International Journal for Parasitology: Parasites and Wildlife 22 (2023) 6–13

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

New species of *Serpinema* (Nematoda: Camallanidae) from the scorpion mud turtle *Kinosternon scorpioides* (Testudines: Kinosternidae) from eastern Amazon, Brazil

Leandro Mauricio Oliveira Silva, Ronald Ferreira Jesus, Ana Nunes Santos, Bianca Nandyara, Yuri Willkens, Jeannie Nascimento Santos, Francisco Tiago Vasconcelos Melo *

Laboratory of Cellular Biology and Helminthology "Profa. Dra. Reinalda Marisa Lanfredi", Institute of Biological Sciences, Federal University of Pará (UFPA), Av. Augusto Correa 01, Guamá, Belém, Pará, 66075-110, Brazil

ARTICLE INFO

Keywords:
Camallanidae
Serpinema
Kinosternon scorpioides
Nematodes
Taxonomy
Neotropical region

ABSTRACT

The genus *Serpinema* Yeh, 1960 allocates parasitic nematodes of freshwater turtles distributed across the Oriental, Neotropical, and Palearctic regions. Five of 10 valid species of the genus *Serpinema* occur in the Neotropical region, and three have been recorded parasitizing *Kinosternon scorpioides* Linnaeus, 1766. In the present work, we describe a new species of *Serpinema*, a gastrointestinal parasite of *K. scorpioides* from the Brazilian Amazon, using light and scanning electron microscopy. *Serpinema pelliculatus* n. sp. differs from other species of the genus by the number and distribution of caudal papillae and spicule morphology. The new species is the seventh recorded for the Neotropical region.

1. Introduction

Nematodes of the family Camallanidae Railliet and Henry, 1915 are parasites of the gastrointestinal tract of amphibians, fish, and reptiles (Moravec, 1998). This family allocates 12 valid genera, commonly identified by the morphology of their buccal capsule and distributed in 2 subfamilies, namely: *Denticamallanus* Moravec and Thatcher, 1997, *Malayocamallanus* Jothy and Fernando, 1970, *Procamallanus* (Baylis, 1922) *Punctocamallanus* Moravec and Scholz, 1991, *Spirocamallanus* (Olsen, 1952) *Spirocamallanoides* Moravec and Sey, 1988 (all Procamallaninae Yeh, 1960) *Camallanides* Baylis and Daubney, 1922, *Camallanus* Railliet and Henry, 1915, *Neocamallanus* Ali, 1957, *Oncophora* Diesing, 1851, *Paracamallanus* Yorke and Maplestone, 1926 and *Serpinema* Yeh, 1960 (all Camallaninae Railliet and Henry, 1915) (Rigby and Rigby, 2013; Pinheiro et al., 2018; Harnoster et al., 2019).

The genus *Serpinema* includes gastrointestinal parasites of freshwater turtles, distributed across the Oriental, Neotropical, and Palearctic regions (Ivashkin et al., 1977; Harnoster et al., 2019). These nematodes have a sclerotized buccal capsule composed by 2 lateral valves with internal longitudinal ridges divided into 2 groups, 1 dorsal and 1 ventral, with a gap between them; they also have tridents and a basal ring (Yeh, 1960).

During a helminthological survey of parasites from freshwater turtles, we collected nematodes from the stomach and intestine of the scorpion mud turtle, *Kinosternon scorpioides* Linnaeus, 1766 from the Brazilian Amazon region. We found nematodes with morphological traits of *Serpinema* and compared them to the known species of the genus. Our analysis revealed that the nematodes have distinct morphological characters representing a new species described in the present work using light and scanning electron microscopy.

2. Material and methods

We analyzed the gastrointestinal tract of 24 specimens of *K. scorpioides* collected in Baía do Capim (municipality of Abaetetuba) and Ilha do Marajó (municipality of Soure), Pará, Brazil (license permission SISBIO: 53527–4). Hosts were anesthetized by injection of 2% ketamine and posteriorly euthanized by ketamine overdosage. We removed and examined all the internal organs under a LEICA EZ4 stereo microscope. Nematodes found in the digestive tracts were rinsed in saline solution (NaCl 0.9%), killed in heated 70% ethanol, and preserved in microtubes containing room-temperature 70% ethanol. For morphological and morphometric analyses, the specimens were cleared in Amann's lactophenol, mounted in temporary slides, and examined

* Corresponding author.
E-mail address: ftiago@ufpa.br (F.T.V. Melo).

https://doi.org/10.1016/j.ijppaw.2023.07.006
Received 26 June 2023; Received in revised form 19 July 2023; Accepted 19 July 2023
Available online 20 July 2023

under an Olympus BX41 microscope (Olympus, Tokyo, Japan) equipped with a drawing tube.

For detailed morphological analyses, we cut the tail of 5 males and positioned ventrally to analyze the caudal papillae distribution pattern. Additionally, we dissected the spicule of 5 specimens to confirm the morphology and number of spicules .

For additional morphological comparisons, we examined *Serpinema magathi* Sprehn, 1932 (codes: CHIOC 28262 a-c, 28263 a-b, 28264 Vouchers) and *Serpinema monospiculatus* Freitasn and Dobbin Jr., 1971 (codes: CHIOC 28265 c-f Paratypes), deposited in the Helminthological Collection of Oswaldo Cruz Institute (CHIOC) - Rio de Janeiro, Brazil.

For Scanning Electron Microscopy (SEM), 6 specimens (3 males and 3 females) were post-fixed in 1% OsO_4 (osmium tetroxide), dehydrated in an increasing ethanol series, dried to the CO_2 critical point, mounted in metallic supports, covered with gold-palladium and analyzed under a Vega3 microscope (TESCAN, Brno, Czech Republic) with an acceleration voltage varying from 10 to 20 kV, at the Laboratory of Structural Biology (LBE) at the Federal University of Pará (UFPa).

All measurements are given in micrometers unless otherwise stated. Holotype/allotype measurements are given followed by minimum and maximum measurements of the paratypes indicated in parentheses. Prevalence and mean intensity were calculated according to Bush et al. (1997). The specimens were deposited in the Other Invertebrate Collection of Museu Paraense Emílio Goeldi (MPEG), Pará, Brazil.

3. Results

3.1. Taxonomy summary

Serpinema pelliculatus Silva, Jesus and Melo n. sp. (Figs. 1–3)

Type host: *Kinosternon scorpioides* Linnaeus, 1766

Type locality: Soure – Ilha de Marajó (00°43′00"S 48°31′24"O), Pará, Brazil.

