A new species of *Serpentirhabdias* Tkach, Kuzmin & Snyder, 2014 (Nematoda: Rhabdiasidae) parasitic in the brown ground snake *Atractus major* Boulenger (Reptilia: Serpentes: Dipsadidae) in Brazil

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Abstract Serpentirhabdias atracti n. sp. is described based on specimens discovered in the lung of Atractus major Boulenger from Caxiuanã National Forest, Pará, Brazil. The new species is assigned to Serpentirhabdias Tkach, Kuzmin & Snyder, 2014 based on morphological characters (comparatively thin body cuticle without prominent inflations, arrangement of circumoral papillae in two lateral groups, pre-equatorial position of vulva, eggs in uteri at early cleavage stages), as well as because of its parasitism in snakes. The new species is most similar to S. vellardi (Pereira, 1928) due to the absence of lips and buccal capsule, similar body dimensions, and the specificity to dipsadid snakes in Brazil. The two species differ in the shape of the tail (bulbous dilatation in the posterior part followed by a threadlike tail tip present in S. atracti n. sp.), the width of the oesophagus, and the size of the excretory glands. Serpentirhabdias atracti n. sp. is the sixth species of this genus found in the Neotropical Region.

Introduction

The colubrid snake genus *Atractus* Wagler (Colubroidea: Dipsadidae) comprises approximately 130 valid species that are widely distributed throughout the Neotropical Region (Myers, 2003). The brown ground snake *Atractus major* Boulenger is distributed in the Brazilian Amazon, on the Amazonian slopes of the Andes in Ecuador and Colombia and in Venezuela. This species of snake is fossorial (occasionally cryptozoic and terrestrial), has both nocturnal and diurnal habits, and feeds mainly on earthworms and occasionally on acari as well as small insects (Martins & Oliveira, 1999).

While studying parasites of amphibians and reptiles from the Caxiuanã National Forest, Pará, Brazil, we discovered lung nematodes in one specimen of *A. major*. The nematodes were identified as belonging to the family Rhabdiasidae Railliet, 1915, based on the morphology of the six specimens found.

The nematode family Rhabdiasidae is distributed worldwide and includes about 100 species parasitic in amphibians or reptiles. Fourteen species of this family were recently transferred from the genus *Rhabdias* Stiles & Hassall, 1905 to the genus *Serpentirhabdias* Tkach, Kuzmin & Snyder, 2014 on the basis of their particular morphological and biological characters, i.e. comparatively thin body cuticle, arrangement of lips in two lateral groups, smaller number of eggs, presence of homogony in the life-cycles, and host specificity (restricted to Serpentes), as well as

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molecular evidence (Tkach et al., 2014). Five species assigned to *Serpentirhabdias* are known to inhabit the Neotropical Region: *Serpentirhabdias* cf. *fuscovenosa* (Railliet, 1899), *S. labiata* (Pereira, 1927), *S. vellardi* (Pereira, 1928), *S. lamothei* (Martínez-Salazar & León-Règagnon, 2006) and *S. filicaudalis* (Barrella, Santos & Silva, 2009); all these are parasitic in colubroidean snakes (Martínez-Salazar & León-Règagnon, 2006; Barrella et al., 2009).

Although the nematodes found in the lung of *A*. *major* in the present study corresponded to the diagnosis of *Serpentirhabdias*, they differed from all known species of the genus by one or more particular morphological characters. Therefore, we describe these nematodes as belonging to a new species of the genus *Serpentirhabdias*.

Materials and methods

During a helminthological survey in March 2014 (under license permission SISBIO 30772-4), one specimen of A. major was collected at Caxiuanã National Forest (1°42'S, 51°31'W) in the Melgaço Municipality of Pará State, Brazil. The snake was euthanised by injection of 2% lidocaine hydrochloride, and its internal organs were examined under a dissection stereo-microscope. The nematodes found in the lung of the snake were washed in saline, fixed in hot 70% ethanol, cleared in lactophenol and examined under an Olympus BX41 microscope (Olympus, Tokyo, Japan) equipped with a drawing tube; drawings were created for subsequent morphometric analysis. Apical section was made manually from one specimen and observed en face. All measurements are reported in micrometres unless otherwise indicated and are presented as those for the holotype followed by the range for the type-series in parentheses.

Order Rhabditida Chitwood, 1933 Family Rhabdiasidae Railliet, 1915

Serpentirhabdias atracti n. sp.

Type-host: Atractus major Boulenger (Reptilia: Squamata: Serpentes: Dipsadidae).

Type-locality: Caxiuanã National Forest, Pará, Brazil (1°42'S, 51°31'W).

Site in host: Lung.

Prevalence and intensity of infection: Six specimens found in a single host examined.

Type-material: Holotype MPEG 000027, paratypes MPEG 000028, deposited in the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará State, Brazil.

Etymology: The new species is named after the generic name of the type-host.

