



Squamasnema amazonica n. gen. n. sp. (Heligmonellinae): A new parasite of *Proechimys roberti* (Rodentia: Echimyidae) in the Brazilian Amazon

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ABSTRACT

A new species of nematode, *Squamasnema amazonica* n. gen. n. sp., is described based on specimens found parasitizing the small intestine of *Proechimys roberti* (Rodentia: Echimyidae) collected during a survey of the fauna of Tapirapé-Aquirí National Forest (Brazil, Eastern Brazilian Amazon). The nematodes were fixed and processed for light microscopy and scanning electron microscopy (SEM). These nematodes were classified under the family Heligmonellidae and the subfamily Heligmonellinae. Although several species in the family Heligmonellidae exhibit discontinuous ridges, *Squamasnema* n. gen. and *Trichotravassosia* are the only genera with columns of scales along their entire body, as an apomorphy of the synlophe. *Squamasnema* n. gen. has columns of cuticular cells along its body, except for on the left flank, and exhibits a synlophe with no size gradient or inclination and does not present chitinized structures supporting the synlophe. Therefore, due to these morphological differences of *Squamasnema* n. gen., the creation of a new genus was necessary.

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1. Introduction

Rodents of the genus *Proechimys* are geographically distributed in different Brazilian biomes, and species *Proechimys roberti*, *P. oris*, *Physalaemus cuvieri*, *Proechimys goeldi*, and *Proechimys guyannensis* can be found in the Amazon region (Weksler et al., 2001; Bonvicino et al., 2008).

The nematodes of the family Heligmonellidae Durette-Desset and Chabaud, 1977 have been reported as parasites of Echimyidae rodents, particularly in the families Heligmonellinae which include the genera *Paraheligmonella* Durette-Desset, 1971; *Trichotravassosia* Lent and Freitas, 1938 and in Pudicinae with the genera *Pudica* Travassos and Darrida, 1929; *Acanthostrongylus* Travassos,

1937 (Durette-Desset, 2009; *Heligmostrongylus* Travassos, 1917 and *Justinema* R'Kha & Durette-Desset, 1991).

These genera are characterized by the presence of a cephalic vesicle, monodelphic females with vulva situated near the anal opening, and tail without spines (Gonçalves et al., 2007; Digiani et al., 2009) and male caudal bursa subsymmetrical with lobes of similar size and pattern 2-2-1 (Durette-Desset and Digiani, 2012). One of the main morphological characters of heligmonellid nematodes is the synlophe (Durette-Desset and Chabaud, 1981). The orientation of the synlophe in transverse sections in relation to sagittal axis the body; the number and continuous or discontinuous pattern of longitudinal ridges along the nematode and the presence of carene are characters to subfamily classification (Durette-Desset and Justine, 1991).

A survey of the helminth fauna of *P. roberti* Thomas, 1901, from Tapirapé-Aquirí National Forest revealed the presence of a new genus and species of nematode belonging to the family Heligmonellidae, parasitizing the small intestine.

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2. Materials and methods

The gastrointestinal tract samples analyzed in this study, were obtained from nine spiny rats (*P. roberti*) collected in Tapirapé-Aquirí National Forrest and fixed in 10% formaldehyde. To search for the presence of helminthes, the samples were analyzed in the Laboratory of Cell Biology and Helminthology Reinalda Marisa Lanfredi at the Institute of Biological Sciences, Federal University of Pará (Universidade Federal do Pará, UFPA). The nematodes found in the small intestine were cleared in an ethanol–glycerin grades for light microscopy analysis.

Morphological analyses were performed using an Olympus BX41 Microscope fitted with a camera lucida (without zoom adjustment), and taxonomic traits were identified according to the descriptions of Durette-Desset (2009). Specimen measurements are expressed in micrometers unless stated otherwise. The results are expressed as the mean and the standard deviation, followed by the minimum and maximum values within parentheses. For additional comparisons, we examined the series type to genera *Trichotravassosia* deposited in Helminthological Collection Oswaldo Cruz (CHIOC) under the number 9766th-i.