Additional locality: Abaetetuba (01°43′05"S 48°52′57"O), Pará, Brazil.

Site of *infection:* Stomach and intestine.

Prevalence and mean intensity: 87,5% (21 infected hosts out of 24 analyzed) and 4,71 (range of infection 1–12; 99 nematodes were found in all hosts, most of the specimens were females).

Specimens deposited: Holotype (MPEG.PLA 000399), allotype (MPEG.PLA 000400), 10 male paratypes (MPEG.PLA 000401) and 10 female paratypes (MPEG.PLA 000402) deposited in the Other Invertebrate Collection of Museu Paraense Emílio Goeldi, Pará, Brazil.

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 *Serpinema pelliculatus* n. sp. is urn: lsid: zoobank.org:act:66B8B1FC-0EE2-419C-9999-97F97E6A5E65.

Etymology: The specific epithet refers to the morphology of the

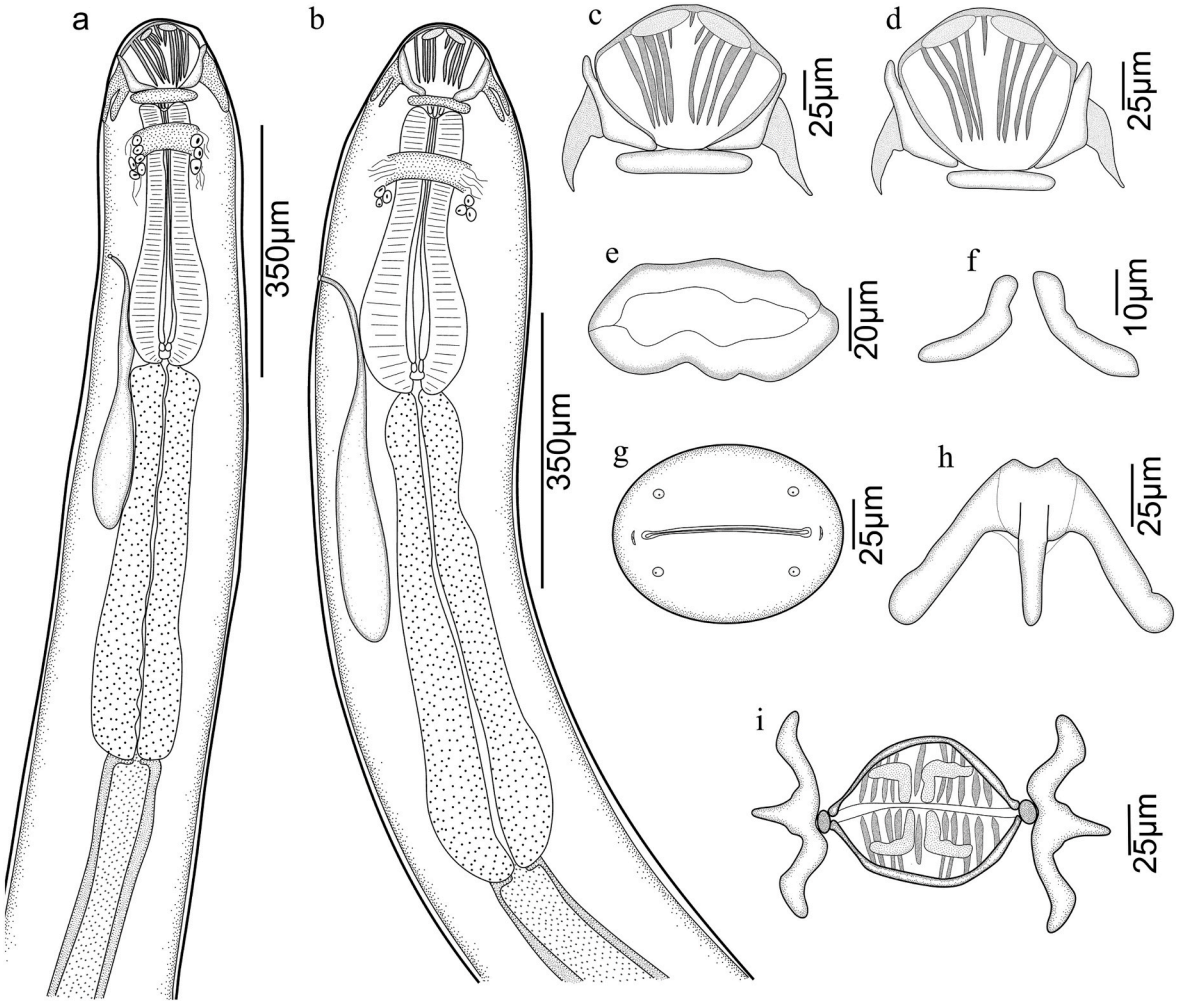

Fig. 1. Line drawings of *Serpinema pelliculatus* n. sp. **(a)** Anterior region of female, lateral view. **(b)** Anterior region of male, lateral view. **(c)** Buccal capsule of female, lateral view. **(d)** Buccal capsule of male, lateral view. **(e)** Basal ring, apical view. **(f)** Anterior curved sclerotized structures, lateral view. **(g)** Cephalic extremity of male, apical view **(h)** Trident of male, ventral view. **(i)** Buccal capsule of male, apical view.

