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Rhabdias paraensis sp. nov.: a parasite of the lungs of *Rhinella marina* (Amphibia: Bufonidae) from Brazilian Amazonia

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The nematode parasites of Rhinella marina *include species of the genus* Rhabdias *(Rhabdiasidae: Rhabditoidea). The present study describes* Rhabdias paraensis *sp. nov., which parasitizes the lungs of* R. marina *in Brazilian Amazonia. Of the more than 70 known species of this genus, 18 are parasites of bufonids, of which, eight are Neotropical. The new species described here is similar to* Rhabdias alabialis *in the absence of lips is different by the presence of conspicuous cephalic papillae. We describe details of the four rows of pores, which are distributed equally along the whole of the length of the body and connected with hypodermal cells, using histology and scanning electron microscopy. Other histological aspects of the internal structure of this nematode are also described.*

Key words: *Rhinella marina* - Bufonidae - *Rhabdias paraensis* sp. nov. - Rhabdiasidae

The nematodes of the genus *Rhabdias* Stiles et Hassall, 1905 are parasites of the lungs of amphibians and reptiles in both tropical and temperate regions. The females are parasites and reproduce predominantly by hermaphroditism and rarely by parthenogenesis (Anderson 2000).

More than 70 species of this genus have been described in Lissanphibia and Squamata. Of these, 18 have been found in bufonid anurans (Bursey et al. 2003, Martinez-Salazar 2006, Junker et al. 2010). Eight species of these have a Neotropical distribution: *Rhabdias fulleborni* Travassos, 1926, *Rhabdias androgyna* Kloss, 1971, *Rhabdias elegans* Kloss, 1971, *Rhabdias hermaphrodita* Kloss, 1971, *Rhabdias americanus* Baker, 1978, *Rhabdias alabialis* Kuzmin, Tkach et Brooks 2007, *Rhabdias kuzmini* Martínez-Calazar et Léon-Règagnon, 2007 and *Rhabdias pseudosphaerocephala* Kuzmin, Tkach et Brooks 2007 (Bursey et al. 2003, Martinez-Salazar & León-Règagnon 2007).

At least 11 *Rhabdias* species appear to be specific to the Bufonidae family (Kuzmin et al. 2007). Four species (*R. americanus*, *R. fulleborni*, *R. alabialis* and *R. pseudosphaerocephala*) have been found parasitizing the lungs of *Rhinella marina* L. 1758 (Kloss 1974, Kuzmin et al. 2003, 2007, Espinoza-Jiménez et al. 2007). *R. marina* is a bufonid toad found throughout much of South and Central America and widely-distributed in Brazil (Kewtt et al. 2006).

Kuzmin et al. (2007) concluded that the reports of *Rhabdias sphaerocephala* being found in *R. marina* from two localities in Nicaragua and three sites in Costa Rica (Kloss 1971, Kloss 1974) actually corresponded to the new species *R. pseudosphaerocephala*. This interpretation was supported by both morphological and molecular analyses, which indicated that the distribution of this species is restricted to the Nearctic region.

Kloss (1971) recorded *R. sphaerocephala*, *R. hermaphrodita*, *R. fuelleborni* and *R. androgyna* parasitizing the lungs of *R. marina* in Brazil. The present paper describes the morphological and ultrastructural aspects of *Rhabdias* found in Brazilian amphibians from the Amazon Region using light microscopy and scanning electron microscopy (SEM), which resulted in the diagnosis of a new species.

MATERIALS AND METHODS

Twenty specimens of *R. marina* were collected from the urban area of Belém, in the Brazilian state of Pará (PA), between August 2007-October 2008. The animals were anesthetized with sodium tiopenthal, euthanized by exsanguination and then examined for helminths. Nematodes encountered in the lungs were fixed in AFA (2% glacial acetic acid, 3% formaldehyde and 95% ethanol 70ºGL), dehydrated in ethanol and then clarified in Aman lactophenol for examination under a light microscope. For SEM, specimens were postfixed in 1% OsO_4 and 0.8% $K_2Fe(CN)_6$ and analyzed in JEOL JSM 5310. For the histological analysis, parasites were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodilate at pH 7.4, dehydrated in ethanol and then embedded in hydroxyethyl methacrylate resin (Leica Historesin Embedding Kit), sectioned at 3 µm intervals and stained with 1% toluidine blue. Illustrations were made using an Olympus BX41 microscope coupled to a *camera lucida*. Photomicrographs were obtained using a Sony Cybershot dsc-5500 camera. Measurements are given in micrometers unless otherwise indicated, with the standard parameter being the mean ± standard deviation, with the range of values followed by holotype measures given in parentheses.

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Ethics - The present study was approved by the Animal Research Ethics Committee of the Federal University of Pará (UFPA), through authorization CEPAE-UFPA (BIO010-10).