For scanning electron microscopy (SEM), the nematodes were post-fixed in 1% osmium tetroxide (OsO₄), washed in 0.1 M sodium phosphate buffer, pH 7.4, and dehydrated in an ethanol series until CO₂ reached a critical point. They were then subjected to gold sputtering and analyzed using a LEO 1450VP Scanning Electron Microscope (Zeiss) (Giese et al., 2010). Type and voucher specimens were deposited in Museu Paraense Emílio Goeldi (MPEG), Belém City, Pará, Brazil.

3. Results

3.1. *Squamasnema n. gen.*

Diagnosis: Heligmonellidae: Heligmonellinae. Small nematodes. Synlophe modified into cuticular scales arranged in a longitudinal series, except on the left flank, without chitinoid struts. Cephalic vesicle reduced followed by evident cuticular dilation, esophageal tooth absent. The males exhibit subequal, long, single-tip spicules, a developed genital cone, gubernaculum absent, well-developed copulatory bursa subsymmetrical divided into two lobes with the same pattern in both lobes (type 1–4) without a distinct dorsal lobe. The monodelphic females exhibit a subterminal vulva near the anus.

Etymology: The name of the genus refers to the cuticular scales on the body of the parasite.

3.2. *Squamasnema amazonica n. gen. n. sp.*

3.2.1. General description

Small and ventrolaterally curved nematodes. Males and females similar in length. Triangular oral opening, without lips, discrete buccal ring and reduced buccal capsule. A pair of amphids and four cephalic papillae (Fig. 1a), with a reduced cephalic vesicle and cuticular dilation in the anterior region (Fig. 1b). Claviform esophagus and a nerve ring located in the middle third of the esophagus (Fig. 2a). Nematodes with a laterally curved body exhibit scale-shaped cuticular projections arranged in longitudinal rows (Fig. 1d), except on the left side of the body (Fig. 1c), cuticular scale starting below the cuticular dilation in the anterior region, ending just before the vulva in females, and before copulatory bursa in males (Fig. 2b–f). Excretory pore and deirids not observed. The posterior region of males exhibits two long and subequal spicules, (Fig. 2b), a well-developed genital cone, gubernaculum absent, and bilobed copulatory bursa supported by lateral and dorsal bursal rays,

without a distinct dorsal lobe (Fig. 2c) and with a smooth dorsal surface and an external papilla at the end of ray 8 (Fig. 1f). The bursa display a type 1–4 ray pattern with a slightly dented margin (Fig. 2d and e). Females have a tapered conical tail (Fig. 1e) and are monodelphic with a subterminal vulva near the anus; ovijector developed; tail short and conical (Fig. 2f); eggs, not observed.

Synlophe (based on cross-sections at the level of the esophagus, midbody and at the posterior end from 2 males and 2 females, Fig. 3): Ridges in both sexes arise just posterior to the cephalic inflation; ridges modified as cuticular scales arranged in a longitudinal series, without chitinoid struts. Left side of the body lacks cuticular scales in both sexes. In females, the scales are distributed along the body until just before the vestibule. In males, the scales are distributed along the body up to half of the spicule length, ending before the copulatory bursa. Scale size gradient and inclination angle absent. Sections revealed the presence of 10 columns of scales (five dorsal and five ventral) (Fig. 3).

3.2.2. Males (based on 1 holotype and 9 paratypes)

Total length, 9.71 ± 0.74 (8.70–10.90) mm, and width, 119.20 ± 10.06 (106.66–133.33). Esophagus, 382.13 ± 18.08 (346.66–413.33) in length. Nerve ring, 200.80 ± 22.83 (152–232) from the anterior end. Subequal spicules: right spicule, 574.39 ± 22.73 (533.33–608), and left spicule, 586.13 ± 23.10 (541.33–610.66) in length. Genital cone, 35.45 ± 3.51 (31.16–41.55) \times 35.32 ± 6.72 (25.97–42.85) (width measured at the base); pre-bursal papillae not observed. Bursal formula: copulatory bursa subsymmetrical (type 1–4): Ray 2 is clearly distinct from ray 3 and extends to the margin of the bursa to create a prominence at the bursal margin. Rays 3, 4, 5 and 6 are grouped into a common trunk, with rays 4, 5 and 6 creating a trident shape. Ray 4 is robust and only separates from ray 5 beginning in the posterior third. Rays 3 and 5 present a similar size, and ray 6 reaches the margin of the bursa (Fig. 2d). Ray 8 originates in the base of dorsal trunk. The dorsal trunk is proximally divided at two-thirds, and distally originating rays 9 and 10, which do not reach the margin of the bursa (Fig. 2e).