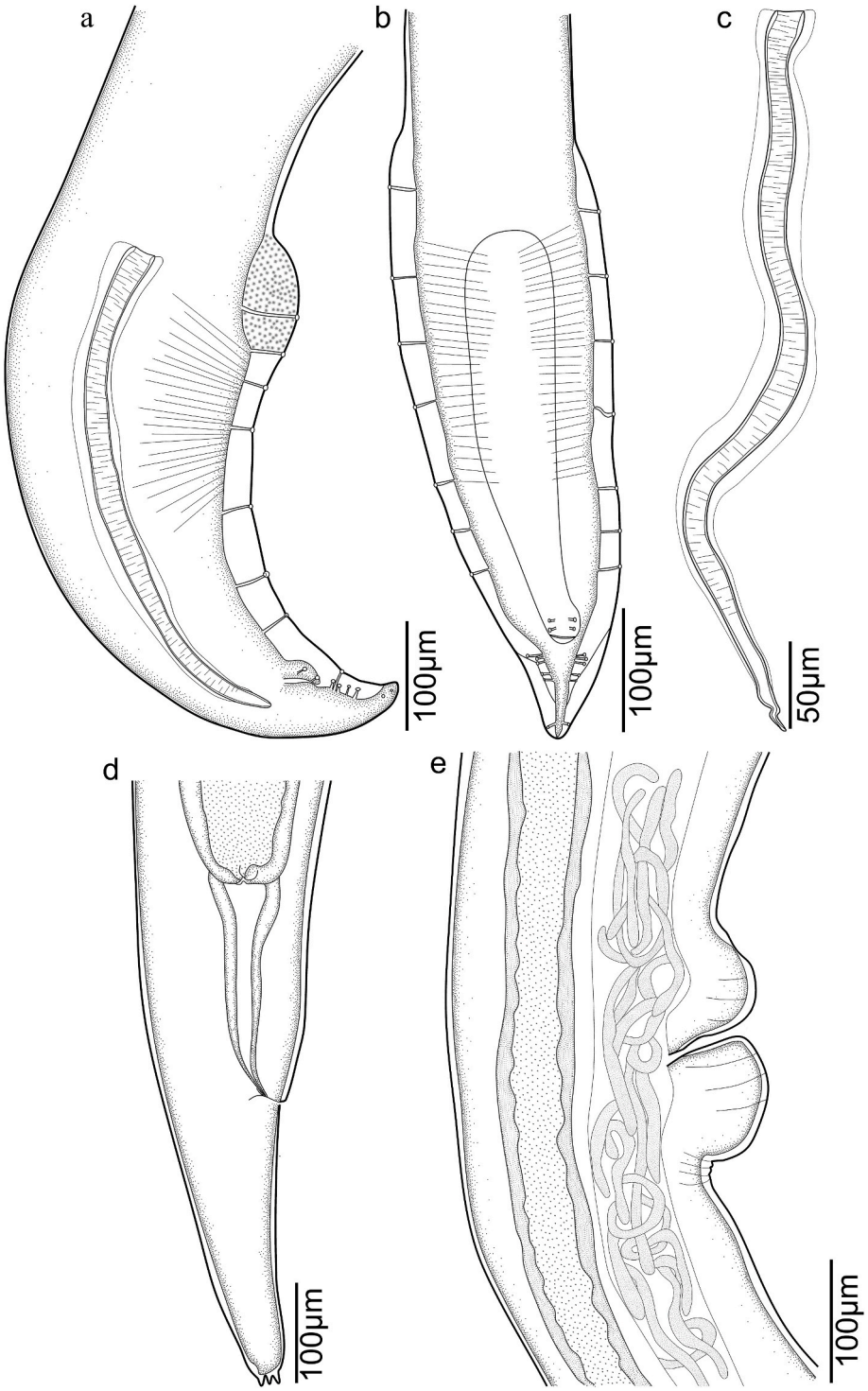

Fig. 2. Line drawings of *Serpinema pelliculatus* n. sp. **(a)** Posterior region of male, lateral view. **(b)** Posterior region of male, ventral view. **(c)** Spicule, ventral view. **(d)** Posterior region of female, lateral view. **(e)** Vulva, lateral view.

spicule that is covered by a sheath. Pelliculatus = covered over with skin or skins or pellicles.

3.2. Description

General: Medium to large-sized nematodes. Translucent red in life, with thin transverse cuticular striations. Body with truncated anterior end (Fig. 1A and B). Mouth opening in transverse slit; 2 pairs of cephalic papillae (1 latero-ventral pair and 1 latero-dorsal pair); a pair of lateral amphids (Fig. 1G). Buccal capsule composed of 2 concave lateral valves; 2 pairs of curved sclerotized structures on the anterior part the valves (Fig. 1F, I; 3A). Valves internally marked by longitudinal complete ridges (extending from anterior to posterior margin of buccal capsule) and incomplete ridges (shortly distant from anterior margin of buccal capsule) (Fig. 1C and D). Tridents, 1 ventral and 1 dorsal, composed of a slightly rounded basal portion and 3 divergent branches; median branch shorter; lateral branches similar in size (Fig. 1H). Chitinous basal ring between buccal capsule and esophagus (Fig. 1E). Esophagus divided into

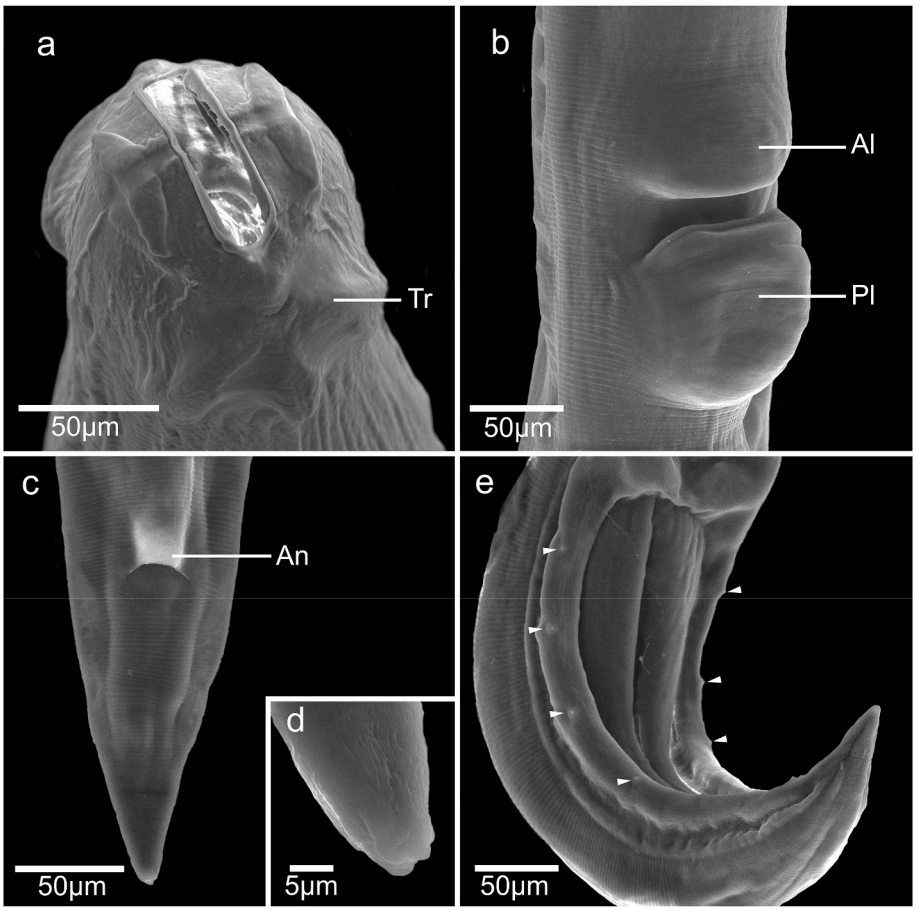

Fig. 3. Scanning electron microscopy of *Serpinema pelliculatus* n. sp. **(a)** Anterior end of male, subventral view; **(b)** Vulva, subventral view. **(c)** Posterior region of female, ventral view. **(d)** Tip tail of female, ventrolateral view. **(e)** Posterior region of male, subventral view (arrowheads indicate papillae). Abbreviations: anterior lip, Al; anus, An; posterior lip, Pl; trident, Tr.

a short muscular anterior portion and a glandular posterior portion (Fig. 1A and B). Nerve ring at anterior part of the muscular esophagus. Excretory pore at posterior half of muscular esophagus (Fig. 1A and B). Deirids not observed.