RESULTS

Rhabdias paraensis sp. nov.
(Fig. 1A-F)

Description (based on 10 adult specimens) - Total length 7.09 ± 0.66 mm (5.92-8.19 mm, 7.19 mm), body width in the vulvar region 422.88 ± 54.53 (346.15-538.46, 423.08), body width in esophageal dilatation 174.35 ± 9.65 (153.90-180.52, 174.68), buccal capsule 8.90 ± 3.71 (3.90-16.88, 8.44) × 20.65 ± 4.00 (14.93-27.92, 20.78) (Fig. 1A, C), labia or pseudolabia absent (Fig. 1D), cuticular swelling with irregular folds starting in the anterior portion of the body, esophagus claviform 421.88 ± 11.97 (394.80-439.61, 415.58) × 82.79 ± 5.34 (74.67-90.91, 78.57) (Fig. 1A) with triangular lumen (Fig. 1E), nerve ring situated at 185.88 ± 21.50 (151.30-234.42, 151.30) from anterior end (Fig. 1A), excretory pore not observed, presence of hypodermal gland cells connected with channels that end in a circular opening in the external surface of cuticular swelling (Fig. 1A), anterior portion of intestine wide and thick-walled, intestinal lumen starts after the esophagus and filled with a dense black material (Fig. 1A), rectum short, vulva post-equatorial with prominent transversal lips situated 4.12 ± 0.67 mm (3.26-5.20 mm, 3.40 mm) from anterior end, vagina 117.07 ± 44.67 (50.67-213.33, 117.33) in length (Fig. 1F), tail conical, 292 ± 22.47 (266.67-346.67, 293.33) long, ending in a thin tip (Fig. 4A, C), cuticular swelling culminating close to tail tip (Fig.

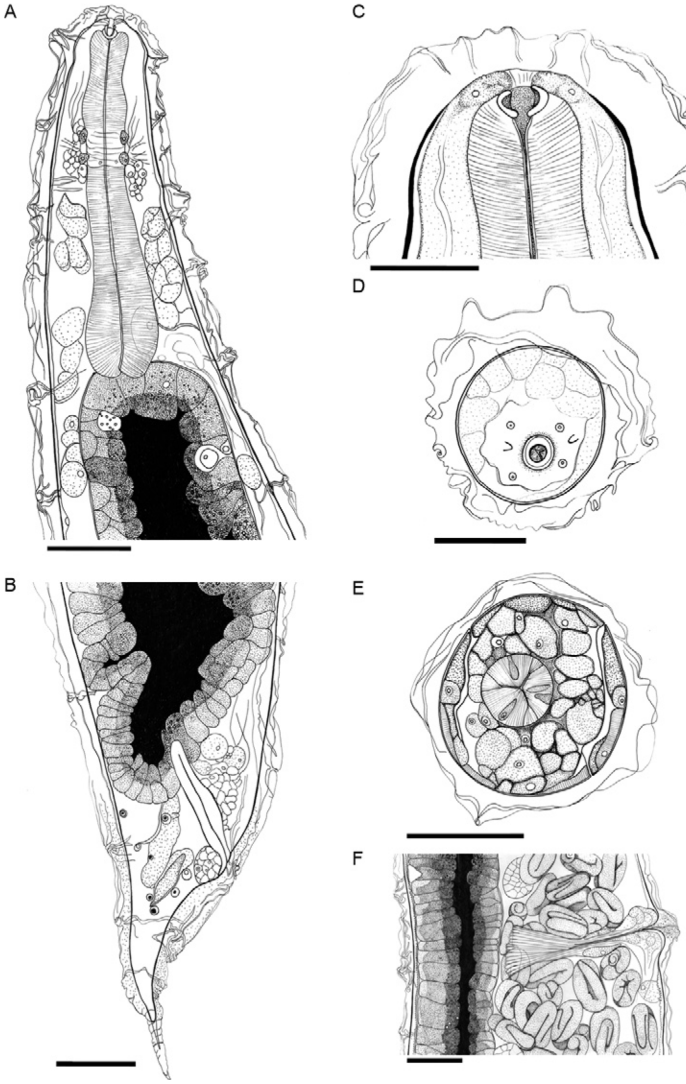

Fig. 1: light microscopy of *Rhabdias paraensis* sp. nov. A: anterior portion showing claviform esophagus, nerve ring, cuticular swelling and insertion points traversing the cuticle. Bar = 100 µm; B: lateral view of conical shape of the tail, anus, rectal ampulla. Bar = 100 µm; C: detail of anterior extremity showing a small, cup-shaped buccal capsule. Bar = 100 µm; D: frontal view of anterior extremity showing the oral opening, two pairs of cephalic papillae and the pair of lateral papillae. Bar = 50 µm; E: transversal section of anterior portion, showing the triangular lumen of esophagus and three pores, seen. Bar = 100 µm; F: lateral view of the vulva, vagina, eggs and larvae at different stages of development. Bar = 100 µm.

1B), uteri long, amphidelphic, thin-walled, filled with eggs and larvae, eggs 41.10 ± 3.49 (36.23-43.44, 43.44) × 25.61 ± 2.48 (21.62-28.38, 25.26). Parasite of the lungs of *R. marina* more numerous in the right lung (mean = 18) than in left lung (mean = 12).

Type host - *R. marina* (Linneaus, 1758) (Syn. *Bufo marinus*) (Amphibia: Bufonidae), Cane Toad.

Site of infection - Lungs.

Type locality - Belém, PA, Brazil (01º28'03"S 48º20'18"W).

Type-data and depository - Holotype, hermaphrodite female. Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Fundação Oswaldo Cruz, in Rio de Janeiro, Brazil [CHIOC 35705a (holotype) and CHIOC 35705b (10 paratypes)].

