

Research Article

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## Two new species of *Rhabdias* Stiles et Hassall, 1905 (Nematoda: Rhabdiasidae) from anuran amphibians in Pará, Brazil

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**Abstract:** Two new lung-dwelling nematode species of the genus *Rhabdias* Stiles et Hassall, 1905 were discovered in Caxiuanã National Forest, Pará state, Brazil. *Rhabdias galactonoti* sp. n. was found in a dendrobatid frog *Adelphobates galactonotus* (Steindachner). The species is characterised by the regularly folded inner surface of the anterior part of the buccal capsule seen in apical view, flask-shaped oesophageal bulb and narrow, elongated tail. *Rhabdias stenocephala* sp. n. from two species of leptodactylid frogs, *Leptodactylus pentadactylus* (Laurenti) (type host) and *L. paraensis* (Heyer), is characterised by a narrow anterior end that is separated from the remaining body by a constriction. Both species possess six small but distinct lips, a cuticle that is inflated along the whole body, a doliiform buccal capsule separated into a longer anterior and a shallow, ring-shaped posterior part, lateral pores in the body cuticle and zones of spermatogenesis in the syngonia. *Rhabdias galactonoti* sp. n. is the first species of the genus found in Dendrobatidae; *R. stenocephala* sp. n. is the second species described from Leptodactylidae in eastern Amazonia.

**Keywords:** taxonomy, *Adelphobates*, *Leptodactylus*, Rhabdiasidae, Anura, eastern Amazonia, South America

Parasitic nematodes of the genus *Rhabdias* Stiles et Hassall, 1905 inhabit the lungs of amphibians and some reptiles. The genus includes approximately 80 species occurring on all continents except Antarctica (Kuzmin and Tkach 2015). Fifteen nominal species are known from amphibian hosts in the Neotropical region: *R. alabialis* Kuzmin, Tkach et Brooks, 2007, *R. androgyna* Kloss, 1971, *R. breviensis* Nascimento, Gonçalves, Melo, Giese, Furtado et Santos, 2013, *R. elegans* Gutiérrez, 1945, *R. fuelleborni* Travassos, 1926, *R. hermaphrodita* Kloss, 1971, *R. kuzmini* Martínez-Salazar et León-Règagnon, 2007, *R. manantlanensis* Martínez-Salazar, 2008, *R. mucronata* Schuurmans Stekhoven, 1952, *R. paraensis* Santos, Melo, Nascimento, Nascimento, Giese et Furtado, 2011, *R. peninsularis* Martínez-Salazar, Falcón-Ordaz, González-Bernal, Parra-Olea et Pérez-Ponce de León, 2013, *R. pseudosphaerocephala* Kuzmin, Tkach et Brooks, 2007, *R. savagei* Bursey et Goldberg, 2005, *R. tobagoensis* Moravec et Kaiser, 1995, and *R. truncata* Schuurmans Stekhoven, 1952 (Kuzmin et al. 2015).

The life cycles of species of *Rhabdias* include a parasitic and a free-living phase; infective larvae dwell in host faeces and soil and enter the amphibian hosts via direct skin penetration (Baker 1979, Anderson 2000, Langford and Janovy 2009). Consequently, species of this genus are commonly parasitic in terrestrial and semi-aquatic amphibians.

During the joined herpetological and parasitological investigations in the Caxiuanã National Forest (Floresta Nacional de Caxiuanã) in Melgaço municipality, Pará, Brazil, we found various amphibian species harbouring lung nematodes, including *Adelphobates galactonotus* (Steindachner) (Dendrobatidae), *Leptodactylus pentadactylus* (Laurenti) and *L. paraensis* (Heyer) (Leptodactylidae).

After examination under a light microscope, the nematodes collected from *A. galactonotus* and *Leptodactylus* spp. were assigned to the genus *Rhabdias*, based on their morphology, infection site and parasitism in amphibians. They appeared to represent two morphological forms and each was found to be different from all known species of *Rhabdias* species. Therefore, we describe them herein as two new species of *Rhabdias*.

