

A new species of *Orientattractis* (Nematoda: Cosmoceroidea: Atractidae) parasite of Yellow-Spotted Amazon River Turtle, *Podocnemis unifilis* Troschel, 1848 (Testudines: Podocnemididae) in Brazilian Amazon

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ARTICLE INFO

Keywords:

Podocnemis unifilis
Atractidae
Orientattractis
Taxonomy
Light microscopy
Scanning electron microscopy

ABSTRACT

The current study describes a new species of nematode, *Orientattractis matosi* n. sp. (Atractidae), from the stomach and large intestine of *Podocnemis unifilis* Troschel, 1848 (Testudinidae), collected in the Tocantins and Xingu rivers in the Brazilian state of Pará. The new species was described using light and scanning electron microscopy and differs from its congeners mainly in the number and distribution of the male caudal papillae and by having a shorter right spicule. *Orientattractis matosi* n. sp. is the eleventh species of the genus *Orientattractis* Petter, 1966, the third reported from the Amazon, and the second described from *P. unifilis*.

1. Introduction

Orientattractis Petter, 1966 comprises nematodes parasitic in fishes, amphibians, and reptiles. Currently, the genus includes 10 valid species, namely: *Orientattractis levanhoai* Petter (1966) (type-species), *Orientattractis leiperi* Buckley (1969), *Orientattractis campechensis* González-Solís and Moravec (2004), *Orientattractis chiapasensis* González-Solís and Moravec (2004), *Orientattractis asymmetrica* Gibbons and Platt (2006), *Orientattractis hamabatrachos* Burse et al., 2014, *Orientattractis mekongensis* Moravec et al., 2015, *Orientattractis brycini* González-Solís and Mariaux (2017), *Orientattractis moraveci* Cavalcante et al. (2017) and *Orientattractis longicaudata* Liu et al., 2018; Jesus et al. (2020). Among these, four species were described from chelonians, *O. asymmetrica* and *O. leiperi* were found in freshwater turtles, and the latter species is the only one reported for *Podocnemis unifilis* Troschel, 1848 (Jesus et al., 2020). The species *O. levanhoai* and *O. longicaudata* were found in the tortoise *Indotestudo elongata* (Blyth, 1854) (Petter, 1966; Liu et al., 2018).

Podocnemis unifilis is a freshwater turtle of the family Podocnemididae Cope, 1868, widely distributed in the hydrographic systems of the Amazon and Orinoco River basins in Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guiana, Peru, Venezuela, and Suriname. This species inhabits various environments, including large rivers, backwaters, lakes,

streams, and flooded forests in black, white, and clear waters (Ferrara et al., 2017).

During a helminthological survey of the parasites of freshwater turtles from the Amazon region we collected a new species of *Orientattractis* from the stomach and large intestine of *Podocnemis unifilis* Troschel, 1848. Thus, in the present work we describe the new species using light and scanning electron microscopy.

2. Materials and methods

We collected three specimens of *P. unifilis* during the years of 2018 and 2019, from the Tocantins (n=2) and Xingu (n=1) Rivers, in the State of Pará, Brazil (SISBIO license: 53527-4). Hosts were anesthetized by injection of 2% ketamine and thereafter euthanized by ketamine overdose. Their internal organs were removed and examined under Leica EZ4 stereo microscopes (Leica Microsystems, Wetzlar, Germany).

Nematodes were washed in 0.9% saline solution (NaCl) and heat-killed in 70% ethanol. Some specimens were fixed in A.F.A. solution (2% glacial acetic acid, 3% formaldehyde, and 70% ethanol) for 24 h and transferred to microtubes containing 70% ethanol at room temperature. We also preserved some nematodes in 70% ethanol at room temperature. For morphological and morphometric analyses, selected specimens were cleared in 20% Aman's Lactophenol, mounted on

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temporary slides according to a protocol adapted from Amato et al. (1991), and analyzed using an Olympus BX41 microscope (Olympus, Tokyo, Japan) equipped with a drawing tube.