Host-parasite data - Prevalence: 45%; intensity of infection: 27 (3-143).

Etymology - The specific name refers to PA, in which the new species was discovered. In Brazilian Portuguese, *paraense* is a native of PA.

SEM - SEM permitted the observation of the following superficial characteristics of *R. paraensis* sp. nov.

The cylindrical body of the parasitic phase of this *Rhabdias* (Fig. 2A) is coated with a voluminous cuticular swelling which begins close to the oral opening and continues along the body until a discreet connection with the tapered extremity of the tail. This gives the surface of the helminth an irregular appearance (Fig. 2B).

A simple, circular oral opening is found at the anterior extremity. Its lipless edges are bordered with tiny cuticular elevations, which radiate outwards from the opening (Fig. 2C, D). Three pairs of head papillae are located around the oral opening. Two of these pairs are large, rounded and submedian, while the third pair are small, lateral papillae (Fig. 2C, D). Both sets of submedian papillae are rounded with a wide base and a small central, spherical structure. The lateral papillae have a flattened conical shape, with a wide base, which tapers progressively towards the extremity (Fig. 2C, D). Amphids not observed.

The external surface of the cuticular swelling presents four longitudinal lines of pores: two ventral-lateral and two dorsal-lateral rows equally-spaced, which ex-

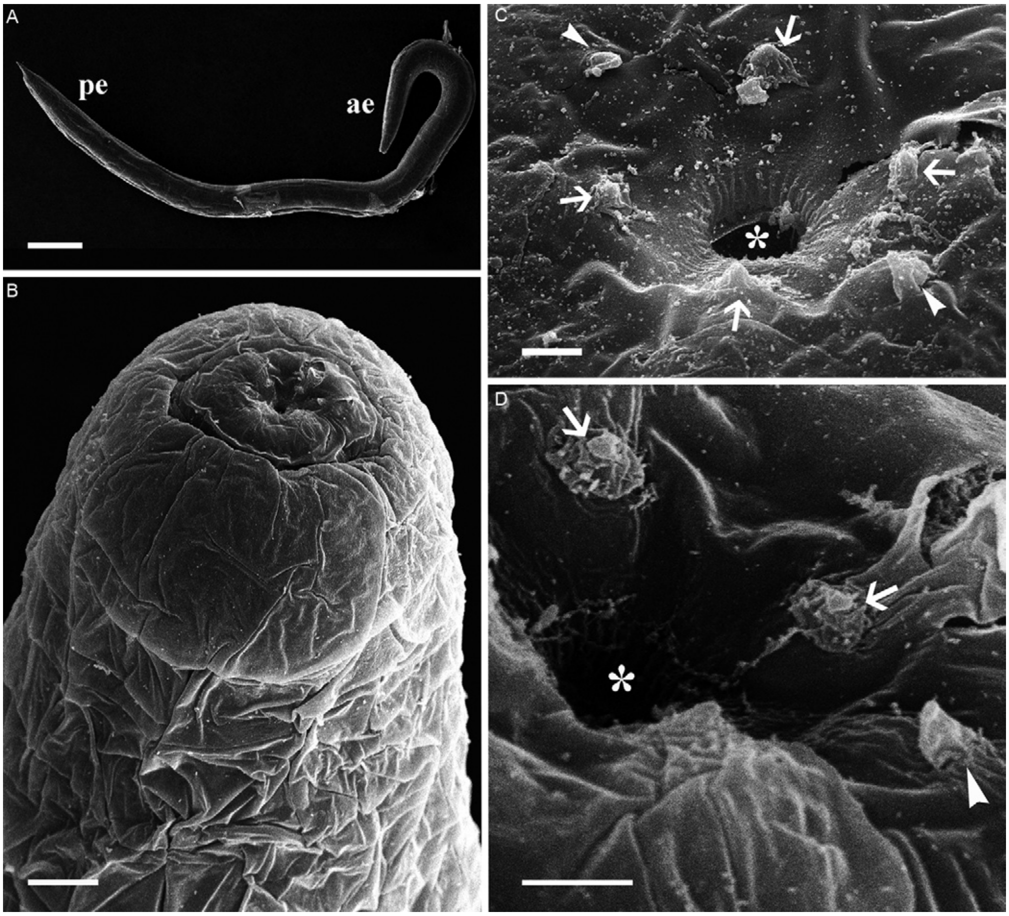

Fig. 2: scanning electron microscopy of *Rhabdias paraensis* sp. nov. A: general view of the body, showing the anterior (ae) and posterior (pe) end. Bar = 500 µm; B: detail of ae and oral aperture without lips. Bar = 5 µm; C: frontal view of the anterior extremity, showing the lateral papillae (arrowhead) and the pairs of cephalic papillae (arrows). Bar = 2 µm. Asterisks mean furrows around the oral opening. Bar = 2 µm; D: detail of two cephalic papillae (arrows), showing the insertion in the cuticular swelling, which is similar to that of the lateral papillae (arrowhead). Bar = 2 µm.

tend along the whole length of the body (Fig. 3A, B). These cuticular pores are coated by the invagination of the cuticular swelling, which is projected inwards in the form of a duct (Fig. 3A, B). These conduits are clearly visible when the internal surface of the cuticular swelling is analyzed by SEM (Fig. 3C, D).