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## MATERIALS AND METHODS

Amphibians were collected manually during day and night time excursions in Caxiuanã National Forest (Floresta Nacional de Caxiuanã), Melgaço municipality, Pará, Brazil, in February 2015. Collections were done under collection permit SISBIO (No. 30772-4) and IBAMA (No. 12420-2). *Leptodactylus pentadactylus* (4 specimens) and *L. paraensis* (11 specimens) were sampled from the same habitat close to the Caxiuanã Research Station. Specimens of *A. galactonotus* originated from two populations separated by the Caxiuanã Bay and belonged to 'orange' (12 specimens) and 'light blue' (6 specimens) colour morphs (after Hoogmoed and Avila-Pires 2012).

All host individuals were killed by injection of lidocaine hydrochloride 2% and their internal organs were examined under a dissecting stereomicroscope. Nematode individuals from the lungs were washed in 0.98% saline and fixed in heated 70% alcohol. Additionally, type specimens of *R. tobagoensis* (holotype and 2 paratypes; collection No. N-619) were borrowed from the Helminthological Collection of the Institute of Parasitology (České Budějovice, Czech Republic) and used for comparative studies. The type series of the two species described were each selected from a single host specimen.

Prior to examination under a light microscope, nematodes were cleared in lactophenol and mounted on temporary slides. Apical sections were made manually, using a razor blade and studied *en face*. Upon completion of our studies, nematodes were transferred to 70% alcohol for storage. Nematodes were examined under an Olympus BX41 microscope equipped with a drawing tube and an Olympus BX53 microscope equipped with differential interference contrast optics and a digital camera. All measurements were made using the Olympus imaging software cellSens Standard 1.9 (Olympus, Japan), and are presented in micrometres unless otherwise indicated. The classification of amphibian hosts follows that of Frost (2016) and de Sá et al. (2014).

## RESULTS

### *Rhabdias galactonoti* sp. n.

Figs. 1, 2

ZooBank number for species:

urn:lsid:zoobank.org:act:1614847C-92BC-4E1A-8642-42638715819C

**Description.** (Based on 5 type specimens, gravid hermaphrodites. Measurements of the holotype are followed by the ranges for the type series in parentheses. Measurements of the entire sample studied are given in Table 1.)

Body elongated, wider in mid-region, gradually tapering in anterior and posterior thirds (Fig. 2A). Anterior end rounded, posterior end pointed. Body length 6.04 mm (5.60–6.04 mm), width at vulva 263 (242–268), width at oesophago-intestinal junction 176 (144–176). Cuticle inflated along entire body, with transverse folds. Lateral pores and ducts distinct along body, ducts in shape of narrow funnels crossing inflated layer of cuticle (Fig. 2C).

Oral opening round (Fig. 1G). Six small lips present on apical surface. Submedian lips located close to edge of oral opening. Lateral lips smaller, located at some distance from oral opening. Each lip with prominent internal labial papilla on its inner edge; external labial papillae not ob-