Description (Fig. 1)

[Based on the holotype and 5 paratypes; gravid females; Fig. 1A-H.] Medium-sized species of the genus. Anterior end rounded; posterior end tapering, sharply-pointed (Fig. 1E, G). Body cuticle thin, slightly thicker in anterior and posterior regions. Body length 3.3 (3.18-3.33) mm, body width at level of vulva 132 (106-132), width at oesophageal-intestinal junction 75 (64–75). Oral opening in shape of triangle with rounded corners in apical view (Fig. 1B). Lips indistinct. Six prominent circumoral papillae arranged in 2 lateral groups. Submedian papillae somewhat larger than lateral papillae (Fig. 1B). Buccal capsule absent. Buccal cavity formed by short vestibulum and funnel-shaped entrance to oesophageal lumen (Fig. 1C, D).

Oesophagus club-shaped, without dilatation in muscular part; posterior bulb elongated (Fig. 1E). Length of oesophagus 245 (213-249), representing 7.4 (6.4-7.7)% of body length. Width of oesophagus 26 (22-27) in anterior part; width of bulb 40 (32-42). Oesophageal lumen somewhat wavy in anterior and middle parts. Nerve-ring surrounding oesophagus at approximately its mid-length, 113 (109-117) from anterior end of body. Excretory pore situated immediately posterior to level of nerve-ring. Excretory glands subventral, prominent, similar in size, shorter than oesophagus (126 and 136 long in holotype); posterior ends of excretory glands situated posterior to and close to oesophageal-intestinal junction (Fig. 1E). Apex of intestine of similar width as oesophageal bulb, but widening rapidly. Intestine thick-walled. Anterior sphincter of intestine short, formed by flattened cells. Posterior sphincter prominently muscular, elongated (Fig. 1G). Contents of intestine black, especially in middle region; intestinal cells brownish in anterior and posterior parts, black in middle part. Rectum thickwalled, elongated, and cuticularised (Fig. 1G).



Fig. 1 Line drawings of *Serpentirhabdias atracti* n. sp. Holotype (A, C, D, F–G); paratype (B, E). A, Overall view of body; B, Anterior end, apical view; C, Anterior end, lateral view; D, Anterior end, ventral view; E, Anterior part of body, lateral view; F, Region of midbody showing vulva and apex of posterior ovary; G, Posterior part of body, lateral view; H, Posterior half of tail, lateral view. *Scalebars*: A, 500 µm; B, 10 µm; C, D, 20 µm; E–G, 100 µm; H, 25 µm

Genital system typical of the family Rhabdiasidae. Vulva pre-equatorial and small; vulval lips not salient. Vulval aperture and short transverse vagina cuticularised (Fig. 1F). Distance from anterior end to vulva 1.52 (1.5–1.65) mm, representing 46.1 (46.1–49.5)% of body length. Uteri comparatively short, sac-like, containing 22 (20–28) eggs. Embryos in eggs at early cleavage stages. Egg size $32-40 \times 60-68$ (n = 6, measured in uteri of holotype, close to vulva). Oviducts short, straight, thick-walled, approximately as long as uteri. Ovaries wide, elongated, and reflected in zone of oocytes, sometimes with 1 or 2 bends closer to oviducts; proximal ends of ovaries overlapping level of vulva (Fig. 1A, F). No testis zones or sperm cells observed in the specimens studied.

Tail narrow, elongated, gradually tapering posteriorly (Fig. 1G). Tail length 155 (112–157), representing 4.7 (3.5–4.9)% of body length. Posterior part of tail with bulb-shaped dilatation and thin, elongated, thread-like cuticular needle on tail tip (Fig. 1H). Phasmids pore-like, situated immediately posterior to mid-length of tail, at 85 from anus in holotype. Single transverse fold of slightly inflated body cuticle present at level of phasmids (Fig. 1H).

Remarks

The new species described herein is assigned to the genus *Serpentirhabdias* based on the following characters: body cuticle thin, without prominent inflations; circumoral papillae arranged in two lateral groups; buccal capsule absent (similarly to some other species of the genus); vulva pre-equatorial; eggs in uteri not numerous; and embryos in eggs at early cleavage stages.

The new species is closely related to *S. vellardi* with regard to the absence of lips and buccal capsule, as well as the body dimensions, egg size, and parasitism in South American colubroidean snakes. However, it differs from *S. vellardi* in the shape of the tail: the tail end in *S. atracti* n. sp. exhibits a bulb-shaped dilatation, followed by a thin, thread-like cuticular needle, whereas in *S. vellardi* the tail is conical, without dilatations. Furthermore, the two species differ in the following metric characters: the width of the oesophageal bulb in *S. atracti* n. sp. is smaller than in *S. vellardi* (32–42 vs 56 μ m), and the new species has a somewhat shorter oesophagus (213–249 vs 260–270 μ m). Additionally, the excretory glands

(approximately 126–136 μ m long) are shorter than the oesophagus in *S. atracti* n. sp., whereas the excretory glands (350–400 μ m long) are longer than the oesophagus in *S. vellardi* (Pereira, 1928).¹