The delicate, threadlike condition of the cuticular swelling along the entire length of the parasite makes this structure vulnerable to fragmentation, which reveals the space between this structure and the cuticular surface (Fig. 4A, B). The material filling this space varies from amorphic to fibrous or filamentous (Fig. 4C, D). In the areas where the cuticular surface is visible, fine and discreet transverse cuticular furrows can be observed (Fig. 4E).

The outermost portion of the vulvar opening presents marked dilation of the cuticular swelling (Fig. 5A), while the anal opening is characterized by a half-moon shaped depression on the body surface (Fig. 5B). The tail tapers abruptly into a "needle-point" tip, with the cuticular swelling, which fades out halfway along this tapering point (Fig. 5C).

Histology - The histological study was based on the analysis of longitudinal sections cut at 3 µm intervals in five specimens of *R. paraensis* sp. nov. Seven slides were prepared per worm, with 20 sections on each slide.

In the esophageal region, the analysis revealed a simple, thick-walled, lightly basophilic and cylindrical epithelium (Fig. 6A). The interstitial region is more intensely basophilic than the esophageal lining, while the amorphic material filling the space within the cuticular swelling presents a subtle metachromatic staining (Fig. 6A, B). The buccal capsule presents a strong affinity with the staining, which revealed that this structure forms a ring around the esophageal opening (Fig. 6A).

The esophagus terminates in the intestinal epithelium, in a structure similar to a muscular valve composed of less chromaffin cells than those of the intestine (Fig. 6A). The intestinal epithelium is formed by a single layer of cells, 20-30 µm thick. These cells are cylindrical, tall and voluminous, with widespread microvilli which are highlighted by an intense metachromatic reaction (Fig. 6B). The nuclei of these cells are voluminous and spherical, with a predominance of euchromatin and a prominent central nucleolus (Fig. 6B). The whole of the cytoplasm is filled with different-sized granules, the largest of which are dense and fringed and are found in the middle and lower portions of the cells, while the apical granules are smaller and more acidophilic (Fig. 6B). The fibrous rectal ampulla has an insertion in the intestinal cells, which has the appearance of a valve (Fig. 6C).

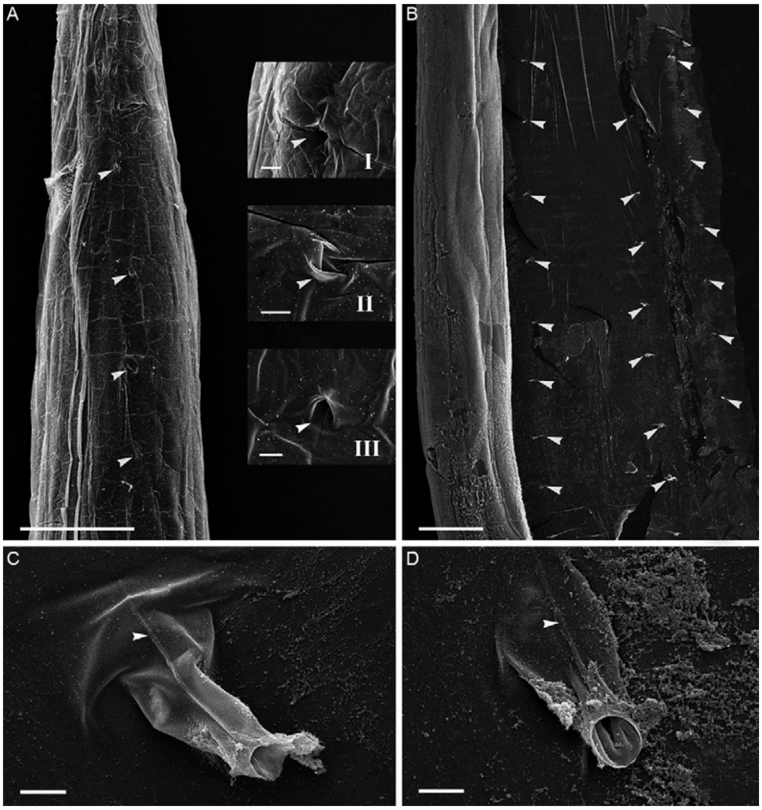

Fig. 3: scanning electron microscopy of *Rhabdias paraensis* sp. nov. A: anterior end of the body showing external surface of cuticular swelling and insertion points (arrowhead). Bar = 100 µm; inserts I, II and III: details of three insertion points. Bar = 10 µm, 5 µm and 5 µm, respectively; B: internal surface of the cuticular swelling where three rows of numerous, regularly-spaced invagination points can be observed (arrowheads). Bar = 200 µm; C, D: details of two points of invagination of cuticular swelling observed from its internal surface. Note the canaliculus, well-defined internal extremity, and a longitudinal line (arrowheads). Bar = 5 µm.

The tubular ovary is strongly basophilic, with gametic cells located all along its periphery (Fig. 6D, F). Sequentially, embryonated eggs can be observed, some of larvated, and larvae are concentrated close to the vaginal canal, vulvar aperture with small labial projections (Fig. 6E, F).