We selected twelve specimens (six males and six females) for scanning electron microscopy (SEM) analyses. The nematodes were post-fixed in 1% OsO₄, dehydrated in a graded ethanol series (30–100%), critical point dried with CO₂, placed on aluminum stubs using carbon tape and sputter coated with gold/palladium. We analyzed the specimens under a Vega3 microscope (TESCAN, Brno, Czech Republic) with an acceleration voltage of 10 kV in the Laboratory of Cellular Structural Biology (LBE) at the Federal University of Pará (UFPA).

Measurements in the text are given in micrometers unless otherwise indicated and are presented as holotype/allotype measurements followed by the range in parenthesis. The specimens were deposited in the Helminthological Collection of Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil.

3. Results

3.1. Taxonomic summary

***Orientatractis matosi* Jesus, Oliveira and Melo n. sp. (Figs. 1 and 2)**

Type host: *Podocnemis unifilis* Troschel, 1848 (Chelonia: Testudinae)

Type-locality: Tocantins River (3°45'44.3"S, 49°39'44.1"W), Tucuruí, Pará, Brazil.

Other localities: Xingu River, (2°41'3.901"S, 52°0'42.379"W), Senador José Porfírio, Pará, Brazil.

Site of infection: Stomach and large intestine.

Prevalence: 100% (3 infected/3 examined)

Type-specimens: Holotype (CHIOC 39301a), allotype (CHIOC 39301b) and 38 paratypes (CHIOC 39302a–39302b) deposited in the Helminthological Collection of Oswaldo Cruz Institute - Rio de Janeiro, Brazil.