In the absence of buccal capsule Serpentirhabdias atracti n. sp. is also similar to S. horigutii (Yamaguti, 1943), S. labiata, S. pearsoni (Kuzmin & Tkach, 2008), and S. vibakari (Kuzmin, 1999), but differs from these species in the absence of lips and in the specific shape of the tail. In addition, in S. atracti n. sp. the nerve-ring is situated at the mid-length of the oesophagus, and the eggs measure $60-68 \times 32-40 \,\mu\text{m}$. Thus, the new species can be easily distinguished from S. horigutii, in which the nerve-ring is posterior to the mid-length of the oesophagus and the eggs are larger, measuring 72–90 \times 42–48 μ m (Yamaguti, 1943; Kuzmin, 2013). The body length of S. atracti n. sp. is larger than that of S. labiata (body length 2.2–2.4 mm; see Pereira, 1927); nevertheless, the oesophagus is slightly longer in S. labiata (250-260 vs 213-249 µm), and the eggs are also larger (84–92 \times 53 µm) (Pereira, 1927). Both S. pearsoni and S. vibakari are much smaller than S. atracti n. sp., having body length of 1.9–2.7 mm and 1.7–2.3 mm, respectively (Kuzmin & Tkach, 2008; Kuzmin, 2013). Moreover, the excretory glands are longer than the oesophagus in S. vibakari (see Kuzmin, 2013), and the nerve-ring is situated posterior to mid-length of the oesophagus in S. pearsoni (see Kuzmin & Tkach, 2008).

In addition to *S. labiata* and *S. vellardi*, three other species of *Serpentirhabdias* are known from the Neotropical Region, i.e. *S. filicaudalis*, *S. lamothei* and *S.* cf. *fuscovenosa*. *Serpentirhabdias atracti* n. sp. differs from these species in the absence of buccal capsule and in the shape of the tail. Furthermore, the new taxon is smaller than *S. filicaudalis* (body length 3.18-3.33 vs 3.89-5.83 mm; see Barrella et al., 2009). Although *S. atracti* n. sp. and *S. lamothei* are similar in the shape of the oral opening and body size, they differ in the length of the oesophagus, which is somewhat smaller in the new species than in *S. lamothei* (213–249 vs 255–317 µm), and in the distance from

¹ In the original description of *S. vellardi* the length of the excretory glands is given as "from 0.035 to 0.040 mm" (p. 13 in Pereira, 1928); apparently, the decimal point was placed erroneously since the excretory glands shown in their figure 2 (p. 19) are longer than the oesophagus described as measuring "from 0.26 to 0.27 mm" (p. 13; Pereira, 1928).

the anterior end of oesophagus to the nerve-ring, which is much greater in *S. lamothei* (155–220 *vs* 109–117 μ m), and the nerve-ring is situated posterior to oesophagus mid-length. The new species is similar to *S.* cf. *fuscovenosa* described from the Neotropical Region by Martínez-Salazar & León-Règagnon (2006) in the body size and relative lengths of oesophagus and tail. In contrast to *S. atracti* n. sp., *S.* cf. *fuscovenosa* possesses rounded (and not triangular) oral opening and distinct lips.

Discussion

The newly discovered specimens correspond to the diagnosis of *Serpentirhabdias* (see Tkach et al., 2014) and resemble *S. vellardi* in the absence of distinct lips and a buccal capsule. Further morphological studies revealed several characters, mainly the specific shape of the tail and the length of the excretory glands, that clearly differentiate the present specimens from *S. vellardi* and other similar species.

Several species of Serpentirhabdias (i.e. S. vellardi, S. horigutii, S. labiata, S. pearsoni, S. vibakari, and S. atracti n. sp.) share the lack of buccal capsule as a common morphological character. Based on this character, these species can be considered to belong to a separate group within the genus Serpentirhabdias. However, they have a rather distinct geographical distribution: S. vellardi, S. labiata and S. atracti n. sp. occur in the Neotropical Region (Pereira, 1927, 1928; present study); S. horigutii and S. vibakari are known from the eastern Palaearctic (Kuzmin, 2013); and S. pearsoni occurs in northern Australia (Kuzmin & Tkach, 2008). In our opinion, neither the morphological data nor the host information unambiguously support or negate an assumption of a common origin for these six species. In all likelihood, only molecular phylogenetic studies, involving as many Serpentirhabdias species as possible, will reveal the precise relationships between the species comprising this genus.

The absence of distinct lips has also been reported in some species of Rhabdiasidae that parasitise two Neotropical amphibians: *R. alabialis* Kuzmin, Tkach & Brooks, 2007 and *R. paraensis* Santos, Melo, Nascimento, Nascimento, Giese & Furtado, 2011 (see Kuzmin et al., 2007; Santos et al., 2011). Presumably, the loss of lips in some species of *Serpentirhabdias* and *Rhabdias* might have occurred independently in these two distant (according to Tkach et al., 2014) lineages of Rhabdiasidae.

Serpentirhabdias atracti n. sp. is the sixth species of the genus to be found in the Neotropical Region. It is likely that additional species of this group will be discovered in this region because the Neotropics are home to rather large numbers of colubroidean and viperid snakes, potential hosts for *Serpentirhabdias* spp. According to Bérnils & Costa (2012), 39 species of Colubroidea and 36 species of Viperidae (Crotalinae) are known in Brazil alone.

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