The numerous and irregularly-shaped hypodermal cells (> 50 µm), which lack basophily, are discreetly acidophilic and have cytoplasm filled with small, uniform and achromatic granules (Fig. 7A-D). These cells have a large, spherical nucleus of approximately 10 µm, which are lightly basophilic, and have a prominent central nucleolus (Fig. 7C, D). These cells also present an irregular area around the nucleus, which is more intensely basophilic than the nucleus itself. All these cells communicate with the host environment through a basophilic duct, which begins at the edge of the lateral hypodermal cord, traverses the cuticle and finishes in a pore with an irregular opening in the external surface of the cuticular swelling (Fig. 7E, F).

DISCUSSION

More than 70 species of *Rhabdias* have been described in the Lissamphibia and Squamata, including 18 that parasitize bufonids. Eight of these are from the Neotropical regions and can be compared directly with *R. paraensis* sp. nov. The new species can be easy distinguishable from its congeners by the differences of cephalic papillae and some morphological measurement.

The post-equatorial position of the vulva in *R. paraensis* sp. nov. differs from that in *R. alabialis*, *R. kuzmini*, *R. pseudosphaerocephala*, *R. americanus* and *R. fuelleborni*. However, this structure is similar to that found in *R. androgyna*, *R. elegans* and *R. hermaphrodita*, although these species differ from new species in a number of different characteristics, such as the length of the esophagus and body and the presence of lips and cephalic papillae.

Few of the studies of Neotropical species have provided data on the dimensions of the buccal capsule, although in *R. kuzmini*, it measures 31-70 × 35-47 µm, while it is 10 × 12-15 µm in *R. americanus*, 7-12 × 15-17 µm in *R. pseudosphaerocephala* and 10 × 10-15 µm in *R. alabialis*. These values, and the shape of the structure, are all distinct from those of *R. paraensis* sp. nov. (8.90 × 20.65 µm).

While most *Rhabdias* species have labia or pseudolabia, some species have no labia. Six of these have a post-equatorial vulva and are parasites of amphibians, like *R. paraensis* sp. nov. However, these species are distinct in terms of bodily dimensions, host species and geographic distribution, being found in the Australian (*Rhabdias australiensis* Moravec et Sey, 1990), Oriental [*Rhabdias shortii* (Singh et Ratnamala 1977), Baker 1980] and Palearctic biogeographic regions (*Rhabdias dossei* Hartwich, 1972, *Rhabdias niponica* Yamaguti, 1943, *Rhabdias rhacophori* Yamaguti, 1941, and *Rhabdias tokyoensis* Wilkie, 1930).

The other nine species have equatorial or pre-equatorial vulvae and are parasites of a variety of host species, including the order Squamata. This group includes species from the Ethiopian (*Rhabdias collaris* Baker, 1987, *Rhabdias gemellipara* Chabaud, Brygoo et Petter, 1961, *Rhabdias madagascariensis* Chabaud, Brygoo et Petter, 1961), Oriental (*Rhabdias escheri* Baer, 1930), and Palearctic regions [*Rhabdias bufonis* (Schrank, 1788) Stiles et Hassall, 1905, *Rhabdias globocephala* Kung et Wu, 1945, *Rhabdias horiguti* Yamaguti, 1943, *Rhabdias incerta* Wilkie, 1930, *Rhabdias martinoi* Kurochkin et Guskov, 1963]. The remaining species (*R. alabialis*) is the only one of the 16 taxa without labia that is found in the Neotropical regions, in according with Martinez-Salazar and León-Règagnon (2007).

Of the Neotropical parasites of *R. marina*, the absence of labia makes *R. paraensis* sp. nov. similar to *R. alabialis* and distinct from *R. kuzmini*, *R. americanus* and *R. pseudosphaerocephala*, which present four submedian labia and two lateral pseudolabia, and *R. fuelleborni*, which has six labia. *R. alabialis* and *R. paraensis* sp. nov. differ in the presence of conspicuous papillae on the head of the new species, which were not cited by Kuzmin et al. (2007) in their description of *R. alabialis*. Similar to *R. paraensis* sp. nov., a number of different