Males [based on holotype and 10 paratypes; measurements of holotype followed by range of paratypes in parenthesis]: Total length 6.45 (6.07–8.20) mm. Width at mid-body 227 (173–240). Buccal capsule 84 (60–91) × 100 (95–109). Buccal valves with 8 (8–8) complete ridges, 1 (1–3) incomplete ridges. Trident middle branch 67 (48–69) long; trident lateral branches 73 (60–85) long. Basal ring 12 (8–13) × 74 (60–79). Esophagus total length 976 (819–976). Muscular esophagus 363 (317–363) × 136 (99–139). Glandular esophagus 613 (499–613) × 152 (115–155). Nerve ring 195 (141–203) and excretory pore 352 (304–357) from the anterior end. Caudal alae well-developed, starting at the level of a vesicular-like globular protrusion, ending near tail tip (Fig. 2A). Fifteen pairs of pedunculate caudal papillae. Seven pairs pre cloacal: all lateral pairs. Two pairs ad cloacal: ventral and anterior to cloaca. Six pairs post cloacal: first, second and third pairs clustered close to cloaca; fourth and fifth pairs grouped, situated near mid-tail length; sixth pair close to tail end (Fig. 2A and B; 3E). One robust, sharp-edged spicule 477 (416–566) long, covered by a sheath (Fig. 2C). Gubernaculum absent. Tail ventrally curved 131 (83–131) long; strong transverse musculature (Figs. 2A and 3E).

Females [based on allotype and 10 paratypes; measurements of allotype followed by range of paratypes in parenthesis]: Total length 8.31 (8.05–9.91) mm. Width at mid-body 227 (227–333). Buccal capsule 96 (84–100) × 119 (97–119). Buccal valves with 8 (8–8) complete ridges, 3 (1–3) incomplete ridges. Trident middle branch 77 (48–80) long; trident lateral branches 92 (81–104) long. Basal ring 13 (10–17) × 86 (74–88). Esophagus total length 0.94 (0.94–1.12) mm. Muscular esophagus 368 (368–416) × 115 (115–141). Glandular esophagus 571 (560–699) × 125 (125–203). Nerve ring 197 (171–221) and excretory pore 339 (339–437) from the anterior end. Vulva close to mid-body; slightly post equatorial; prominent lips; more developed posterior lip; situated 3.71 (3.71–4.64) mm from the posterior end (Fig. 2E; 3B), at 55.37% (51.02%–56.06%) of the total length. Tail, 140 (140–197) long (Fig. 2D; 3C), ending with 3 small projections, 1 dorsal and 2 ventrolateral (Fig. 3D). Vulva-anus distance 3.55 (3.55–4.44) mm. Viviparous. Uterus with several larvae (Fig. 2E).

3.3. Examination of Serpinema magathi and Serpinema monospiculatus

We evaluated specimens of *S. magathi* and *S. monospiculatus* deposited at CHIOC. The morphology of these specimens agrees with Freitas and Dobbin Jr. (1971) descriptions. *Serpinema magathi* has 9–11 ridges on the buccal capsule, 14 pairs of caudal papillae, and a single spicule ending in a bifid tip; while *S. monospiculatus* has 10–12 ridges on the buccal capsule, 10 pairs of caudal papillae and a single spicule with sharpened end. We did not observe a sheath covering the spicule in both species.

4. Discussion

The new species was assigned to the genus *Serpinema* based on the following morphological characteristics: buccal capsule consisting of 2 lateral valves with internal longitudinal ridges divided into 2 groups,

with a gap between them; tridents and sclerotized basal ring, in addition it was found parasitizing the gastrointestinal tract of a freshwater turtle. According to Yeh (1960) these are the diagnostic characters of *Serpinema*. However, several authors have discussed the validity of the genus, mainly due to its morphological similarity with *Camallanus* (Stromberg and Crites, 1974; Baker, 1983; Rigby and Rigby, 2013).

Despite the complex taxonomic history, we considered *Serpinema* as a valid taxon. Harnoster et al. (2019) provides molecular data that support the validation of the generic status of *Serpinema*. Additionally, Ailán-Choke and Pereira (2021) demonstrated that, although *Serpinema* and *Camallanus* are phylogenetically close, they are different taxa whose morphological characteristics are very similar due to the divergence of a common ancestor that occurred more recently. Thus, we describe and propose a new species of *Serpinema* based on differences found in spicule morphology and the number and distribution of male caudal papillae.

We considered the genus *Serpinema* accommodate 10 valid species. Harnoster et al. (2019) listed 9 valid species of the genus: *Serpinema amazonicus* Ribeiro, 1941, *Serpinema cayannensis* Harnoster, Svitin and Du Preez, 2019, *Serpinema intermedius* Hsü and Hoeppli (1931), *Serpinema kachugae* Baylis and Daubney, 1922, *Serpinema lissemysus* Gupta and Singh, 1959, *S. magathi, Serpinema microcephalus* Dujardin, 1845, *Serpinema trispinosus* Leidy, 1852, and *Serpinema octorugatum* (Baylis, 1933). We also considered *S. monospiculatus* a valid species (see below).

In the present work, we reexamined specimens of *S. magathi* and *S. monospiculatus* collected and analyzed by Freitas and Dobbin Jr. (1971). These nematodes were found in *K. scorpioides* and *Mesoclemmys tuberculata* (Luederwaldt, 1926) in Brazil and are species of *Serpinema* frequently found parasitizing chelonians in the country (Mascarenhas and Müller, 2021). Additionally, the material collected and analyzed by Freitas and Dobbin Jr. (1971) is deposited in the Helminthological Collection of Oswaldo Cruz Institute (Brazil), and both species are known to have a single spicule. For those reasons, we decided to reexamine the deposited material, and based on our analysis and Freitas and Dobbin Jr. (1971) descriptions, we reinforce the validity of these 2 species.