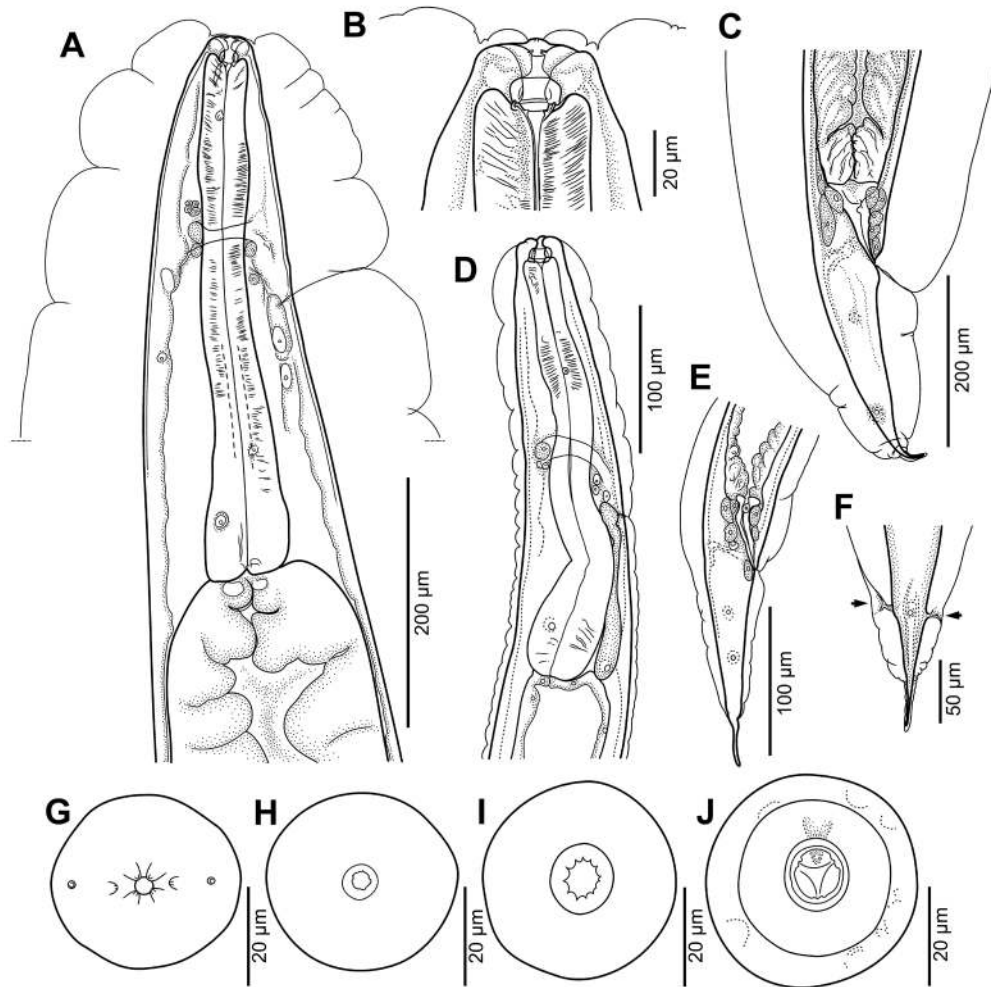
served. Amphidial openings located posterior to lateral lips (Fig. 1G). Vestibulum narrow, cuticularised, with folds on inner surface seen in apical view (Fig. 1H). Buccal capsule doliiform, widest at mid-depth, consisting of thick-walled anterior part and shallow, thin-walled posterior part (Fig. 1B). Anterior part transparent, circular in apical view, with regularly folded inner surface (Fig. 1I). Posterior part surrounded by apex of oesophagus, with 3 paired thickenings directed into lumen (Fig. 1J), tooth-shaped in lateral view (Fig. 1B), associated with borders between oesophageal sectors. Maximum diameter of buccal capsule 16 (14–17), total depth of both parts 12 (10–12); depth to width ratio 0.8 (0.7–0.8).

Oesophagus club-shaped (Fig. 1A), 443 (419–459) long [7.3% (7.3–8.1%) of body length], with slight dilatation in middle of anterior half. Width of oesophagus anterior end 32 (27–32), maximum width of anterior dilatation 43 (39–43), width posterior to dilatation 37 (31–37). In posterior half, oesophagus gradually widening towards flask-shaped bulb; bulb with flattened posterior edge, 73 (61–73) wide. Nerve-ring encircling oesophagus posterior to its anterior dilatation, anterior to its mid-length (Fig. 1A), at 165 (165–184) from anterior end, this distance corresponding to 37% (37–43%) of oesophagus length. Excretory pore distinct in some specimens posterior to nerve ring (Fig. 1A), at 250 (195–250) from anterior end. Excretory duct narrow, straight. Excretory glands absent in gravid specimens. Intestine thick-walled, its anterior part much wider than oesophageal bulb, occupying entire space in pseudocoelome (Figs 1A, 2A). Rectum funnel-shaped, wide in anterior part, tapering posteriorly, prominently cuticularised (Fig. 1C).