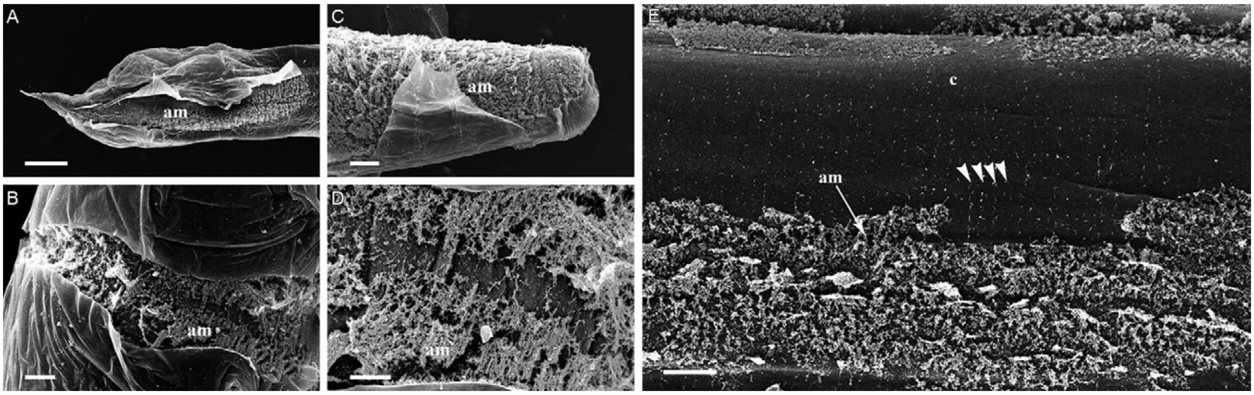

Fig. 4: scanning electron microscopy of *Rhabdias paraensis* sp. nov. A, B: details of posterior and anterior end showing the amorphic material (am) between the external surface of cuticle (c) and the cuticular swelling. Bar = 100 µm and 20 µm, respectively; C: detail of the mid-portion of the body with a rupture in cuticular swelling, exposing the amorphous material (am). Bar = 20 µm; D: detail of fibrous material attached to c. Bar = 10 µm; E: surface of the helminth, showing transversal furrows (arrowheads) in c and am. Bar = 20 µm.

studies have referred to papillae surrounding the oral opening of the species *Rhabdias* in both anuran (Baker 1987, Moravec & Kaiser 1995, Tkach et al. 2006, Kuzmin et al. 2007, Martinez-Salazar 2008, Junker et al. 2010) and squamate hosts (Martinez-Salazar 2006, Lhermitte-Vallarino et al. 2009, 2010, Moravec 2010).

R. paraensis sp. nov. also differs from *R. alabialis* in a number of morphometric parameters, such as the shorter esophagus (340-445 µm) and reduced distance of the nerve ring in relation to the anterior extremity of the body (146.2 µm) in *R. alabialis*, while in *R. paraensis* sp. nov., the vulva is located closer to the posterior extremity of the helminth. The sum of this evidence indicates clearly that *R. paraensis* sp. nov. is unlike any other known species of the genus.

Kuzmin et al. (2007) compared species of *Rhabdias* (*R. pseudosphaerocephala*, *R. sphaerocephala* and *R. fuelleborni)* using relationship of the relative length of esophagus (percentage of total length) to the total length. In this work these parameters aid in differentiation of species studied. However, those morphometric values in *R. paraensis* n. sp compared with these other three species showed similarities with *R. pseudosphaerocephala,* but the latter species differs by the presence of four labia and two lateral pseudolabia.

R. paraensis sp. nov. is thus a distinct species, which can be differentiated from the others of the genus due to its unique set of morphometric parameters, such as the difference in the location of the vulva, anal opening and nerve ring and the length of the body and the esophagus. In particular, it can be distinguished from the most closely-related species, *R. alabialis*, based on its set of conspicuous head papillae.

The external morphology of some *Rhabdias* species has been analyzed using SEM, including *R. kuzmini* (Martinez-Salazar & León-Règagnon 2007), *R. alabialis*, *R. pseudosphaerocephala*, *R. sphaerocephala* (Kuzmin et al. 2007), *Rhabdias joaquinensis*, *Rhabdias eustreptos* (Kuzmin et al. 2003), *Rhabdias bakeri*, *Rhabdias ranae* (Tkach et al. 2006), *Rhabdias leonae* (Martinez-Salazar 2006), and *Rhabdias manantlanensis* (Martinez-Salazar 2008). In *R. paraensis* sp. nov. there is a conspicuous presence of a cuticular swelling linked to the external surface through caniculi, which traverse the cuticle and are arranged in two ventral-lateral and two dorsal-lateral rows of regularly-spaced pores. These insertion points, which are linked to hypodermal cells, when observed in the histological sections, constitute a pathway of communication between the parasite and the internal environment of the lungs of the host.

Kloss (1974) observed the presence of a number of cells with large nuclei arranged sequentially in the anterior portion of *R. hermaphodita* and *R. fuelleborni*, although these cells lead into a single excretory canal, which reaches the exterior of the organism through an excretory pore in the cuticle. The author interpreted those cells as belonging to excretory system. In *R. paraensis* sp. nov., by contrast, the analysis of 202 sequential histological sections and clarified specimens found no evidence of an excretory pore, nor of an excretory gland, other than the dozens of pores associated with the hypodermal cells.

Junker et al. (2010) observed no excretory pore in their description of the tropical African species *Rhabdias picardiae* and *Rhabdias ohlerae*, although in *R. picardiae*, lateral cells impeded observation of the pore, while in *R. ohlerae*, a long excretory gland was identified. These authors also analyzed *Rhabdias vencesi* and reported that the excretory pore was difficult to observe in the fixed specimens.