We did not consider the other 3 species of the genus *Serpinema*: *Serpinema magnorugosus* (Caballero, 1939), *Serpinema ptychozondis* (MacCallum, 1918), and *Serpinema undulatus* (Railliet and Henry, 1915) listed by Yeh (1960). First, *S. magnorugosus* represents a synonym of *S. trispinosus* (Moravec and Vargas-Vazquez, 1998). Additionally, the descriptions of *S. ptychozondis* and *S. undulatus* are incomplete and considered invalid by Sharma et al. (2002).

We can separate *Serpinema* into 2 species groups based on the number of spicules, a group with a single spicule: *S. lissemysus*, *S. magathi* and *S. monospiculatus*. The second group includes species having 2 unequal spicules: *S. amazonicus*, *S. cayannensis*, *S. intermedius*, *S. kachugae*, *S. microcephalus*, *S. trispinosus*, and *S. octorugatum*. Therefore, *Serpinema pelliculatus* n. sp. belongs to the first species group, with a single spicule. Also, the new species differs from its congeners in the number of ridges on the valves of the buccal capsule, the number and distribution of male caudal papillae, and the length and morphology of the spicule. We also highlight that the new taxon is the only species of the genus to present a spicule covered by a sheath.

Serpinema magathi is a parasite of *K. scorpioides* (Kinosternidae) and differs from *S. pelliculatus* n. sp. in having a thin spicule with a bifid tip distal end with unequal branches *vs*. a robust spicule with a simple tip (without branches), different number and distribution of pairs of papillae (7 pre cloacal and 7 post cloacal *vs*. 7 pre cloacal, 2 ad cloacal, and 6 post cloacal), larger females (12.58 mm *vs*. 8.05–9.91 mm), and position of vulva (is located 7.13 mm from the posterior *vs*. 3.76–4.64 mm).

Serpinema monospiculatus parasitizing *M. tuberculata* (Chelidae) differs from the new species by having a larger and wider buccal capsule (108–112 × 137–144 *vs*. 60–91 × 95–109), longer esophagus length (1.16–1.24 mm *vs*. 0.82–0.98 mm), longer basal ring (25–29 *vs*. 8–13), and by the number of caudal papillae (6 pre cloacal, 1 ad cloacal and 3 post cloacal *vs*. 7 pre cloacal, 2 ad cloacal and 6 post cloacal). Furthermore, *S. monospiculatus* has non-prominent vulvar lips *vs*. 2 prominent vulvar lips. Additionally, they differ by the distance from vulva to the posterior end (6.21–8.87 mm *vs*. 3.76–4.64 mm) and distance from vulva to anus (6.29 mm *vs*. 3.55–4.44 mm) (Freitas and Dobbin Jr., 1962).

The other species with a single spicule is *S. lissemysus*, a parasite of *Lissemys punctata* (Bonnaterre, 1789) (Tryonichidae). *Serpinema lissemysus* is smaller than the new species (4.75–5.00 mm *vs*. 6.07–8.20 mm) and has 14–16 ridges on buccal capsule *vs*. 9–11 ridges. In *S. lissemysus* the anterior vulvar lip is larger than the posterior and each lip is formed by a cuticular protuberance with granular content, internally divided into several lobes, whereas in *S. pelliculatus* n. sp. have posterior vulvar lip larger than the anterior, and the internal lobes are absent. Furthermore, the new species has 15 pairs of caudal papillae (7 pre cloacal, 2 ad cloacal and 6 post cloacal), while *S. lissemysus* (Gupta and Singh, 1959) has 18 pairs of caudal papillae (6 pre cloacal, 2 ad cloacal and 10 post cloacal).

Among the species with 2 spicules, *Serpinema pelliculatus* n. sp. resembles *S. kachugae* in the number of pairs of caudal papillae (15 pairs in both species); however, they differ in the arrangement of the post cloacal papillae. The first species has pairs 1, 2 and 3 grouped close to the cloaca; pairs 4 and 5 clustered near the middle of the tail; and the isolated par 6 nearby to the posterior end; while *S. kachugae* have the pair 1 being isolated right after the cloaca; pairs 2, 3 and 4 grouped lateral; pair 5 isolated; and pair 6 isolated near the end of the tail. Furthermore, the new taxon is smaller than *S. kachugae* in both sexes, showing similarities only in the length of the glandular esophagus (560–699 *vs*. 540–990) in males and distance from nerve ring to anterior end (171–221 *vs*. 200–230) in females. Those species also differ in vulva morphology; the females of both species have a vulva with prominent lips, but in *S. kachugae,* the vulva has a larger anterior lip, overlapping the posterior lip. In contrast, new species have the posterior lip of vulva more developed than the anterior ones, and vulvar lips do not overlap. The additional morphometric and morphological data of all valid species of *Serpinema* and the new taxon are presented in Table 1.

Until now, 6 species of *Serpinema* have been recorded in Neotropical region: *S. amazonicus* (Ribeiro, 1941), *S. cayannensis* (Harnoster et al., 2019), *S. kachugae* (Hungría, 1978), *S. magathi* (Freitas and Dobbin Jr., 1971), *S. monospiculatus* (Freitas and Dobbin Jr., 1962) and *S. trispinosus* (Moravec and Vargas-Vazquez, 1998). For the host *K. scorpioides,* we have the following species registred: *S. kachugae* (Hungría, 1978), *S. magathi* (Alho, 1965; Freitas and Dobbin Jr., 1971; Viana et al., 2016), and *S. monospiculatus* (Pereira et al., 2018). We have the report of *S. amazonicus*, *S. magathi* and *S. monospiculatus* in Brazil. Furthermore, 2 species were registered in the Amazon Biome: *S. amazonicus*, described parasitizing *Podocnemis expansa* (Schweigger, 1812) from Belém, Pará; and *S. magathi* found parasitizing *K. scorpioides* from Anajás, Pará (Ribeiro, 1941; Alho, 1965; Freitas and Dobbin Jr., 1971; Viana et al., 2016). *Serpinema pelliculatus* n. sp. is the seventh species recorded for the Neotropical region, and the fourth species of the genus reported in Brazil.

The record of different nematodes of the same genus parasitizing one host species is not common. However, González-Solís and Moravec (2004) suggest that morphological variability among parasitic nematodes results from the ecological conditions to which the host is exposed. *Kinosternon scorpioides* is widely distributed from southern Mexico to northern Argentina (Berry and Iverson, 2011; Ferrara et al., 2017), and these hosts are exposed to different environmental conditions. In addition, chelonians are animals whose diversification occurred long ago, and their parasites must have suffered evolutionary and ecological pressures leading to parasite speciation. Those factors could explain the diversity of *Serpinema* (4 species) reported for *K. scorpioides*.

