially considering the last three pairs of pre-cloacal papillae, since these are located lateroventrally on the anterior border of the cloacal aperture (Fig. 2E, G, 3D), which is not observed in other species of this genus (see Sharpilo 1974, Baker, 1980).

***Oxyascaris caatingae* n. sp.**

(Figs. 4 and 5)

General. Sexual dimorphism evident, with females up to four times larger than males. Males with numerous somatic papillae (Fig. 5C), females without somatic papillae. Lateral body alae absent in both sexes. Cephalic end with three lips and triangular buccal aperture (Fig. 4C, 5A, B). Dorsal lip with two large papillae on the edge (Fig. 4C, 5A, B). Lateroventral lips with two papillae on the edge, one large on the ventral edge, and another small on dorsal edge of each lip (Fig. 4C, 5A, B). Lateral amphids (Fig. 4C, 5A, B). Oesophagus oxyuriform, divided into four parts: pharynx, corpus, isthmus and bulb (Fig. 4A–B). Nerve ring anterior to excretory pore, located between the anterior third and middle third of the oesophagus (Fig. 4A–B). Excretory pore located at the level of oesophageal isthmus, between the middle third and the posterior third of the oesophagus (Fig. 4A–B).

Male (based on 4 specimens): Total body length 4.4–4.8 (4.6±0.2) mm, body width at level of oesophagus-intestinal junction 150–175 (159±12). Total oesophagus length 396–575 (458±84); pharynx 34–38 (36±2) long, corpus 251–396 (337±76) long, isthmus 59–83 (75±13) long, bulb 50–62 (57±6) long. Nerve ring 197–211 (205±8) and excretory pore 385–451 (420±27) from anterior end. Caudal papillae slightly larger in size than somatic papillae. Caudal papillae slightly larger in size than somatic papillae (5+1:1:9) (Fig. 4D–E). Five pairs of lateroventral precloacal papillae (Fig. 4D–E, 5C), with the first located anterior to proximal end of spicules and the fifth pair immediately anterior to cloacal aperture (Fig. 4D–E). One large unpaired medial precloacal papilla on the anterior border of cloacal aperture (Fig. 4D–G, 5E, F). One pair of lateral adcloacal papillae located at the commissure of the cloacal aperture (Fig. 4D–G, 5E, F). Nine pairs of postcloacal papillae; the first lateroventral pair just below the adcloacal papillae; second pair lateroventral and posterior to the first pair; third pair lateroventral posterior to the second pair, but more external to the second pair; fourth pair lateral; fifth pair lateroventral; sixth pair lateral; seventh pair lateroventral; eighth pair lateral, slightly posterior than the seventh pair; and ninth pair laterodorsal, aligned transversely with the eighth pair (Fig. 4D–G, 5E–G). Small lateral phasmids, posterior to the eighth pair of postcloacal papillae (Fig. 4F, 5G). Spicules equal and slender, with rounded proximal end and slightly rounded distal end, not alate, 95–109 (103±7) long (Fig. 4D–G, I). Gubernaculum present, slender in ventral view, 30–50 (41±10) long (Fig. 4D–G, J). Tail conical (Fig. 4D–G, 5C, E, G), 205–265 (238±25) long.

Female (based on 8 specimens): Total body length 9.7–21.9 (13.8±4.5) mm, body width at level of oesophagus-intestinal junction 187–291 (225±39). Total oesophagus length 620–884 (759±99); pharynx 38–52 (43±5) long, corpus 507–761 (625±98) long, isthmus 60–96 (80±13) long, bulb 56–103 (73±15) long. Nerve ring 242–375 (292±52) and excretory pore 466–830 (613±114) from anterior end. Didelphic and amphidelphic, with the ovary of the anterior uterus located anterior to the vulvar opening and the ovary of the posterior uterus located posterior to the vulvar opening. Vulva preequatorial, not protruded, 4.1–9.6 (6.1±2.0) mm from anterior end. Ovijector thin and muscular along its entire length, anteriorly directed (Fig. 4K). Eggs oval, larvated in ovijector (Fig. 4K), 68–103 (85±11) long and 37–68 (54±8) width (Fig. 4K). Tail conical elongated, 810–1,539 (997±241) (Fig. 4H, 5D).