Genital system amphidelphic, typical of the genus. Vulva usually pre-equatorial, situated at 2.8 mm (2.5–2.8 mm) from anterior end [46% (43–50%) of body length]. Lips of vulva not salient (Fig. 2D). Vagina short, transverse, cuticularised. Uteri joined, thin-walled, containing numerous eggs (>100 in total); eggs near vulva with fully-developed embryos. Egg size 103–114 × 57–63 (N = 11, measured in uteri of paratype). Both genital tubes U-bent in proximal parts of seminal receptacles. Distance from anterior end of body to anterior U-bend 1 041 (593–1 155), distance from posterior U-bend to tail end 1 077 (694–1 077). Testis zones observed in all specimens, in at least one syngonium (Fig. 2B). Numerous sperm cells usually observed in seminal receptacles (Fig. 2E). Proximal parts of syngonia overlapping level of vulva.

Tail narrow, gradually tapering (Fig. 1C). Slight elevation of body wall present on ventral side posterior to anus. In caudal region, cuticular inflation not reaching tail tip (Fig. 1C,F). Tail length 229 (229–333), or 3.8% (3.8–6.0%) of body length. Phasmids located at 91 (91–120) from tail end (Fig. 1F).

**Immature specimens.** One immature specimen (Fig. 1D,E) was found in the lungs of its host (orange morph). It differed from gravid specimens by the following characters: smaller size (body length 2.17 mm), post-equatorial vulva (distance from anterior end to vulva corresponded to 53% of body length), absence of eggs in uteri,



**Fig. 1.** *Rhabdias galactonoti* sp. n. from *Adelphobates galactonotus* (Steindachner), line drawings. **A** – anterior part of body, lateral view (holotype); **B** – anterior end, lateral view (holotype); **C** – posterior part of body, lateral view (holotype); **D** – immature specimen, anterior part of body, dorso-lateral view; **E** – immature specimen, posterior part of body, lateral view; **F** – caudal end, dorsal view (paratype), showing phasmids (arrowheads); **G** – anterior end of body, *en face* view; **H** – optical section through vestibulum; **I** – optical section through anterior part of buccal capsule; **J** – optical section through posterior part of buccal capsule.

less inflated body cuticle and presence of distinct excretory glands (Fig. 1D). Size of buccal capsule (15 × 9), as well as shape of oesophagus and tail (Fig. 1E) in immature specimen similar to those in gravid worms.

**Variability.** In the two samples of mature worms studied, the specimens collected from the blue colour morph of *A. galactonotus* were somewhat different from those from the orange colour morph (Table 1). In the former specimens body length and all related metrical characters (body width, oesophagus dimensions, tail length, distance to nerve ring, distance to vulva), as well as buccal capsule width exceeded those seen in the orange colour morph, whereas buccal capsule length was rather similar in the two samples. The qualitative characters, however, were found not to be different in the two samples.

**Type specimens:** Holotype (MPEG.NEM 000062) and 4 paratypes (MPEG.NEM 000063) are deposited in the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil.

**Type locality:** Caxiuanã National Forest (Floresta Nacional de Caxiuanã), Melgaço municipality, Pará, Brazil; 1°47'32"S; 51°26'03"E.