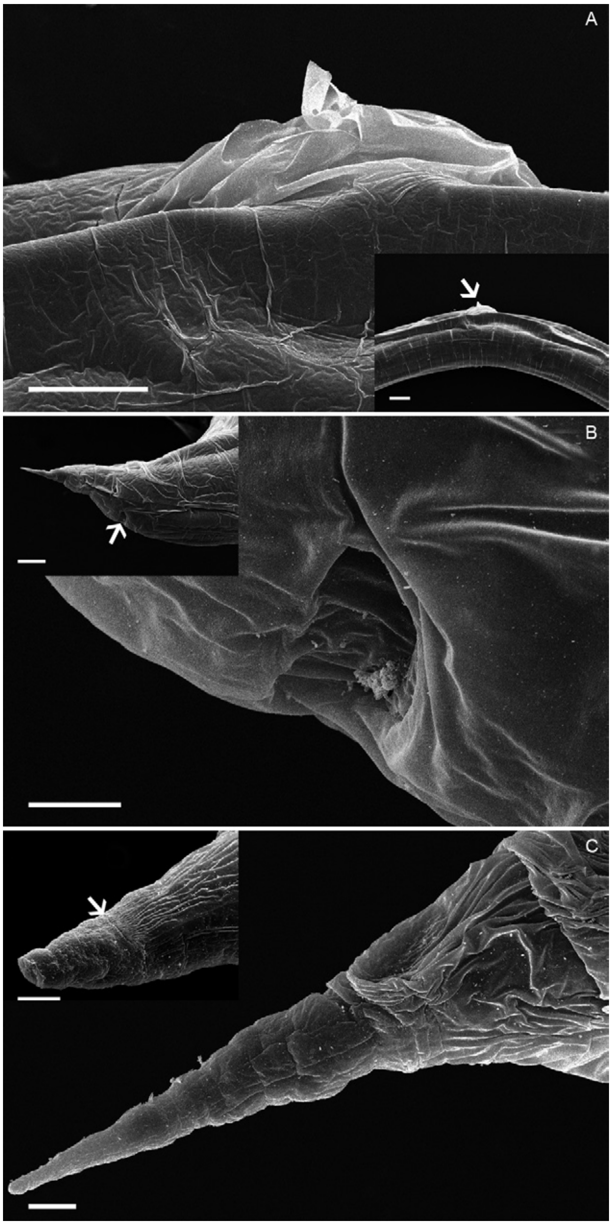

Fig. 5: scanning electron microscopy of *Rhabdias paraensis* sp. nov. A: mid-portion of body, showing the vulvar opening (arrow). Bar = 100 µm; insert: lateral view of the vulvar opening. The expanded cuticular swelling can be observed. Bar = 50 µm; B: detail of the anal opening (arrow in insert) covered with the cuticular swelling. Bar = 50 µm and 10 µm, respectively; C: view of the sharp point of the tail. Bar = 20 µm; insert: note cuticular swelling terminates before the point, where it is inserted into a well-defined transversal crease (arrow). Bar = 10 µm.

In *R. collaris*, Baker (1987) observed a cuticle inflated with ducts leading to hypodermal glands all along the body. In *R. picardiae*, Junker et al. (2010) recorded the cuticle vesicle is attached laterally to the inner layer of the cuticle via conspicuous fibers associated with subcuticular pores. Using histology and SEM, we observed similar features in *R. paraensis* sp. nov., although the structures and the connections of the hypodermal glandular cells with the outer surface were shown in greater detail.

In the superfamily Rhabditoidea, each lateral and sublateral line of the hypodermal cord is formed by 12-14 hypodermal cells (Chitwood & Chitwood 1974). In *R.*

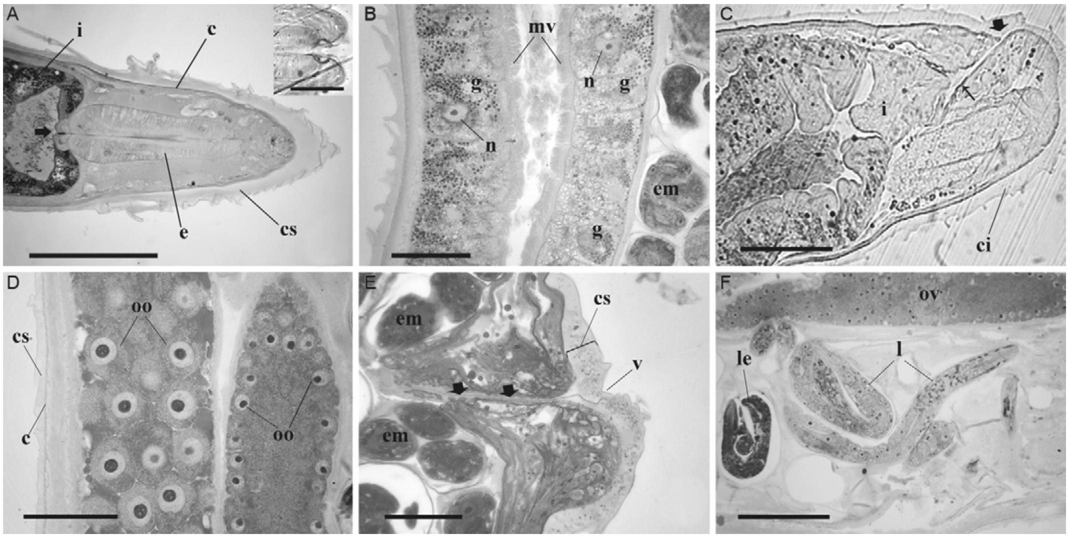

Fig. 6: histology of *Rhabdias paraensis* sp. nov. stained with 1% toluidine blue. A: anterior end showing basophilic cuticular swelling (cs), cuticle outlining the body (c), basophilic esophagus (e), valve (large arrow) and intestine (i). Basophilic buccal capsule (insert). Bar = 200 µm, insert: 30 µm; B: body mid-portion showing the cylindrical intestinal epithelium with nuclei (n) and nucleoli, cytoplasmatic granules (g) and microvilli (mv). Bar = 50 µm; C: anus (large arrow), cs and the transition i - rectal ampulla (arrow). Bar = 50 µm; D: details of segments of the ovary with oogonia (oo). Bar = 50 µm; E: vulva (v) and vagina (large arrows). The labial projections covered by cs. Bar = 50 µm; F: detail of larvated eggs (le) and larvae (l). Bar = 100 µm. em: embryonated eggs.