All *Serpinema* species have been described as parasitizing freshwater turtles, and the genus dispersion among these animals is probably associated with the life cycle of these nematodes. Some studies suggest

Table 1
Morphometric and morphological data of the valid species of *Serpinema*. Measurements are in micrometers, unless otherwise stated.

Species	*Serpinema pelliculatus* n. sp.	*Serpinema amazonicus*	*Serpinema cayannensis*	*Serpinema intermedius*
Reference	This study	Ribeiro (1941)	Harnoster et al. (2019)	Hsu and Hoeppli (1931)
Type host	*Kinosternon scorpioides*	*Podocnemis expansa*	*Rhinoclemmys punctularia*	*Mauremys reevesi*
Type locality	Brazil	Brazil	French Guiana	China
Number of ridges in valves	9–11	17–20	8–11	10
Pairs of male caudal papillae	15	9	13	14
Total length (mm)	♂ 6.07–8.20	♂ 11.95	♂ 9.7–12.2	♂ 5.65–7.90
	♀ 8.05–9.91	♀ 15.08–17.92	♀ 13.7–18.5	♀ 11.05–12.10
Width at mid-body	♂ 173–240	♂ 400	♂ 332–477	♂ 200–230
	♀ 227–333	♀ 530–680	♀ 487–688	♀ 165–175
Buccal capsule	♂ 60–91 × 95–109	♂ 170 × 180	♂ 129–150 × 170–203	♂ 130–150[a]
	♀ 84–100 × 97–119	♀ 204–208 × 226–239	♀ 121–179 × 213–245	♀ 110–130[a]
Trident middle branch length	♂ 48–69	♂ 69	♂ 105–138	♂ 60–85
	♀ 48–80	♀ 78–87	♀ 104–187	♀ 100–120
Trident lateral branches length	♂ 60–85	♂ –	♂ –	♂ –
	♀ 81–104	♀ –	♀ –	♀ –
Basal ring	♂ 8–13 × 60–79	♂ 43 × 104	♂ 11–17 × 106–113	♂ 35–90[a]
	♀ 10–17 × 74–88	♀ 53 × 139	♀ 13–20 × 118–150	♀ 82–140[a]
Muscular esophagus	♂ 317–360 × 99–139	♂ 610[b]	♂ 258–458 × 148–171	♂ 450–510[b]
	♀ 368–416 × 115–141	♀ 730–750[b]	♀ 541–620 × 167–200	♀ 520–560[b]
Glandular esophagus	♂ 499–613 × 115–155	♂ 530[b]	♂ 804–926 × 148–171	♂ 490–550[b]
	♀ 560–699 × 125–203	♀ 630–650[b]	♀ 895–1040 × 152–220	♀ 600–690[b]
Nerve ring	♂ 141–203	♂ 400	♂ 253–335	♂ 160–165
	♀ 171–221	♀ 426	♀ 303–378	♀ 170–215
Excretory pore	♂ 304–357	♂ –	♂ 428–510	♂ 180–260
	♀ 381-437	♀ –	♀ –	♀ –
Deirids	♂ –	♂ –	♂ –	♂ 310–410
	♀ –	♀ –	♀ –	♀ 410–535
Tail	♂ 83–128	♂ 130	♂ 184–365	♂ 550–560
	♀ 155–197	♀ 210	♀ 215–560	♀ 170–210
Right spicule	416–566	570	360–806	490–630
Left spicule	–	240	208–456	200–260
Vulva (mm)	3.76–4.64	8.64–9.17	6.28–10.06	5.25–6.00

Species	*Serpinema kachugae*	*Serpinema lissemysus*	*Serpinema magathi*	*Serpinema microcephalus*
Reference	Baylis and Daubney (1922)	Gupta and Singh (1959)	Freitas and Dobbin Jr. (1971)	Dujardin (1845)
Type host	*Pangshura smithii*	*Lissemys punctata*	*K. scorpioides*	*Emys orbicularis*
Type locality	India	India	Bolivia	France
Number of ridges in valves	8–10	14–16	9–11	10
Pairs of male caudal papillae	15	18	14	12
Total length (mm)	♂ 10.9–14.5	♂ 4.75–5.00	♂ 8.34–8.93	♂ 6.93–10.84
	♀ 20.8–22.0	♀ 10.5	♀ 12.58	♀ 11.68–12.73
Width at mid-body	♂ 300–370	♂ 210–330	♂ 260–290	♂ 265
	♀ 450–500	♀ 310	♀ 290–360	♀ 390
Buccal capsule	♂ 110–130 × 140–160	♂ 110–130 × 130–140	♂ 97 × 119–133	♂ 109[b]
	♀ 110–130 × 140–160	♀ 120 × 140	♀ 108 × 144	♀ 125[b]
Trident middle branch length	♂ 80–100	♂ 50–100	♂ 61	♂ –
	♀ 80–100	♀ 50–110	♀ 51‡	♀ –
Trident lateral branches length	♂ –	♂ –	♂ 76–79	♂ –
	♀ –	♀ –	♀ 86	♀ –
Basal ring	♂ 100[a]	♂ –	♂ 18–22 × 86–90	♂ –
	♀ 100[a]	♀ –	♀ 18–22 × 101–108	♀ 22–29 × 72–90
Muscular esophagus	♂ 540–660[b]	♂ 380–440[b]	♂ 410–420[b]	♂ 359[b]
	♀ 540–660[b]	♀ 490[b]	♀ 430–450[b]	♀ 437[b]
Glandular esophagus	♂ 640–990[b]	♂ –	♂ 660–710[b]	♂ 203[b]
	♀ 540–990[b]	♀ –	♀ 710–780[b]	♀ 687[b]

(*continued on next page*)

Table 1 (*continued*)

Species	*Serpinema kachugae*	*Serpinema lissemysus*	*Serpinema magathi*	*Serpinema microcephalus*
Nerve ring	♂ 200–230	♂ 200–220	♂ 99–132	♂ 203
	♀ 200–230	♀ 250	♀ 139	♀ –
Excretory pore	♂ 500	♂ –	♂ –	♂ 393
	♀ 500	♀ –	♀ –	♀ –
Deirids	♂ 500-550	♂ –	♂ –	♂ 338–363
	♀ –	♀ –	♀ –	♀ –
Tail	♂ 210	♂ 130–170	♂ 107–120[c]	♂ –
	♀ 300	♀ –	♀ 220	♀ 172
Right spicule	970	–	540–590	800
Left spicule	430	–	–	380
Vulva (mm)	9.10–10.40	–	7.13	5.65