**Type host:** *Adelphobates galactonotus* (Steindachner) (Amphibia: Anura: Dendrobatidae), orange colour morph.

**Localisation in host:** Lungs.

**Prevalence and intensity:** 61% (11 of 18); 2.4 (1–5).

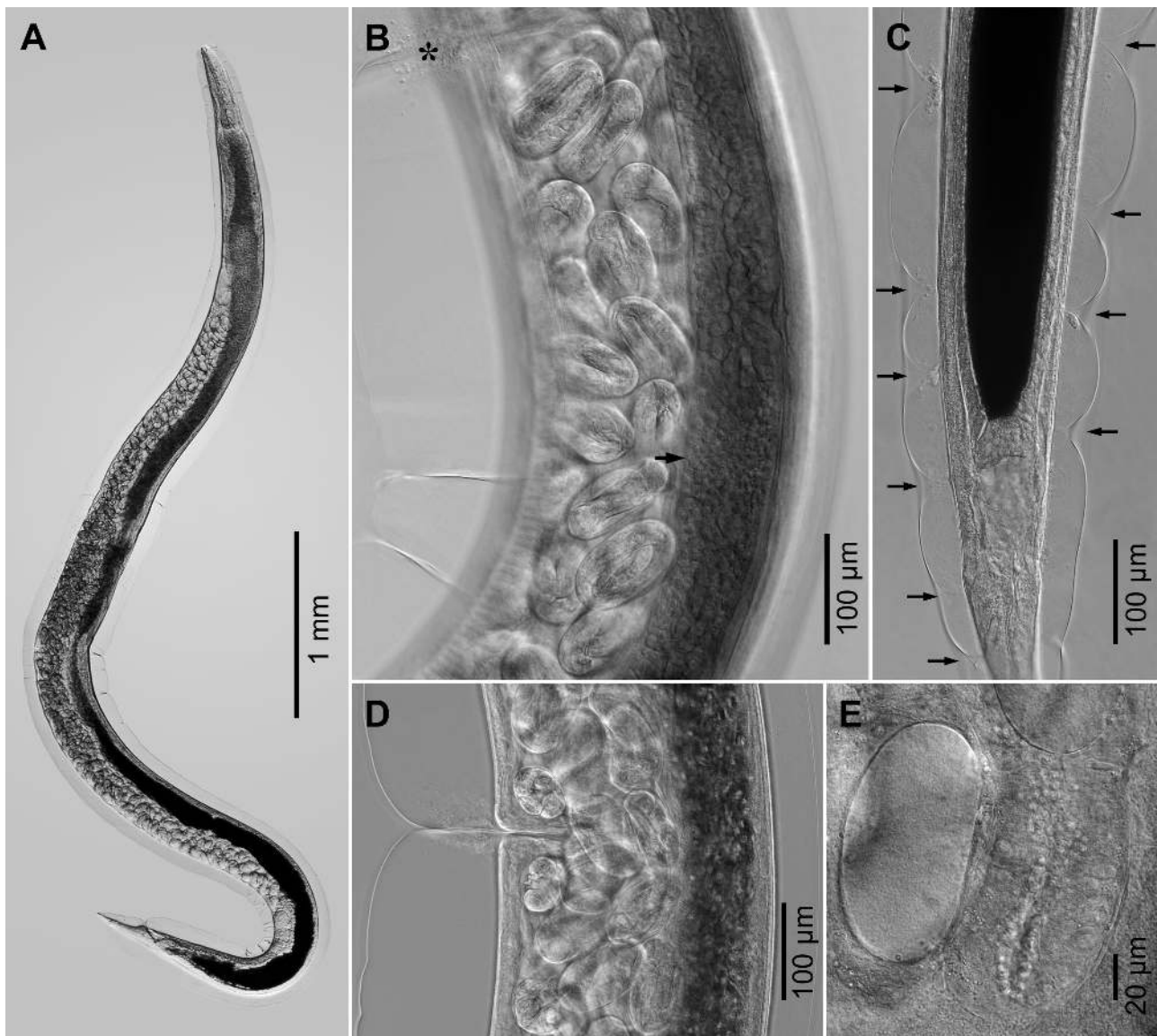
**Etymology:** The species is named after the species name of the type host.

**Remarks.** The new species is assigned to the genus *Rhabdias* due to the following morphological characters: inflated body cuticle, small buccal capsule, amphidelphic genital system with short transverse vagina and joined uteri, and based on its lung parasitism in an amphibian host.