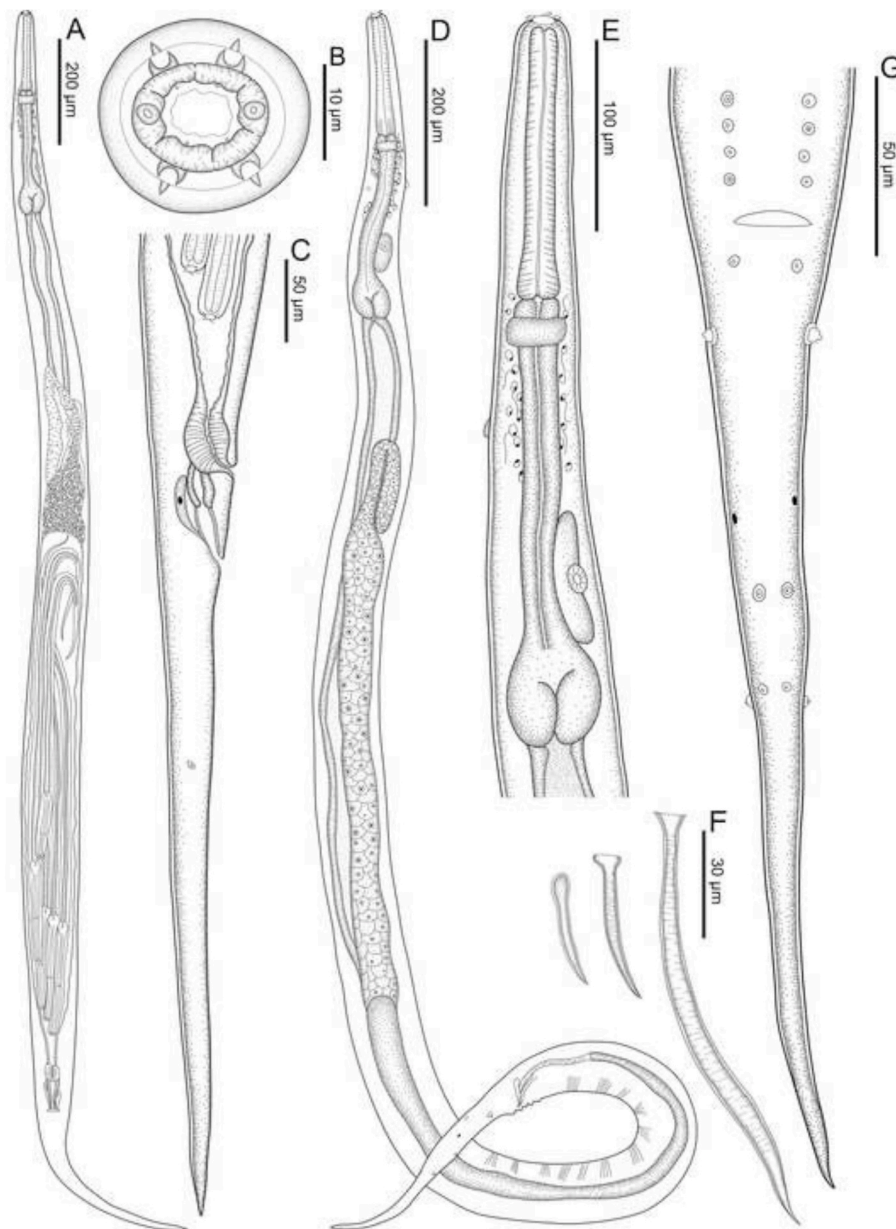


Fig. 1. Line drawings of *Orientatractis matosi* n. sp. (A) Female, whole body, ventral view. (B) Female, cephalic extremity, apical view (C) Posterior end of female, lateral view. (D) Male, whole body, lateral view. (E) Anterior extremity of body, ventrolateral view. (F) Details of spicules and gubernaculum. (G) Male, posterior end, ventral view.