3.2.3. Females (based on 1 allotype and 9 paratypes)

Total length, 9.16 ± 1.71 mm (6–11) \times 134.13 ± 23.88 (93.33–178.66). Esophagus, 378.93 ± 33.69 (290.66–410.66) in length. Nerve ring, 192 ± 39.61 (160–237.33), from the anterior end. Vulva at 211.20 ± 24.10 (189.33–272) and anus at 58.39 ± 3.65 (53.33–64) from the posterior end. Vagina vera, 16.88 ± 4.26 (12.98–24.67); vestibule measuring 147.24 ± 13.65 (133.76–174.02); sphincter measuring 63.34 ± 5.04 (22.07–38.96); and infundibulum 141.55 ± 16.90 (110.38–171.42) in length.

3.3. *Squamasnema amazonica n. gen. n. sp.*

Host type: *P. roberti* Thomas, 1901.

Infection site: Small intestine.

Location: Tapirapé-Aquirí National Forrest, Carajás Reserve, State of Pará, Brazil (5°35' to 6°00'S and 50°24' to 51°06'W).

Prevalence: 50% (5 infected rodents/10 rodents analyzed).

Infection intensity: 3.3 ± 4.21 (3–12).

Deposition of the species: “Museu Paraense Emílio Goeldi” collection, Holotype male (MPEG no. 000029), allotype female (MPEG no. 000030) and 2 paratypes (1 male MPEG 000031 and 1 female MPEG 000032).

Comparative material examined: *Trichotravassosia travassosi* Lent & Freitas, 1938 CHIOC 9766h-i deposited at Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC).

Etymology: The species name references the biome where the host was collected.