By the possession of six distinct lips, *R. galactonoti* is similar to eight Neotropical *Rhabdias* spp. from anuran amphibians: *R. androgyna*, *R. breviensis*, *R. fuelleborni*, *R. kuzmini*, *R. manantlanensis*, *R. peninsularis*, *R. savagei*, and *R. tobagoensis*.

*Rhabdias galactonoti* differs from *R. androgyna*, since the latter has a larger body (up to 15.4 mm long in adult specimens), an extended cuticular swelling consisting of





**Fig. 2.** *Rhabdias galactonoti* sp. n. from *Adelphobates galactonotus* (Steindachner), photomicrographs (paratypes). **A** – general view; **B** – mid-region of body, ventrolateral view showing vulva (asterisk) and testis zone in posterior syngonium (arrowhead); **C** – posterior part of body, dorsal view showing lateral pores (arrows); **D** – region of vulva, lateral view; **E** – U-bend of posterior seminal receptacle.

two layers at the anterior end, a particular shape of the anterior part of the body with a shoulder-like circular dilatation and a wider but shallower buccal capsule, 19–27 µm wide and 0.29–0.47 depth to width ratio (according to Kloss 1971, Kuzmin et al. 2015).

*Rhabdias galactonoti* differs from *R. breviensis* by having a longer but narrower body (in *R. breviensis* the body is 2.63–3.63 mm long and 370–543 µm wide near the vulva after Nascimento et al. 2013). Additionally, *R. breviensis* has a smaller buccal capsule, 4–9 µm deep and 7–13 µm wide, and the vulva is distinctly postequatorial: distance from anterior end to vulva corresponds to 65–71% of body length (Nascimento et al. 2013).

*Rhabdias fuelleborni* is similar to *R. galactonoti* in the relative length of the oesophagus and tail and in the position of the vulva. However, *R. fuelleborni* attains a larger body size than *R. galactonoti*, up to 10–12 mm (Travassos 1926) or 16.3 mm (Kloss 1971). Additionally, the lateral

lips in *R. galactonoti* are reduced, whereas in *R. fuelleborni* lips are similar in shape and size; the internal surface of the anterior part of the buccal capsule is folded in the former species and smooth in the latter; in *R. fuelleborni* the posterior part of the buccal capsule lacks the tooth-like projections, according to Kuzmin et al. (2015).

*Rhabdias galactonoti* is smaller than *R. kuzmini*, which is 14.2–19.2 mm long. In the latter species, a cuticular swelling is present mostly in the anterior and posterior parts, the lateral lips are modified to lateral pseudolabia, the buccal capsule is larger, 35–47 µm wide, and the vulva is mostly postequatorial (Martínez-Salazar and León-Règagnon 2007).

In contrast to *R. galactonoti*, *R. manantlanensis* is characterised by a body cuticle that is either not or only slightly inflated, a long and narrow vestibulum, a larger buccal capsule, 11–19 µm deep and 19–27 µm wide and completely surrounded by the anterior part of the oesophagus, as well

**Table 1.** Metrical characters of *Rhabdias galactonoti* sp. n. from *Adelphobates galactonotus*. All measurements are in micrometres unless otherwise indicated.

