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

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A B S T R A C T

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 Pudiciniae with the genera Padica 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.
2. Materials and methods

The gastrointestinal tract samples analyzed in this study, were obtained from nine spiny rats (P. roberti) collected in Tapirapé-Aquiri National Forest 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 Trichotравассосия 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. Synlophoe modified into cuticular scales arranged in a longitudinal series, except on the left flank, without chitinid struts. Cephalic vesicle reduced followed by evident cuticular dilation, esophageal tooth absent. The males exhibit subequal, long, single-tip spicules, a developed cephalic 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). Claviworm 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; ovjector developed; tail short and conical (Fig. 2f); eggs, not observed.

Synlophoe (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 chitinid 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 spike 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. Subepical 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) × 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) × 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-Aquiri National Forrest, Carajás Reserve, State of Pará, Brazil (5°3′5′′ 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: Trichotравассосия travassosi Lent & Freitas, 1938 CHIOC 9766th-i deposited at Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC).

Etymology: The species name references the biome where the host was collected.
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 synlophe (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 sagital 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 Brevistriatinia 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 sagital 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 sagital 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 synapomorphy 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 Trichotavassosia, 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.
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 Heligmolidae 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.
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References