Taxonomic summary

Type host: *Leptodactylus macrosternum* Miranda-Ribeiro (Anura, Leptodactylidae) (Miranda's White-lipped Frog, Caçote).

Type locality: Caatinga *stricto sensu* area (9°7'35.58"S, 40°21'30.79"W), in the municipality of Petrolina, state of Pernambuco, Brazil.

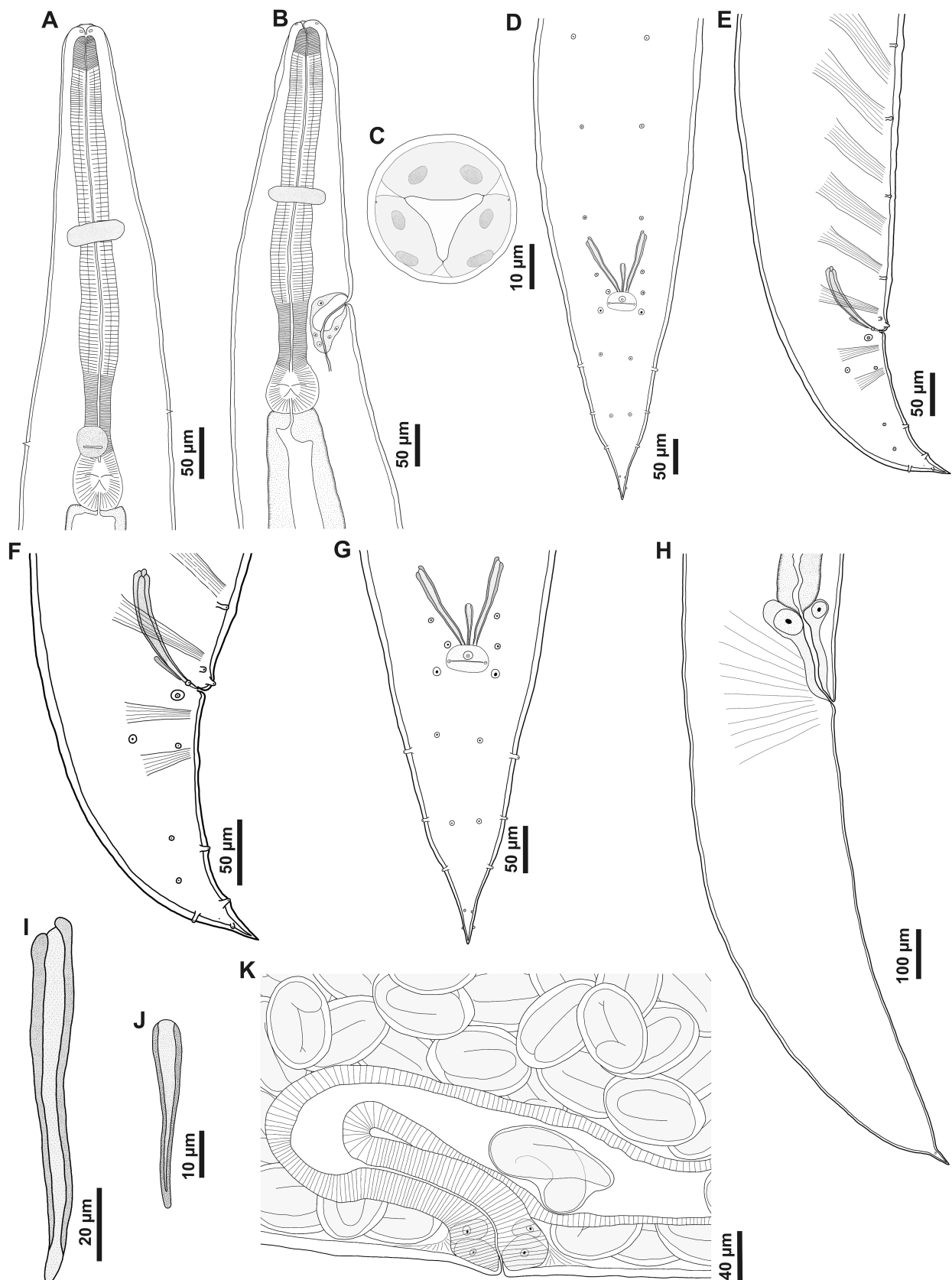


FIGURE 4. *Oxyascaris caatingae* n. sp. (A) anterior region, male, ventral view. (B) anterior region, male, lateral view. (C) anterior end, male, apical view. (D) posterior region, male, ventral view. (E) posterior region, male, lateral view. (F) tail, male, lateral view. (G) tail, male, ventral view; (H) tail of female, lateral view. (I) left spicule, ventral view. (J) gubernaculum, ventral view. (K) vulva, ovijector, and eggs, lateral view.