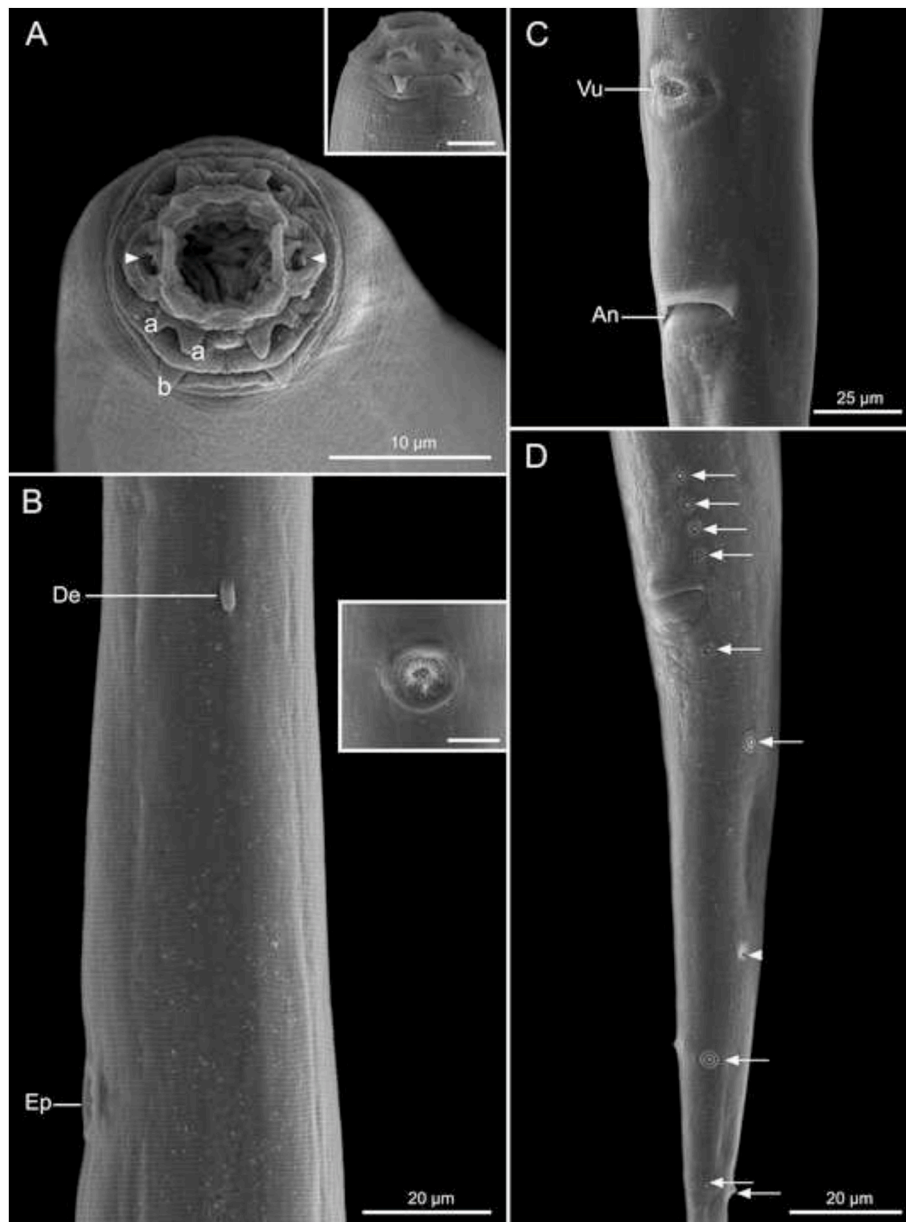


Fig. 2. Scanning electron micrographs of *Orientatractis matosi* n. sp. (A) Cephalic extremity, apical view (amphidial pores, arrowheads). (B) Anterior extremity of body, lateral view, lines indicate deirid and excretory pore. (C) Posterior extremity of female, ventrolateral view, lines indicate vulva and anus. (D) Posterior extremity of male, ventrolateral, distribution of caudal papillae (arrows) and phasmids (arrowheads). Abbreviations: anus, An; deirid, De; excretory pore, Ep; vulva, Vu; single large submedian spine, a; submedian pointed spines, b. Inset: Cephalic end details, ventrolateral view (Scale-bar: 5 µm), Detail of excretory pore (Scale-bar: 5 µm).

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 *Orientatractis matosi* n. sp. is urn:lsid:zoobank.org:act:623D54BF-3023-4864-9849-F884EC0B3E38

Etymology: The specific epithet is given in honor of Professor Edilson Rodrigues Matos from the Federal Rural University of Amazonia, Brazil, in recognition of his valuable contributions to our knowledge of the parasites of aquatic animals in the Amazon.

3.2. Description

General. Small and slender nematodes, tapering anteriorly. Lateral alae absent. Cuticle finely transversely striated. Males and females

similar in length (Fig. 1A and D). Oral aperture hexagonal, with six lips fused, surrounded by four submedian papillae in one circle. Four submedian lips (two subdorsal and two subventral) and two lateral lips supporting large amphids. Each submedian lip bearing a chitinoid piece formed by two well-sclerotized, recurved pointed spines connected at their base and a single large median spine, lateral lips with two small spines posterior to each amphidial pore (Fig. 1B and 2A). Esophagus divided into three parts; anterior esophagus (corpus), posterior esophagus (isthmus) and valved bulb. Nerve ring formed by numerous nerve fibers, some connected by nervous cells distributed from mid-length of esophagus to beginning of isthmus. Deirids small, lappet-like in shape, somewhat posterior to nerve ring level; excretory pore large, surrounded by cuticular striations, anterior to esophageal bulb (Fig. 1E and 2B). Tail long and sharply pointed (Fig. 1A, C, D and G).