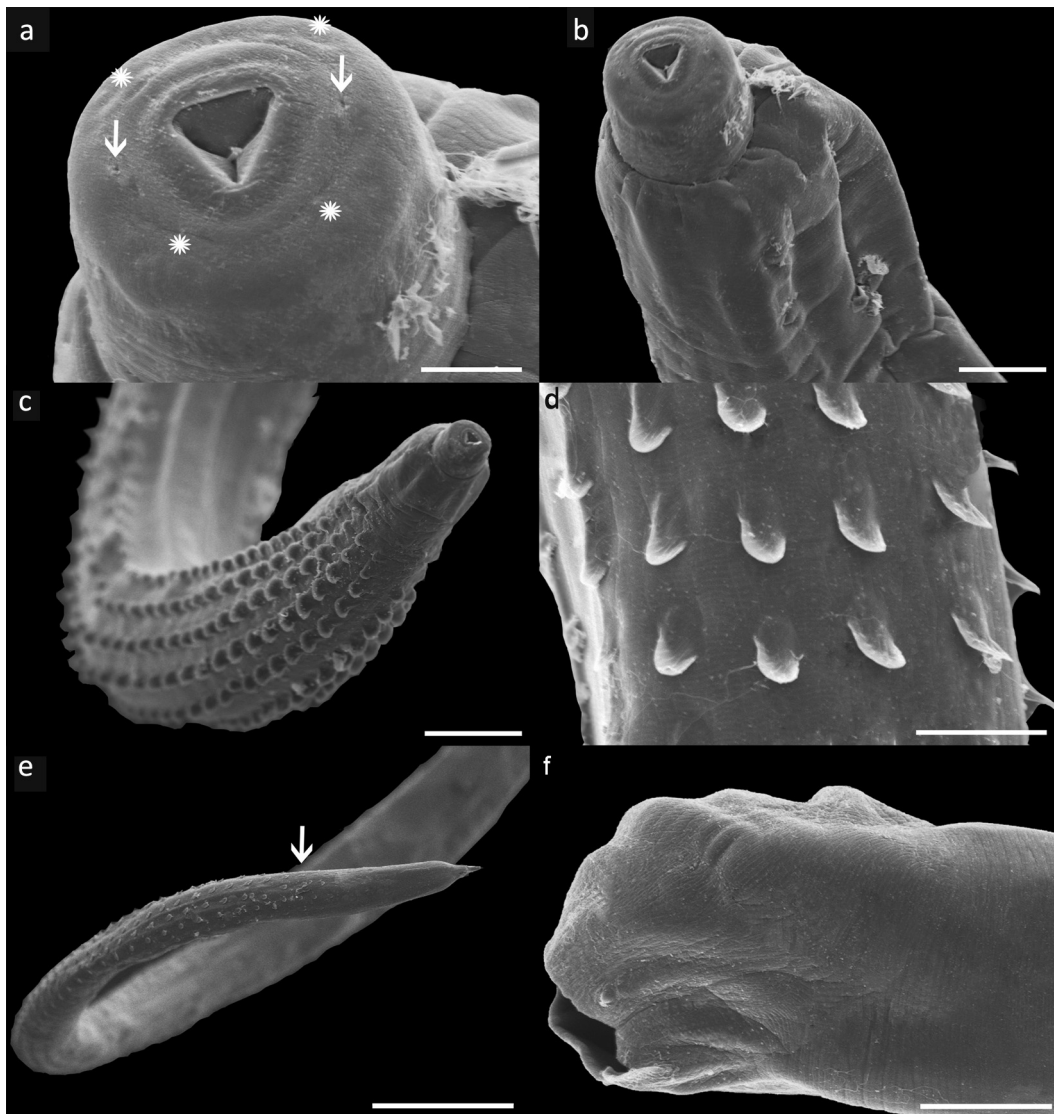


Fig. 1. Scanning electron microscopy (SEM) results. (a) An apical view detailing the triangular buccal opening, two amphids (arrows) and four papillae (stars) (bar = 8 μm). (b) The anterior region, emphasizing the cephalic vesicle (bar = 30 μm). (c) The scales arranged in a longitudinal series begin below the cephalic vesicle, and the left lateral side is lacking scales (stars) (bar = 60 μm). (d) A detailed image of the cuticular formations (bar = 30 μm). (e) A view of the posterior region of the females, showing the ends of the columns of scales (arrow) (bar = 100 μm). (f) A view of the posterior region of the males, emphasizing the bursal contour and the external papillae (bar = 30 μm).

4. Discussion

The new genus described in this study is classified within the family Heligmonellidae, as it exhibits the taxonomic elements typical of the family, including the presence of a cephalic vesicle and monodelphic females with a subterminal vulva (Gonçalves et al., 2007; Digiani et al., 2009). One of the main characteristics of this family is the presence of a synlophes (Durette-Desset and Chabaud, 1981), which is the ensemble of longitudinal ridges that extend along the body of the nematode. Interrupted ridges formed by cuticular scales are observed in *Squamasnema* n. gen.

Durette-Desset and Chabaud (1981) describes the family Heligmonellidae as composed by four subfamilies, classified according to ridges inclination angle in relation to sagittal axis. The subfamily Heligmonellinae comprises species that have axis of orientation of ridges inclined no more than 45°, the subfamily Nippostrongylinae has axis of orientation range from 45° to 67° and the subfamilies Pudicinae and Brevistriatinae ranges from 67° to 90°.

According to Durette-Desset and Digiani (2005) the axis of orientation is defined as the axis that separates the ridges in two

groups represented in opposite positions. It's possible to observe in *Squamasnema* n. gen. that the scales are disposed perpendicularly to the body surface, then, the inclination in relation of the sagittal axis is absent, character that allow us to classify the genus in Heligmonellinae.

Despite the specimens studied here in present some morphological characters of the subfamily Pudicinae, as less than 14 ridges and absence of size gradation of the ridges, they can be classified in Heligmonellinae by the absence of the inclination of the scales in relation of the sagittal axis, sub symmetrical cupulatory bursa with the pattern 1–4, discussed by Durette-Desset and Digiani (2012), and the absence of carene and comaretes, both synapomorphies of subfamily Pudicinae (Durette-Desset and Justine, 1991).

In the subfamily Heligmonellinae, only two genera display discontinuous ridges: *Paraheligmonella* Durette-Desset, 1971, with four discontinuous ridges, and *Trichotravassosia*, Lent and Freitas, 1938, with all ridges discontinuous.