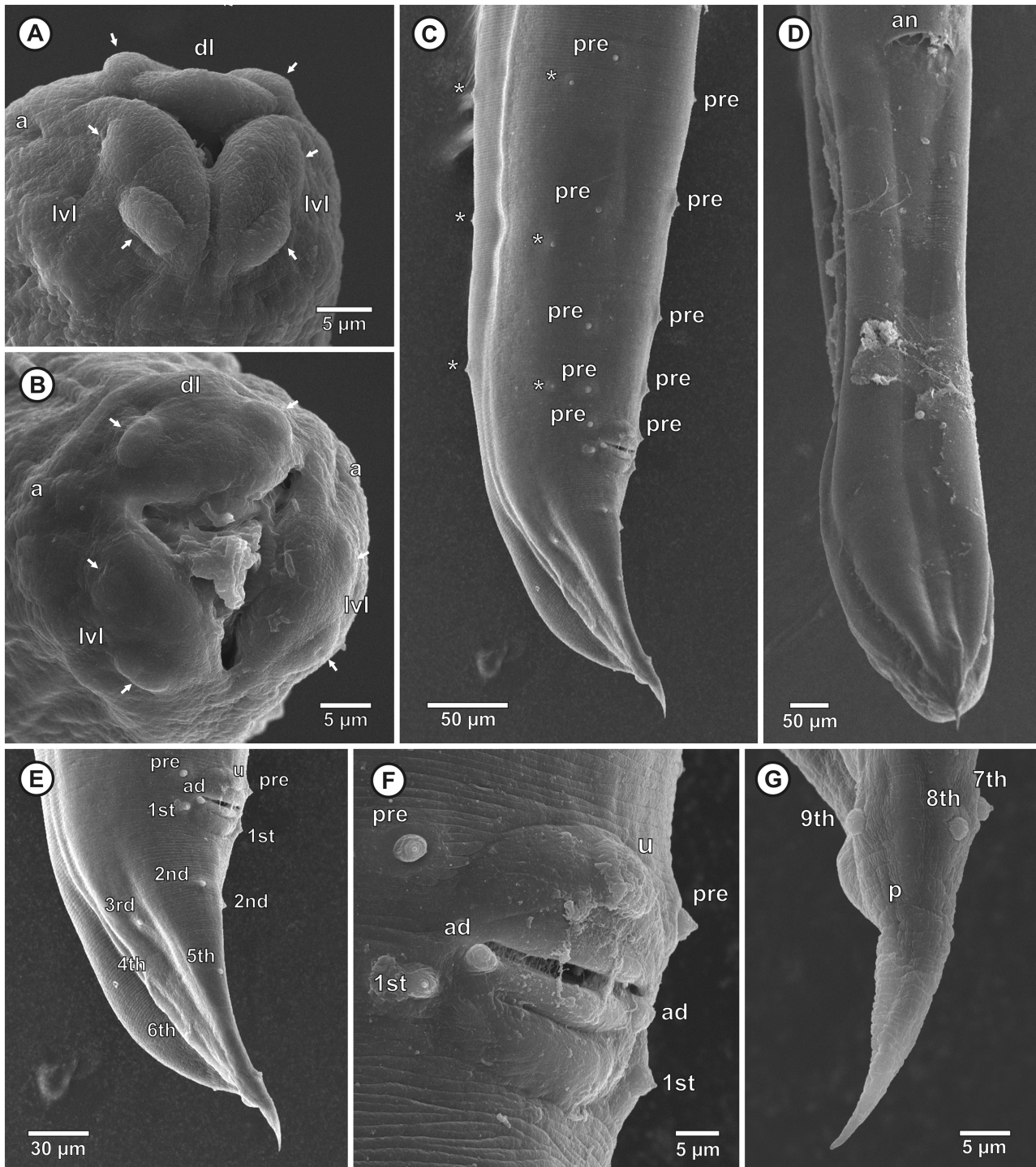


FIGURE 5. *Oxyascaris caatingae* n. sp. (A) anterior end, male, apical view (white arrows indicate the cephalic papillae). (B) anterior end, female, apical view (white arrows indicate the cephalic papillae). (C) posterior region, male, latero ventral view. (D) tail, female, latero ventral view. (E) tail, male, latero ventral view. (F) cloacal region, male, latero ventral view. (G) tip of tail, male, latero ventral view. (*—somatic papillae; 1st–9th—first to ninth post cloacal papillae; a—amphid; ad—ad cloacal papillae; an—anus; dl—dorsal lip; lvi—latero ventral lip; pre—pre cloacal papillae; u—unpaired pre cloacal papillae).

Site of infection: Small intestine

Prevalence: 30.4% (14 infected hosts)

Mean intensity: 5.6±3.3 parasites per infected hosts

Mean abundance: 1.1±0.4 parasites per analyzed hosts

TABLE 2. Comparative morphometrics of *Oxyascaris* Travassos species

	<i>O. caatingae</i> n. sp.		<i>O. caudacutus</i> (Freitas)		<i>O. oxyascaris</i> Travassos		<i>O. similis</i> (Travassos)		<i>O. mcclarni</i> Bursley & Goldberg	
Type hosts	<i>Leptodactylus macrosternum</i> Miranda-Ribeiro (Anura, Leptodactylidae)		<i>Scinax nasicus</i> (Cope) (= <i>Hyla nasicca</i>) (Anura, Hylidae)		<i>Mastigodryas bifossatus</i> (Rad-di) (= <i>Dryadophis bifossatus</i>) (Squamata, Colubridae)		<i>Leptodactylus ocellatus</i> Girard (Anura, Leptodactylidae)		<i>Duellmanohyla uranochroa</i> (Cope) (Anura, Hylidae)	