Characters	11 specimens from orange colour morph*, mean (min.–max.)	7 specimens from blue colour morph, mean (min.–max.)	Entire sample, 18 specimens, mean ± SD
Body length (mm)	5.6 (4.9–6.2)	6.9 (6.3–7.2)	6.1 ± 0.8
Body width at vulva	236 (189–268)	247 (227–270)	240 ± 25
Body width at oesophago-intestinal junction	142 (123–176)	160 (142–169)	149 ± 15
Buccal capsule maximum width	15 (14–17)	18 (16–20)	16 ± 2
Buccal capsule depth	11 (10–12)	11 (11–12)	11 ± 1
Buccal capsule depth/width ratio	0.7 (0.7–0.8)	0.6 (0.5–0.7)	0.7 ± 0.1
Oesophagus length	417 (349–459)	462 (440–487)	434 ± 36
Oesophagus length in % of body length	7.5 (6.8–8.2)	6.7 (6.3–7.0)	7.2 ± 0.6
Width of oesophagus anterior end	30 (27–32)	38 (35–40)	33 ± 4
Width of oesophagus anterior dilatation	39 (39–43)	45 (39–48)	41 ± 3
Width of oesophagus posterior to dilatation	32 (31–37)	37 (35–39)	34 ± 3
Width of oesophageal bulb	64 (59–73)	77 (70–84)	69 ± 8
Distance from anterior end to nerve-ring	170 (149–184)	195 (173–226)	180 ± 18
Distance from anterior end to vulva (mm)	2.6 (2.3–2.9)	3.2 (2.8–3.5)	2.9 ± 0.4
Distance from anterior end to vulva in % of body length	47.5 (43.5–50.0)	46.5 (43.7–49.2)	47 ± 2
Distance from anterior end to anterior bend of genital system	875 (593–1 066)	1 189 (1 090–1 490)	997 ± 201
Distance from posterior bend of genital system to tail end	832 (733–1 040)	1 069 (830–1 190)	936 ± 159
Tail length	277 (229–333)	321 (298–353)	240 ± 37
Tail length in % of body length	5.0 (3.8–6.5)	4.6 (4.2–5.0)	4.9 ± 0.7
Distance from phasmids to tail end	103 (67–120)	138 (128–158)	118 ± 23

\* – including type specimens.

as a shorter tail, 143–232 µm long, occupying 1.5–3.3% of body length (after Martínez-Salazar 2008) vs 3.8–6.5% in *R. galactonoti*.

Based on the description of Martínez-Salazar et al. (2013), *R. peninsularis* can be recognised as distinct from *R. galactonoti* by a cuticular inflation that is mainly restricted to the anterior and posterior parts, a shorter vestibulum and a wide lumen of the buccal capsule. While the body length is similar in the two species, the oesophagus is shorter in *R. peninsularis*, representing 5.6–6.4% of body length vs 6.3–8.2% in *R. galactonoti*. In addition, the vulva in *R. peninsularis* is postequatorial, but mostly pre-equatorial in *R. galactonoti*.

*Rhabdias galactonoti* is larger than *R. savagei*, which, according to Bursey and Goldberg (2005), is 4.2–5.3 mm long. Additionally, the latter species lacks the dilatation in the anterior part of the oesophagus, and its oesophageal bulb is 43–52 µm wide vs 59–84 µm in *R. galactonoti*; the position of the vulva in gravid *R. savagei* is post-equatorial.

*Rhabdias tobagoensis* is similar to *R. galactonoti* in body length but is much wider at mid-body, 408–476 µm (after Moravec and Kaiser 1995) vs 189–270 µm. Lips in *R. tobagoensis* are more distinct in lateral view, and the anterior end is rounded and distinctly wider at the level of the buccal capsule than in *R. galactonoti* (present observations).

*Rhabdias galactonoti* has six small but distinct lips and by this character it can be distinguished from *R. alabialis*, *R. elegans* and *R. paraensis*. The latter three species have no lips and parasitise mostly bufonid hosts (Kuzmin et al. 2007, González and Hamann 2008, Santos et al. 2011). Details of the morphology of the anterior end are not known for *R. hermaphrodita*. This species, however, is larger than

*R. galactonoti*, up to 12.9 mm long, and parasitises bufonid hosts in southern Brazil (Kloss 1971, 1974), whereas *R. galactonoti* is described from a dendrobatid host endemic to eastern Amazonia.