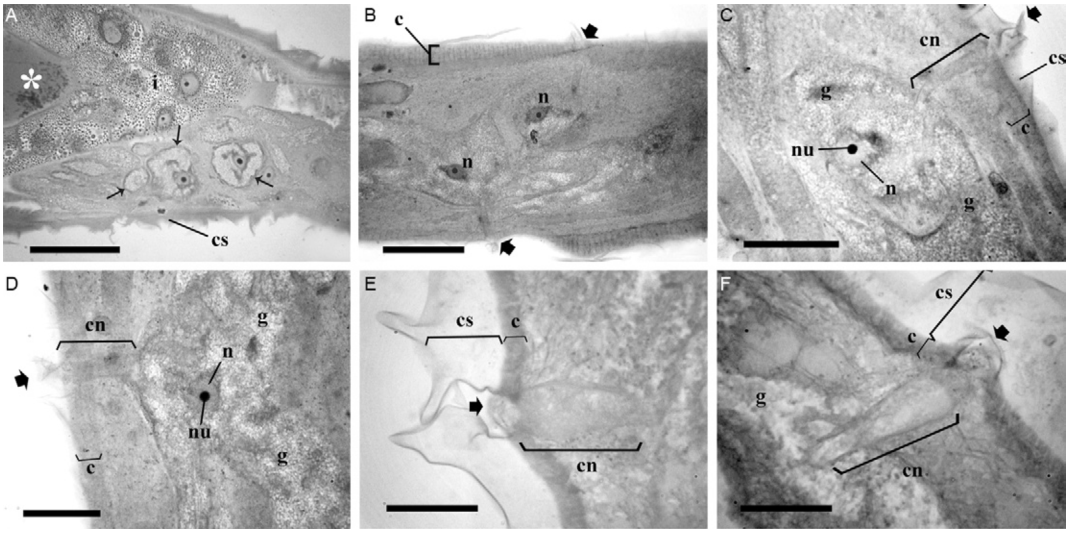

Fig. 7: histology of *Rhabdias paraensis* sp. nov., stained with 1% toluidine blue. Hypodermal cells, canaliculi and the pores. A: mid-portion of the body. Note a voluminous, chromophobic cells between intestine (i) and cuticle (c) with grainy cytoplasm, nucleus and nucleolus (arrows). Asterisk means intestinal lumen. Bar = 100 µm; B: tangential section at the posterior end show chromophobic cells with large nuclei (n), canaliculi (c) and pores (large arrows) in cuticular swelling (cs). Bar = 100 µm; C, D: detail of chromophobic cell, grainy cytoplasm (g), n and nucleoli (nu). Canaliculus (cn) traverses c. Bar = 50 µm; E, F: detail of two cn with pore opening (large arrow) through c in E and the folding of cs at the pore opening; F: note cn enveloped in cytoplasm rich of chromophobic granules (g). Bar = 20 µm.

paraensis sp. nov., we observed (SEM) four lines of 14-16 sublateral pores connected to hypodermal cells. We believe that these pores represent the distribution of the cells of the hypodermal cord.

The ultrastructural characteristics of these specialized cells of the hypodermis were described by Chitwood and Chitwood (1974). These cells contain numerous small granules and present a strong affinity with alkaline stains. By contrast, *R. paraensis* sp. nov. is clearly chromophobic.

In *R. paraensis* sp. nov., in addition, the space between the cuticular swelling and the external surface of the cuticles is filled with an amorphous and basophilic material not found in any other *Rhabdias* species described to date. Chitwood and Chitwood (1974) point out that this swelling is caused by the separation of the cortical layer of the cuticle from the remaining layers, which is normally caused by the liquefying of the matrix. While the cuticular swelling described by these authors is a specialization of the external layer of the cuticle, it is important to note that we observed delicate transverse striae at uniform intervals on the surface of the helminth - below this swelling - a common feature in many forms of parasitic invertebrates.

The analysis of serial histological sections of samples embedded in methacrylate resin may provide an important complementary approach and essentials comparative parameters for future studies of both microanatomical details and the parasite-host interaction analysis of *Rhabdias* specimens. This technique, associated with SEM, has provided new insights into the morphology of the genus and contributed to the development of new taxonomic parameters for the characterization of the principal internal structures, which are essential for the development of comparative studies and the understanding of host-parasite interactions. This study increases our knowledge of the diversity of the helminths that parasitize *R. marina* in the Amazon Region, expanding on the work of Espinoza-Jiménez et al. (2007), Espinola-Novelo & Guillén-Hernandez (2008) and Santos et al. (2008).

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