Type localities	Petrolina, Pernambuco, Brazil		Ilha Seca, São Paulo, Brazil		Manguinhos, Rio de Janeiro, Brazil		Manguinhos, Rio de Janeiro, Brazil		Heredia Province, Costa Rica	
References	Current study		Freitas (1958)		Freitas (1958)		Freitas (1958)		Bursley & Goldberg (2007)	
Body length (mm)	Male 4.4–4.8 (4.6±0.2)	Female 9.7–21.9 (13.8±4.5)	Male 2.78–3.62	Female 5.63–16.21	Male 4.96–6.06	Female 9.71–23.28	Male 4.69–7.40	Female 6.87–32.53	Male 11.2–14.9 (13.05±1.35)	Female 18.56
Body width (µm)	150–175 (159±12)	187–291 (225±39)	170–200	130–330	270–330	250–600	230–430	230–740	230–319 (273±39)	459
Oesophagus length (µm)	396–575 (458±84)	620–884 (759±99)	360–440	430–630	600–830	700–1,410	560–860	660–2,190	1,122–1,530 (1,321±138)	1,670
Pharyngeal region length (µm)	34–38 (36±2)	38–52 (43±5)	35	43–52	43–61	52–140	52–78	61–113	102–128 (112±11)	128
Isthmus length (µm)	59–83 (75±13)	60–96 (80±13)	-	-	-	-	-	-	256–344 (298±38)	382
Oesophagus corpus (µm)	251–396 (337±76)	507–761 (625±98)	230–350	270–470	470–660	500–1,190	460–710	530–1,890	612–892 (765±120)	969
Oesophagus bulb length (µm)	50–62 (57±6)	56–103 (73±15)	-	-	-	-	-	-	128–179 (146±20)	191
Oesophagus bulb width (µm)	56–62 (57±4)	61–107 (80±16)	61–70	70–113	87–130	96–157	70–113	87–191	92–143 (113±22)	158
Nerve ring (µm)	197–211 (205±8)	242–375 (292±52)	180–240	270–280	260–350	300–450	270–460	310–700	319–357 (339±14)	395
Excretory pore (µm)	385–451 (420±27)	466–830 (613±114)	210–300	310–510	330–530	410–780	330–630	500–1,340	969–1,148 (1,076±66)	1,352
Lateral alae	Absent	Absent	Present	Present	Absent	Absent	Present	Present	Absent	Absent