In specimens belonging to *Squamasnema* n. gen., all of the ridges are discontinuous, formed by cuticular scales and exhibit no size gradient, which are characteristics similar to those of the genus

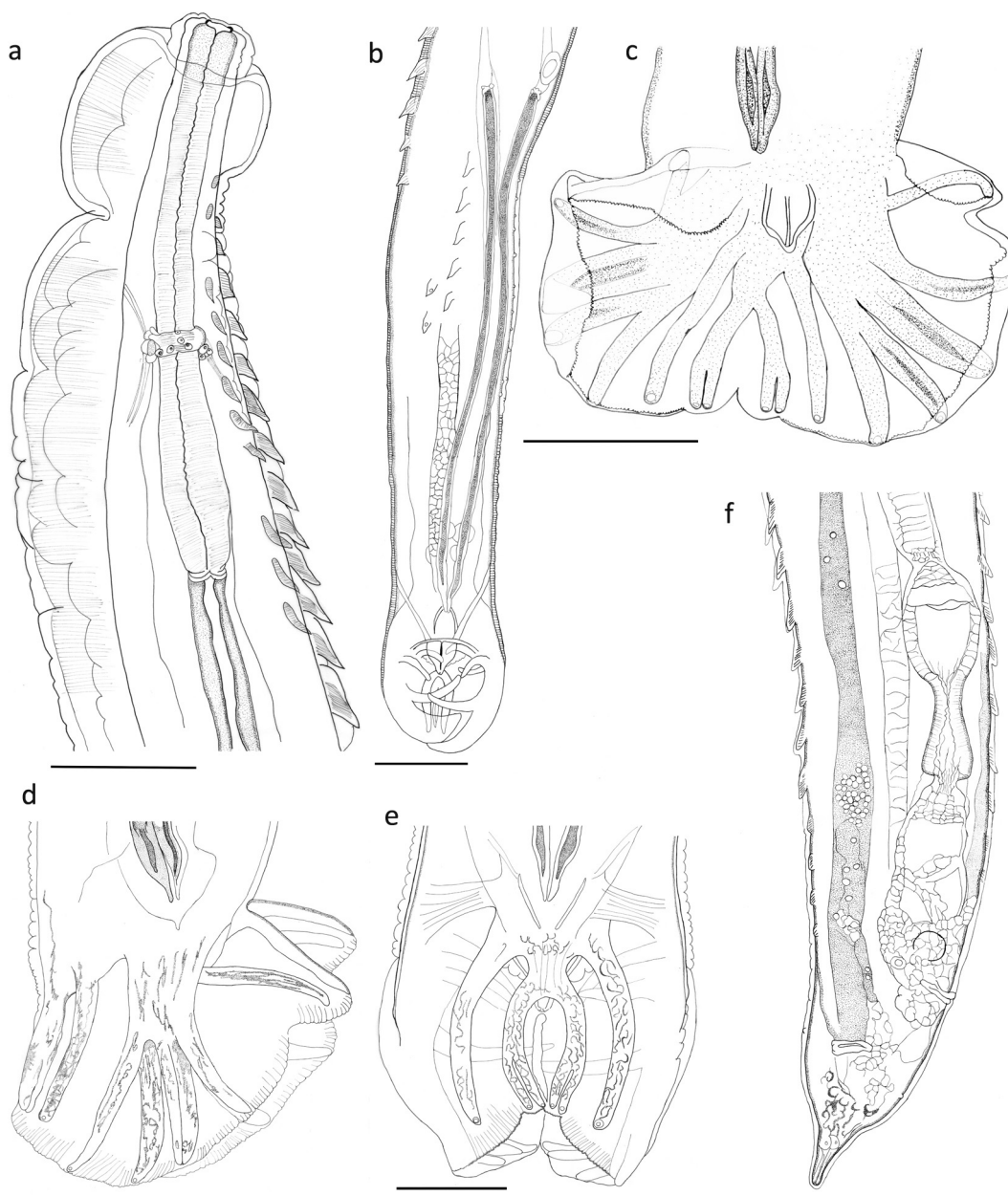


Fig. 2. *Squamasnema amazonica* n. gen. n. sp. (a) A view of the anterior region, including a complete view of the esophagus, the location of the nerve ring, the cuticular inflation and the beginning of the columns of scales (bar = 100 μ m). (b) A ventral view of the posterior region of a male, highlighting the spicules and the ends of the columns of scales (bar = 100 μ m). (c) A ventral view of the bursa showing the position of the bursal rays and genital cone (bar = 100 μ m). (d) A lateral view of the copulatory bursa emphasizing the bursal contour (bar = 50 μ m). (e) A dorsal view of the bursa highlighting the external dorsal rays, which originate in the trunk of the dorsal ray that ends in bifurcated tips (bar = 50 μ m). (f) Lateral views of the terminal end of the female, showing the ends of the columns of scales and the ovjector (bar = 100 μ m).