Species	***Serpinema monospiculatus***	***Serpinema trispinosus***	***Serpinema octorugatum***
Reference	Freitas and Dobbin Jr. (1962)	Leidy (1852)	Sharma et al. (2002)
Type host	*Mesoclemmys tuberculata*	*Trachemys decussata*	*Heosemys grandis*
Type locality	Brazil	Cuba	Malaysia
Number of ridges in valves	10–12	14–15	8–12
Pairs of male caudal papillae	10	12	14
Total length (mm)	♂ 9.55–10.92	♂ 6.93–7.95	♂ 7.46–11.50
	♀17.08–18.69	♀ 10.6–13.0	♀ 7.58–21.05
Width at mid-body	♂ 230–260	♂ 270–280	♂ 225–375
	♀ 300–360	♀ 270–280	♀ 275–675
Buccal capsule	♂ 108–112 × 137–144	♂ 129–135 × 150–171	♂ 130–150 × 125–150
	♀115–137 × 173–198	♀ 144–150 × 189–198	♀ 145–190 × 140–195
Trident middle branch length	♂ 72–76	♂ 75-81	♂ 50–90
	♀ –	♀ 75–111	♀ 65–105
Trident lateral branches length	♂ 58–65	♂ –	♂ –
	♀ –	♀ –	♀ –
Basal ring	♂ 25–29 × 76–79	♂ –	♂ 15–20 × 70–100
	♀ 22–29 × 72–90	♀ –	♀ 15–20 × 90–130
Muscular esophagus	♂ 500–530[b]	♂ 440–460 × 150	♂ 370–520 × 110–165
	♀ 550–740[b]	♀ 480–550[b]	♀ 410–620[b]
Glandular esophagus	♂ 630–710[b]	♂ 500–540[b]	♂ 495–690 × 110-230
	♀ 710–1000[b]	♀ 430–610[b]	♀ 490–930[b]
Nerve ring	♂ 112–132	♂ 200–230	♂ 200–240
	♀ 132–165	♀ 240–250	♀ 210–310
Excretory pore	♂ –	♂ –	♂ 350–505
	♀ –	♀ –	♀ 380–680
Deirids	♂ –	♂ 360	♂ –
	♀ –	♀ 380	♀ 490–710
Tail	♂ 133[c]	♂ –	♂ –
	♀ 200–230	♀ 160–270	♀ 100–325
Right spicule	430	788–816	320–370
Left spicule	–	360–385	140–190
Vulva (mm)	6.21–8.87	5.16–5.65	4.03–10.50

[a] Only width.
[b] Only length.
[c] Measurements obtained through access to slides deposited at CHIOC.

that the parasite larvae infect crustacean copepods which are intermediate hosts and can be ingested by paratenic hosts such as fish, amphibians, and aquatic snails. The parasite reaches the definitive host when the freshwater turtle feeds directly on the intermediate host or the paratenic host (Bartlett and Anderson, 1985; González and Hamann, 2007; Wiles and Bolek, 2015). Since *K. scorpioides* is an omnivorous species that feed on algae, plants, seeds, and a variety of insects, mollusks, fish, and small amphibians (Berry and Ivwerson, 2001; Berry and Iverson, 2011), its feeding habits facilitate the infection of this freshwater turtle by different parasitic nematode larvae present in aquatic environments, such as camallanids.

Due to the complex taxonomic history of the family Camallanidae, we reinforced the need for future studies using molecular biology approaches. Those new data will aid the identification of camalanid species and possible cryptic species or synonymies, unraveling the evolutionary history and clarifying the systematics of this family of nematodes.

Ethics approval

All procedures contributing to this work comply with all applicable institutional, national, and international guidelines for animal care and use Animal Research Ethics Committee, Federal University of Pará, under license N8341260821CEUA/UFPa. 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 CAPES/PPGBAIP/UFPa, Fundação Amazônia de Amparo a Estudos e Pesquisa (FAPESPA) (01/2021 – PROGRAMA DE APOIO A NUCLEOS EMERGENTES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Research productivity scholarship of CNPq to MELO, F.T.V. (Process number 314116/2021–4) and SANTOS, J.N. (Process number 305552/2019–8).

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 Fred Gabriel Haick de Moura and Emanuelle Gabriela Gualberto de Argôlo for their support in host necropsies and nematode collection; to 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. This study is part of the Ph.D thesis of Leandro Mauricio Oliveira da Silva in Postgraduate Program in the Biology of Infectious and Parasitic Agents (ICB-UFPa).

References

Ailán-Choke, L.G., Pereira, F.B., 2021. Deep in systematics of Camallanidae (Nematoda): using integrative taxonomy to better understand the phylogeny and consistency of diagnostic traits. Parasitology 148 (8), 962–974. https://doi.org/10.1017/S0031182021000706.

Alho, C.J.R., 1965. Contribuição ao conhecimento da fauna helmintológica de quelônios do estado do Pará, Brasil. Bol. Mus. Para. Emílio Goeldi 58, 1–13.

Baker, M.R., 1983. Nematodes parasites of the turtle *Pelusios sinuatus* (pelomedusidae: pleurodira) from southern africa. Syst. Parasitol. 5 (3), 161–167.

Bartlett, C.M., Anderson, R.C., 1985. Larval nematodes (ascaridida and spirurida) in the aquatic snail, *Lymnaea stagnalis*. J. Invertebr. Pathol. 46, 153–159.

Baylis, H.A., Daubney, R., 1922. Report on the parasitic nematodes in the collection of the Zoological survey of India. Mem. Indian Mus. 7 (4), 264–342.

Berry, J.F., Iverson, J.B., 2011. *Kinosternon scorpioides* (Linnaeus, 1766) – scorpion mud turtle. In: Rhodin, A.G.F., Pritchard, P.C.H., Van Dijk, P.P., Saumure, R.A., Buhlman, K.A., Iverson, J.B., Mittermeier, R.A. (Eds.), Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtles Specialist Group. Chelonian Research Monographs, p. 63, 1–63.15.

Berry, J.F., Ivwerson, J.B., 2001. Kinosternon Scorpioides. Catalogue of American Amphibians and Reptiles, 725.1–725.11.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: margolis et al. Revisited. J. Parasitol. 83 (4), 575–583.

Dujardin, M.F., 1845. Histoire naturelle des helminthes ou vers intestinaux (Paris).