.....continued on the next page

TABLE 2. (Continued)

	<i>O. caatingae</i> n. sp.	<i>O. caudacutus</i> (Freitas)	<i>O. oxyascaris</i> Travassos	<i>O. similis</i> (Travassos)	<i>O. mcclari</i> Burse & Goldberg
Spicule length (µm)	95–109 (103±7)	122–134	150–206	160–193	612–689 (642±38)
Relative size of spicule (%)	2.1–2.3	3.7–4.4	3.0–3.4	2.6–3.4	4.6–5.5
Gubernaculum length (µm)	30–50 (41±10)	Absent	Absent	Absent	79–98 (87±7)
Total number of pairs of papillae	15+1	13+1	8	13+1	11
Number and position of pairs of caudal papillae (pre:ad:post)	5:1:9	6:0:7	4:0:3	6:0:7	5:0:6
Unpaired papillae	Yes	Yes	No	Yes	No
Vulva from anterior end (mm)	4.1–9.6 (6.1±2.0)	2.01–4.19	4.56–9.78	3.52–14.07	14.08
Vulva position	Pre-equatorial	Pre-equatorial	Pre-equatorial	Pre-equatorial	Post-equatorial
Relative vulva position (%)	42.3–43.8	25.8–35.7	41.9–47.0	43.25–51.23	75.9
Egg length (µm)	68–103 (85±11)	67–80	84–105	89–109	49–55 (51±3)
Egg width (µm)	37–68 (54±8)	42–50	50–67	42–67	37–40 (38±2)
Tail (µm)	205–265 (238±25)	170–230	200–310	180–270	344–383 (362±15)
Tail filament	short	long	short	short	absent
Relative size of tail (%)	7.0–8.3	5.7–7.3	2.9–4.5	6.0–7.3	5.5

Range of infections: 1–16

Type material: Holotype male: CHIOC 38969a, allotype female: CHIOC 38969b, paratypes: CHIOC 38969c.

Etymology: The specific name refers to the first species of this genus described in a host in the Caatinga biome.

Remarks. The genus *Oxyascaris* Travassos currently is composed of four species (Table 2). The main characters used to differentiate species in this genus are the presence/absence of lateral body alae in males and females, number and distribution of caudal papillae, presence/absence of unpaired medial precloacal papillae, presence/absence of gubernaculum, and size of spicules in males; and positioning of the vulva regarding the anterior end of the body in females (Freitas 1958, Baker & Vaucher 1985, Bursey & Goldberg 2007).

Oxyascaris caatingae n. sp. and *O. mcdiarmidi* Bursey & Goldberg differ from *O. caudacutus* (Freitas), *O. oxyascaris* Travassos, and *O. similis* (Travassos) in having a gubernaculum, while in the other species this structure is absent (Freitas 1958, Bursey & Goldberg 2007) (Table 2). However, the vulva is pre-equatorial in *O. caatingae* n. sp., while in *O. mcdiarmidi* it is post-equatorial (Bursey & Goldberg 2007) (Table 2). The absolute size of the spicules and the number of caudal papillae are also differentiating characters between these two species. Males of *O. caatingae* n. sp. have smaller spicules with a mean of 103 μm of mean size, whereas in *O. mcdiarmidi* the spicules present a mean size of 642 μm (Table 2). Considering caudal papillae, *O. caatingae* n. sp. presents an unpaired precloacal papilla on the anterior border of cloaca. This papilla was not described in *O. mcdiarmidi* (Bursey & Goldberg 2007). These two species also differ in the number of postcloacal papillae pairs, with nine pairs in *O. caatingae* n. sp. and six pairs in *O. mcdiarmidi* (Bursey & Goldberg 2007) (Table 2).

Discussion

The family Cosmocercidae is composed of viviparous and oviparous nematodes, with laying of the first larval stage, the presence of an elongated rather than spherical oesophageal isthmus, and males without a precloacal sucker (Chabaud 1978). Among its 11 genera, mammiliform caudal papillae are present in males of *Aplectana*, *Oxysomatium*, *Oxyascaris*, *Raillietnema* and *Paraplesiohedruris*, without rosettes or plectanes (Chabaud 1978, Bursey *et al.* 2012).