Male [Based on holotype and 19 paratypes; all adult males] Total length

2.30 (2.30–2.58) mm, width at esophago-intestinal junction 65 (48–69). Entire esophagus 369 (353–391) long. Corpus 138 (131–149) long; isthmus and esophageal bulb 231 (213–242) long. Width of bulb 45 (38–52). Nerve ring, excretory pore, and deirids 138 (143–164), 286 (277–303), and 205 (195–227), respectively, from anterior end of body. Nine pairs of caudal papillae: four subventral precloacal pairs forming a group of papillae close to each other, and five postcloacal pairs (first subventral, second ventrolateral, third subventral, fourth subventral and fifth dorsolateral). Unpaired papilla on anterior cloacal lip absent. Phasmids lateral, located between second and third pairs of postcloacal papillae at 179 (177–209) from posterior end (Fig. 1G and 2D). Spicules unequal in size, lanceolate, with transverse striations, proximal ends slightly expanded, distal ends sharply pointed, larger (left) spicule 108 (101–126) long, smaller (right) spicule 37 (33–40) long; gubernaculum with proximal end slightly expanded, distal end pointed, 31 (28–33) long (Fig. 1F). Tail 261 (271–300) long.

Female [Based on allotype and 19 paratypes; all adult females with well developed larvae in uterus] Total length 2.54 (2.41–2.70) mm, width at esophago-intestinal junction 61 (51–70). Entire esophagus 382 (353–387) long. Corpus 143 (130–152) long; isthmus and esophageal bulb 239 (217–245) long. Width of bulb 48 (40–52). Nerve ring, excretory pore, and deirids 160 (147–166), 304 (274–317), and 210 (200–225), respectively, from anterior end of body. Vulva close to anus, 447 (381–515) from posterior end of body; distance anus-vulva 57 (53–67) (Fig. 1C and 2C). Vagina short, muscular, directed anteriorly from vulva (Fig. 1C). Uterus monodelphic and prodelphic. Viviparous, larvae present, often filling uterus (larvae total length 0.94–1.13 mm based on four larvae measured in uterus of allotype). Phasmids located at 271 (235–339) from posterior end. Tail 388 (331–462) long.

4. Discussion

The new species was assigned to the genus *Orientattractis* based on the presence of four well-sclerotized cuticular formations (two subdorsal and two subventral) at the cephalic end. These formations comprise two spines connected at the base and a single median spine below and between those spines. According to González-Solis and Mariaux (2017) and Liu et al. (2018), this is the main diagnostic character of the genus *Orientattractis*.

Orientattractis spp. can be divided into two groups based on the number of male caudal papillae (Liu et al., 2018). The first group comprises species whose males have 10 pairs of caudal papillae: *O. moravec* and *O. longicaudata*. The second group includes species in which males have 9 or 8 pairs of caudal papillae: *O. levanhoai*, *O. leiperi*, *O. campechensis*, *O. chiapasensis*, *O. asymmetrica*, *O. hamabatrachos*, *O. mekongensis*, and *O. brycini*. Males of *O. matosi* n. sp. have 9 pairs of caudal papillae and belong to the second species group.

We did not make detailed comparisons between the new species and *O. brycini*, *O. hamabatrachos*, *O. levanhoai*, and *O. mekongensis* because they have eight pairs of caudal papillae and are not neotropical species. We did, however, provide a table including morphometric and morphological data of all valid species of *Orientattractis* for comparison (See Table 1).

Within the group of species with 9 pairs of caudal papillae, the new species can be easily distinguished from *O. campechensis*, a parasite of cichlids, by the following characters: spicule length (right spicule 84–106, left spicule 430–506 *O. campechensis* vs. right spicule 33–40, left spicule 101–126 *O. matosi* n. sp.), gubernaculum length (62–74 *O. campechensis* vs. 28–33 *O. matosi* n. sp.); male tail length (608–722 *O. campechensis* vs. 271–300 *O. matosi* n. sp.), and the distribution of caudal papillae (5 adcloacal pairs, 4 postcloacal pairs and 1 unpaired papilla on upper lip of cloaca *O. campechensis* vs. 4 precloacal pairs and 5 postcloacal pairs *O. matosi* n. sp.) (González-Solis and Moravec, 2004).