Ferrara, C.R., Fagundes, C.K., Morcatty, T.Q., Vogt, R.C., 2017. Quelônios Amazônicos: Guia de Identificação e Distribuição (Manaus).

Freitas, J.F.T., Dobbin Jr., J.E., 1962. Novo nematódeo camalanídeo parasito de quelônio. Atas Soc. Biol. Rio de Janeiro 6 (1–2), 5–7.

Freitas, J.F.T., Dobbin Jr., J.E., 1971. Contribuição ao conhecimento da fauna helmintológica de quelônios no estado de Pernambuco, Brasil. Mem. Ins. Oswaldo Cruz 69 (1), 33–39.

González, C.E., Hamann, M.I., 2007. The first record of amphibians as paratenic hosts of *Serpinema* larvae (Nematoda; Camallanidae). Braz. J. Biol. 67 (3), 579–580. https://doi.org/10.1590/s1519-69842007000300026.

González-Solís, D., Moravec, F., 2004. Two new nematode species, *Orientatractis campechensis* n. sp. and *Orientatractis chiapasensis* n. sp. (Nematoda: Atractidae) from cichlid fishes in southern Mexico and Nicaragua. J. Parasitol. 90 (6), 1443–1449.

Gupta, N.K., Singh, S., 1959. On a new *Camallanus* nematode from *Lissemys - (Punctata) punctata*. Res. Bull. Panjab University 10 (3–4), 285–289.

Harnoster, F., Svittin, R., Du Preez, L., 2019. *Serpinema cayennensis* n. sp. (Nematoda: Camallanidae), a parasite of the freshwater turtle *Rhinoclemmys punctularia* Daudin (Reptilia: Testudines: geoemydidae) from French Guiana: morphology and phylogenetic relationships with other turtle-parasitising camallanids. Zootaxa 4679 (1), 181–193. https://doi.org/10.11646/zootaxa.4679.1.11.

Hsu, H.F., Hoeppli, R., 1931. Parasitic nematodes mostly from snakes collected in China. Natl. Med. J. China (Peking) 17 (4/5), 567–588.

Hungría, C.D., 1978. Helmintos parásitos de vertebrados en el estado Zulia (Venezuela) algunas especies nuevas para Venezuela. Vet. Trop. 3, 15–37.

ICZN, 2012. International Commission on Zoological Nomenclature: Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. Zootaxa 3450, 1–7. https://doi.org/10.3897/zookeys.219.3994.

Ivashkin, V.M., Soboloev, A.A., Khromova, L.A., 1977. Camallanata of Animals and Man and Diseases Caused by Them. Israel Program for Scientific Translations, Jerusalem.

Leidy, J., 1852. Description of new species of entozoa. Proc. Acad. Nat. Sci. Phila. 5 (7), 155–156.

Mascarenhas, C.S., Müller, G., 2021. Checklist of helminths associated with continental testudines from South America. Neotrop. Helminthol. 15 (1), 97–126. https://doi.org/10.24039/rnh20201511047.

Moravec, F., 1998. Nematodes of Freshwater Fishes of the Neotropical Region. Academia, Prague.

Moravec, F., Vargas-Vazquez, J., 1998. Some endohelminths from the freshwater turtle *Trachemys scripta* from Yucatan, Mexico. J. Nat. Hist. 32, 455–468. https://doi.org/10.1080/00222939800770241.

Pereira, A.M.A., Brito, S.V., Araujo Filho, J.A., Teixeira, A.A.M., Teles, D.A., Santana, D. O., Lima, V.F., Almeida, W.O., 2018. Diet and helminth parasites of freshwater turtles *Mesoclemmys tuberculata, Phrynops geoffroanus* (Pleurodira: Chelidae) and *Kinosternon scorpioides* (Criptodyra: kinosternidae) in a semiarid region, Northeast of Brazil. Acta Herpetol. 13 (1), 21–32. https://doi.org/10.13128/Acta_Herpetol-20323.

Pinheiro, R.H.S., Melo, F.T.V., Monks, S., Santos, J.N., Giese, E.G., 2018. A new species of *Procamallanus* Baylis, 1923 (nematoda, Camallanidae) from *Astronotus ocellatus* (agassiz, 1831) (perciformes, cichilidae) in Brazil. ZooKeys 790, 21–33. https://doi.org/10.3897/zookeys.790.24745.

Ribeiro, D.J., 1941. Pesquisas helmintológicas realizadas no Estado do Pará. VIII – *Camallanus amazonicus* n. sp. parasito de *Podocnemis expansa* (Schw). Mem. Inst. Oswaldo Cruz 35 (4), 723–731.

Rigby, M.C., Rigby, E., 2013. Order camallanida: superfamilies anguillicoloidea and camallanoidea. In: Nematoda Schmidt-Rhaesa, A. (Ed.), Handbook of Zoology: Gastrotricha, Cycloneuralia and Gnathifera, vol. 2. Walter De Gruyter, Berlin, pp. 637–659.

Sharma, R.S.K., Rigby, M.C., Sumita, S., Sanira, R.A., Vidyadaran, M.K., Jasni, S., Dailey, M.D., 2002. Redescription of *Serpinema octorugatum* (Baylis, 1933) (nematoda: Camallanidae) from the malayan box turtle *Cuora amboinensis* (daudin) (Chelonia: bataguridae). Syst. Parasitol. 53, 19–28. https://doi.org/10.1023/a:1019997922052.

Sprehn, L.V.C., 1932. Über einige von Dr. Eisentraut in Bolivien gesammelte Nematoden. Zool. Anz. 11/12, 273–284.

Stromberg, P.C., Crites, J.L., 1974. Specialization, body volume, and geographical distribution of Camallanidae (Nematoda). Syst. Zool. 23 (2), 189–201.

Viana, D.C., Rodrigues, J.F.M., Madelaire, C.B., Santos, A.C.G., Sousa, A.L., 2016. Nematoda of *Kinosternon scorpioides* (Testudines: kinosternidae) from northeastern Brazil. J. Parasitol. 102 (1), 165–166. https://doi.org/10.1645/15-788.

Wiles, C.M., Bolek, M.G., 2015. Damselflies (Zygoptera) as paratenic hosts for *Serpinema trispinosum* and its report from turtle hosts from Oklahoma, USA. Folia Parasitol. 62 (19), 1–8. https://doi.org/10.14411/fp.2015.019.

Yeh, L., 1960. On a reconstruction of the genus *Camallanus* Railliet & Henry, 1915. J. Helminthol. 34, 117–124.