Paraplesiohedruris is distinguished from all Cosmocercidae genera for presenting females with a tail with a sclerotized hook (Bursey *et al.* 2012). *Raillietnema* has females with small ovaries and uteri, containing few and large eggs (larger than 100 μm) (Chabaud 1978). *Aplectana*, *Oxysomatium* and *Oxyascaris* differs by the genital system morphology in females (Bursey *et al.* 2012). *Aplectana* is prodelphic, with both ovaries located anterior to the vulvar opening; *Oxysomatium* is amphidelphic, with the ovary of the anterior uterus located posterior to the vulva and the ovary of the posterior uterus positioned anterior to the vulva; and *Oxyascaris* can be opisthodelphic, with both ovaries located posterior to the vulva (Bursey *et al.* 2018), or amphidelphic, with the ovary of the anterior uterus located anterior to the vulva and the ovary of the posterior uterus located posterior to the vulva. Also, in *Oxyascaris* the ovaries do not cross, as occurs in *Oxysomatium* (Freitas 1958).

Oxysomatium petrolinensis n. sp. was included in this genus by having amphidelphic females, with crossed ovaries, because the ovary of the anterior uterus is located posterior to the vulva and the ovary of the posterior uterus is positioned anterior to the vulva. *Oxyascaris caatingae* n. sp. was included in this genus because it has amphidelphic females, without crossed ovaries, where the ovary of the anterior uterus is located anterior to the vulva and the ovary of the posterior uterus is located posterior to the vulva.

Baker (1980), in a review study of the genus *Oxysomatium*, allocated several species previously attributed to this genus in other genera of Cosmocercidae (e.g. *Aplectana*, *Cosmocercoides*, and *Raillietnema*), based on differences in their genital system compared to *Oxysomatium* females and/or due to the presence of rosettes or plectanes in males. This author also considered *Oxysomatium* as a senior synonym for *Neoxysomatium* Ballesteros-Márquez, and highlighted the difficulties in differentiating between species of the genera *Aplectana* and *Oxysomatium*, since some species have been insufficiently described in terms of morphology.

Some *Oxysomatium* species proposed after Baker (1980), are considered doubtful in the present study, since their descriptions are deficient in data and graphic representations, besides not presenting type material deposited in zoological collections, impairing the complement of their original descriptions.

Oxysomatium bareilliana Gupta, Chandra & Shalaby, was described in the bufonid toad *Duttaphrynus melanostictus* (Schneider) (= *Bufo melanostictus*) from India. The original description of this cosmocercid contains some errors, such as the statement that the specimens do not have a nerve ring. The drawings do not detail the relevant taxonomic characters for correct identification of the species, and the authors used only body size to differentiate the new species from another species of the genus *Aplectana* (Gupta *et al.* 2014). For these reasons, we consider this parasite as a *species inquirenda*.

Oxysomatium medhii Ilyas is a parasite of the microglossid frog *Hoplobatrachus tigerinus* (Daudin) (= *Rana tigerina*), from India. We consider the description insufficient because Ilyas (1980) does not describe the position of the ovaries and uterus of the analyzed females, which hamper the nematodes identification at a generic level, since the uterus and ovaries' position is a distinguishing feature between the genera *Aplectana*, *Oxysomatium* and *Oxyascaris*, in the family Cosmocercidae (Chabaud 1978, Baker 1980, Bursey *et al.* 2012). Other problems were observed, such as the proposal of a new species based on only one male specimen; the incomplete drawings that do not clearly show the species' diagnostic characters; and the absence of information regarding the zoological collection where the type material was deposited. Thus, we also consider this nematode as *species inquirenda*.

Oxysomatium teraensis Sonia, Gambhir, Tarnita, Zenith & Lakshmiyari is also considered a *species inquirenda*, since its description was published in the Proceedings of a local conference in India, hindering its access. We obtained the original description of the species from Sonia *et al.* (2011), and found inconsistencies such as the low quality of the species drawings, which lacks the species diagnostic characters; and the lack of the female genital system description, hampering the correct identification of specimens up to the generic level in Cosmocercidae.