Orientattractis chiapasensis described from cichlids differs from the new species in the male body length (2.70–2.97 mm *O. chiapasensis* vs. 2.30–2.59 mm *O. matosi* n. sp.), spicule length (right spicule 60–68, left

Table 1 Selected measurements (in µm) and morphological characters of valid species of *Orientattractis*.

Species	Type-Host	Body length (mm)		Spicule length		Gubernaculum length	Caudal papillae ^a	Vulva from posterior end	Distance anus-vulva	Tail length		References
		male	female	right	left					male	female	
<i>O. matosi</i> n. sp.	<i>Podocnemis unifilis</i>	2.30–2.58	2.41–2.70	33–40	101–126	28–33	4: 0: 5	381–515	53–67	271–300	331–462	This study
<i>O. asymmetrica</i>	<i>Rhinoclemmys pulcherrima</i>	4.05–4.29	3.74–5.5	86–104	220–239	56–66	4: 2: 3 + 1	–	100–112	836–952	820–1,080	Gibbons and Platt (2006)
<i>O. brycini</i>	<i>Brycinus macroleptodorus</i>	2.58–3.07	2.50–3.61	75–90	130–158	29–39	1: 3: 4	2.19–3.18	39–72	207–257	270–388	González-Solis and Mariaux (2017)
<i>O. campechensis</i>	<i>Vieja bifasciata</i>	3.02–3.52	3.31–4.33	84–106	430–506	62–74	0: 5: 4 + 1	946–1.32	76–106	608–722	866–1.246	González-Solis and Moravec (2004)
<i>O. chiapasensis</i>	<i>Vieja bifasciata</i>	2.70–2.96	2.02–3.80	60–68	204–238	44–49	0: 5: 4 + 1	616–851	92–129	460–505	380–722	González-Solis and Moravec (2004)
<i>O. hamabatrachos</i>	<i>Austrochaperina basipalmata</i>	3.20–4.35	3.39–4.86	79–98	146–165	55–67	2: 1: 5: + 1	612–740	102–153	306–408	510–612	Bursev (2004)
<i>O. leiperi</i>	<i>Podocnemis vogli</i>	3.0–3.9	2.7–4.3	160	470	76	3: 1: 5: + 1	960	–	670	880	Buckley (1969)
<i>O. levanhoai</i>	<i>Indostedo elongata</i>	3.3	3.4	90	170	40	3: 0: 5	–	50	800	1,000	Petter (1966)
<i>O. longicaudata</i>	<i>Indostedo elongata</i>	4.64–5.24	4.60–5.36	108–157	325–383	92–125	1: 4: 5 + 1	1.98–2.62	71–98	1,98–2.26	1.88–2.52	Liu et al. (2018)
<i>O. mekongensis</i>	<i>Pangasius bocourti</i>	5.35–6.66	7.75–8.95	90–105	306–384	33–51	2: 1: 5	6.71–7.75	108–150	435–680	952–1,074	Moravec et al. (2015)
<i>O. moravec</i>	<i>Pimelodus blochii</i>	2.09–3.13	1.98–2.71	69–100	161–198	38–58	3: 2: 5	–	57–76	225–270	237–294	Cavalcante et al. (2017)

^a Caudal papillae distribution represented as = precloacal: adcloacal: postcloacal + median unpaired.

spicule 204–238 *O. chiapasensis* vs. right spicule 33–40, left spicule 101–126 *O. matosi* n. sp.), in the position of the vulva from the tail tip and the vulva-anus distance (vulva: 616–851 *O. chiapasensis* vs. 381–515 *O. matosi* n. sp.; vulva-anus distance: 92–129 *O. chiapasensis* vs. 53–67 *O. matosi* n. sp.) as well as in the length of tail in males (460–505 *O. chiapasensis* vs. 271–300 *O. matosi* n. sp.) (González-Solís and Moravec, 2004).