Trichotravassosia described from *Kannabateomys amblyonyx*, a rodent belonging to the family Echimyidae, collected in the state of Rio de Janeiro, Brazil (Lent and Freitas, 1938). However, scale placement differs between these genera, since in *Squamasnema* n. gen the scales, are spaced, arising soon after the cuticular inflation and no scales are found on the left side of the body, while according to our observations and the description of Lent and Freitas (1938), the scales in *Trichotravassosia* are juxtaposed and cover the whole body of the nematode beginning at the end of the esophagus. Additionally, the scales of *Squamasnema* n. gen. do not have cuticular struts, and immediately differ from *Trichotravassosia*.

The copulatory bursa also distinguished *Squamasnema* n. gen., which has a pattern of type 1–4, subsymmetrical, where ray 2

extends to the bursal edge forming a prominence, and rays 9 and 10 show similar sizes. Additionally, a telamon was not observed. Conversely, we observed that *Trichotravassosia* features includes a slightly asymmetrical bursa of pattern of 2–2–1, wherein the ray 3 is larger than ray 2, extending to the bursal margin, while ray 9 is longer than ray 10 and faces outward, and a telamon is present.

Trichotravassosia is the genus closely related *Squamasnema* n. gen by the presence of scales covering the body surface, however the differences presented here justify the creation of a new genus. As described in this work, although several species in the Heligmonellidae display discontinuous ridges, these two genera are the only ones that exhibit columns of cuticular scales along the entire length of their body, as an synapomorphy of the synlophe.

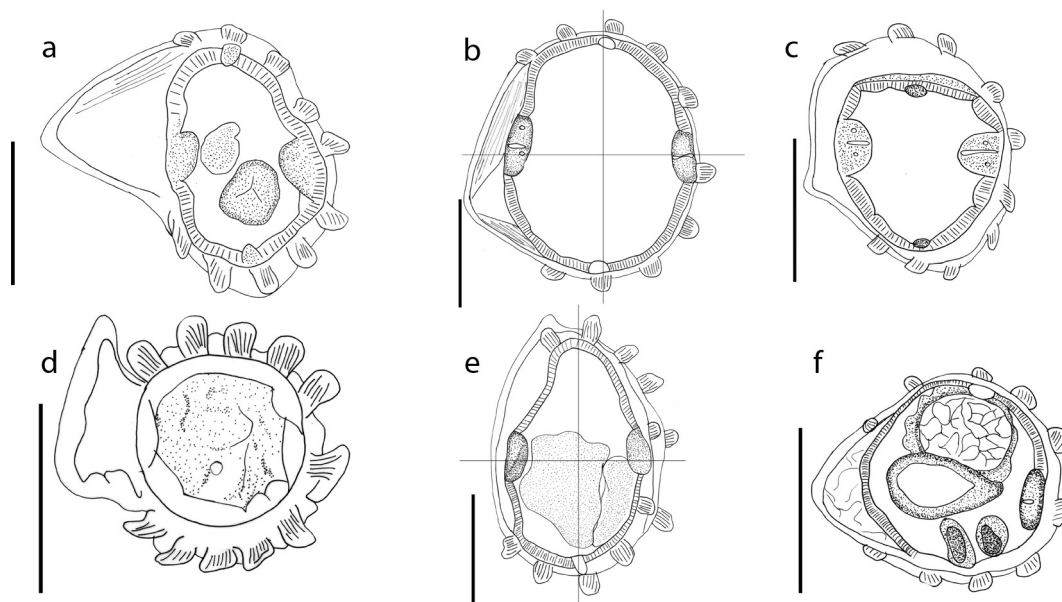


Fig. 3. Line drawings of cross-sections of *Squamasnema amazonica* n. gen. n. sp. (a–c) Anterior, medial and posterior regions of males, respectively. (e–g) Anterior, medial and posterior regions of females, respectively (all bars are 50 μ m).

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