Records of *Oxysomatium* species in the Neotropical region, including Brazil, were absent until the present study (Ávila & Silva 2010, Campião *et al.* 2014). Species of this genus parasitize snakes, lizards and frogs in Asia, Africa and Europe (Sharpilo 1974, 2003, Baker 1980, Shimalov 2009, Düşen *et al.* 2010a, b, Düşen & Oğduz 2010, Düşen 2011, Düşen & Öz 2013, Sümer *et al.* 2019). Thus, we provide the first record of an *Oxysomatium* species in a neotropical host.

The genus *Oxyascaris* Travassos is composed of few species, all occurring in Neotropical hosts (Travassos 1920, Freitas 1958, Baker & Vaucher 1985, Bursey & Goldberg 2007) (Table 2). Baker & Vaucher (1985) described *O. oxyascaris* and *O. caudacutus* collected from different anurans species in Paraguay, both presenting a gubernaculum in males. However, the original description of *O. oxyascaris*, made by Travassos (1920), and redescription, made by Freitas (1958), do not mention the presence of a gubernaculum in this species. Later, Fabio (1980) also did not mention the presence of gubernaculum in males of this nematode, collected in four Brazilian species of anurans. The same occurred with *O. caudacutus* (= *Pteroxyascaris caudacutus*), described by Freitas (1958), who did not report the presence of a gubernaculum in males. Therefore, we observe a paradox between the original description and redescription of *O. oxyascaris* (Travassos 1920, Freitas 1958, respectively), the original description *O. caudacutus* (Freitas 1958), and the specimens assigned to these two species in Baker & Vaucher (1985). It is important to highlight that Baker & Vaucher (1985) did not consult the type material of these two species, deposited at CHIOC, Rio de Janeiro, Brazil. We consider that the gubernaculum in males of *Oxyascaris* is not a difficult structure to observe in light microscopy, and certainly did not go unnoticed in the descriptions of Travassos (1920) and Freitas (1958). Thus, for comparison purposes with the new species of *Oxyascaris* described herein, we believe it is more consistent to consider the detailed redescription of *O. oxyascaris* and description of *O. caudacutus*, both made by Freitas (1958) (Table 2).

The genus *Oxyascaris* is distributed only in Neotropical hosts, with reports in Costa Rica, Central America (Bursey & Goldberg 2007, Goldberg & Bursey 2008) and Paraguay (Baker & Vaucher 1985), Argentina, and Brazil (Camião *et al.* 2014), in South America, presenting anurans as their main hosts. In Brazil, species were recorded in all regions and in anurans from the families Bufonidae, Hylidae and Leptodactylidae, including a report of *O. oxyascaris* in *L. macrosternum* of unspecified locality (Camião *et al.* 2014). In northeastern Brazil, an unidentified species of the genus *Oxyascaris* was recorded parasitizing the snake *Leptodeira annulata* (Linnaeus) (Squamata: Colubridae), in the State of Ceará (Carvalho *et al.* 2018). *Oxyascaris caudacutus* was recorded in the anuran *Pithecopus nordestinus* (Caramaschi) (Anura: Phyllomedusidae), in an Atlantic Forest remnant in the state of Pernambuco (Sena *et al.* 2018). *Oxyascaris oxyascaris* was recorded in *Pleurodema diplolister* (Peters) (Anura: Leiuperidae) in the state of Bahia (Camião *et al.* 2014) and *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad & Garda (Anura: Leptodactylidae) in the State of Ceará (Silva *et al.* 2018). However, none of these records provided morphological data to confirm the correct identification of *Oxyascaris* species.

We found that most studies with records of *Oxysomatium* and *Oxyascaris* species lack morphological and morphometric data that allow the correct identification of the studied specimens, with some lacking the deposit of representative material in scientific collections. In general, the records indicate the geographical occurrence, data regarding the host species and parasitic ecology. Additional important information is also omitted, such as the parasite site of infection and the methodology and bibliographic references used in the nematode identification. We hypothesize that the species richness of *Oxysomatium* and *Oxyascaris* in Brazil are thus likely to be greater than what is currently known, since the possible new species are possibly neglected by the lack of a proper morphological study.

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