Orientattractis asymmetrica was described from *Rhinoclemmys pulcherrima* (Gray, 1855) (Geoemydidae) in Costa Rica and is easily distinguished from the new species by having a larger body in males (4.05–4.29 mm *O. asymmetrica* vs. 2.30–2.58 mm *O. matosi* n. sp.), larger spicules (right spicule 86–104, left spicule 220–239 *O. asymmetrica* vs. right spicule 33–40, left spicule 101–126 *O. matosi* n. sp.) and longer gubernaculum (56–66 *O. asymmetrica* vs. 28–33 *O. matosi* n. sp.). Furthermore, *O. asymmetrica* has a greater vulva-anus distance (100–112 in *O. asymmetrica* vs. 53–67 in *O. matosi* n. sp.) and a longer tail in males (836–952 *O. asymmetrica* vs. 271–300 *O. matosi* n. sp.). The species also differ in the distribution of caudal papillae (4 precloacal pairs, 1 adcloacal pair, 3 postcloacal pairs, and 1 unpaired papilla on the upper lip of cloaca in *O. asymmetrica* vs. 4 precloacal pairs and 5 postcloacal pairs in *O. matosi* n. sp.) (Gibbons and Platt, 2006).

Finally, the new species can be distinguished from *O. leiperi* parasite of *Podocnemis vogli* Müller, 1935; Buckley (1969) and also reported in *P. unifilis* (Podocnemididae) (Jesus et al., 2020) by the spicule length (right spicule 160, left spicule 470 *O. leiperi* vs. right spicule 33–40, left spicule 101–126 *O. matosi* n. sp.), gubernaculum length (76 *O. leiperi* vs. 28–33 *O. matosi* n. sp.), the position of the excretory pore from the anterior extremity and the vulva from the posterior extremity (pore: 460 *O. leiperi* vs. 277–303 *O. matosi* n. sp.; vulva: 960 *O. leiperi* vs. 381–515 *O. matosi* n. sp.), male tail length (670 *O. leiperi* vs. 271–300 *O. matosi* n. sp.). Additionally, they differ in the distribution of caudal papillae (3 precloacal pairs, 1 adcloacal pair, 5 postcloacal pairs and 1 unpaired papilla on the upper lip of cloaca *O. leiperi* vs. 4 precloacal pairs and 5 postcloacal pairs *O. matosi* n. sp.).

Thus, *Orientattractis matosi* n. sp. differs from all congeners mainly in the distribution of caudal papillae (4 precloacal pairs and 5 postcloacal pairs) and by having a shorter right spicule. This is the second known species of the genus *Orientattractis* in *P. unifilis* and the third reported from the Amazon region.

Ethics approval

We followed all applicable institutional, national, and international guidelines for animal care and use. The study is registered with the Animal Ethics Committee from the UFPA under code 8341260821. The present study was approved by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Brazil, and host specimens were collected under license number SISBIO: 53527-4.

Funding

This study was supported by Coordination for the Improvement of High Higher Education Personnel (CAPES), Postgraduate Program in the Biology of Infectious and Parasitic Agents (PPGBAIP)/UFPA, PROPESP/UFPA (PAPQ); Amazon Foundation for Research and Studies Support (FAPESPA), Programa de Apoio a Nucleos Emergentes (FAPESPA/CNPq PRONEM 01/2021, process number 51/2021); Research productivity

scholarship of CNPq to F.T.V. Melo (Process number 314116/2021-4). This study is part of the Ph.D. thesis of Ronald Ferreira de Jesus in PPGBAIP (Institute of Biological Sciences-UFPA).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Ana Nunes Santos for her support in host necropsies and collection of nematodes; to Yuri Willkens for his technical support with SEM analyses and Dra. Edilene Oliveira da Silva, from Laboratory of Cellular Structural Biology at the Federal University of Pará, Belém, for her technical support with the SEM analyses.

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