***Rhabdias stenocephala* sp. n.**

Figs. 3, 4

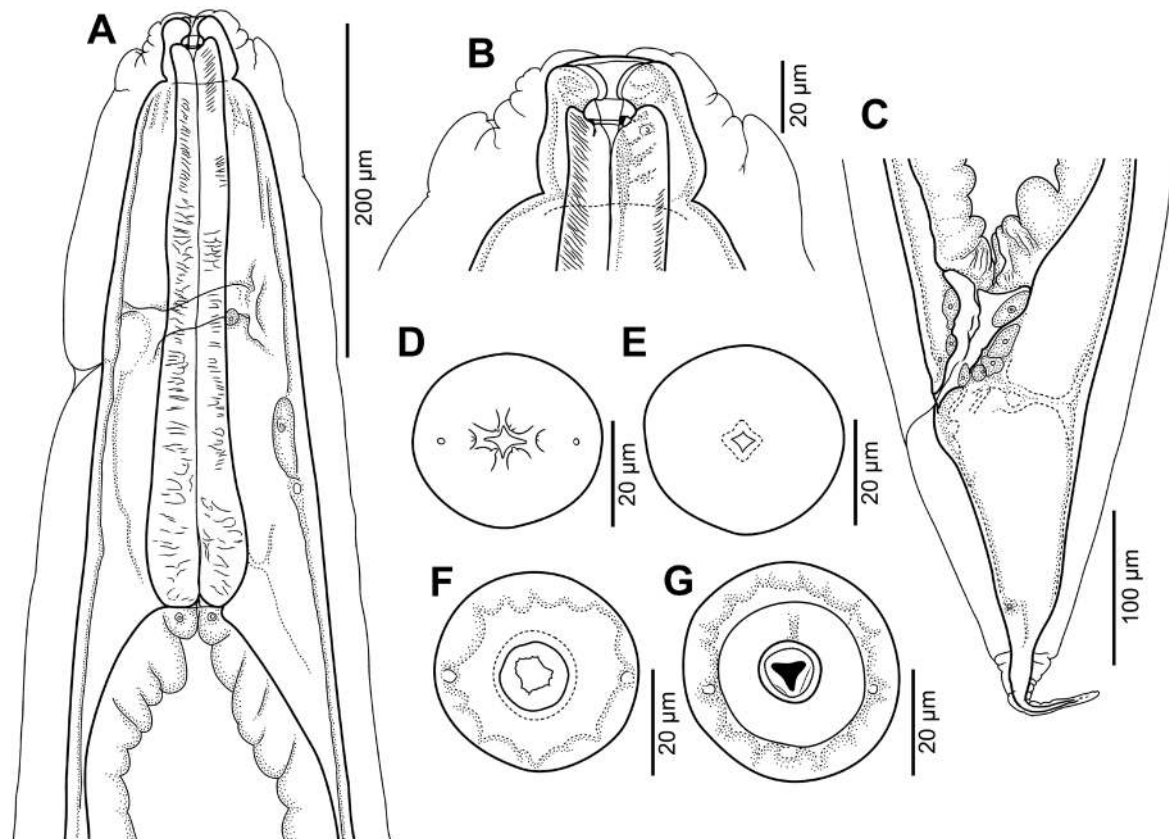
ZooBank number for species:

<http://www.zoobank.org/act:EE8EA365-9E8D-4170-9316-9309FEB763A0>

**Description.** (Based on 11 type specimens, gravid hermaphrodites. Measurements are given for the holotype followed by the ranges for the type series in parentheses. Measurements of the whole studied sample are given in Table 2.)

Body elongated, gradually tapering from mid-length towards anterior and posterior extremities (Fig. 4A). Body length 7.7 (6.9–8.1) mm, width at vulva 414 (325–439), width at oesophago-intestinal junction 184 (164–188). Body cuticle inflated along entire body, with folds; no separated inflations observed in any part of body. Lateral pores present along whole body, lateral ducts in inflated cuticle funnel-shaped (Fig. 4B,E). Anteriormost part of body (excluding inflated cuticle) narrow, 45 (39–58) long and a maximum of 56 (48–61) wide in its posterior part, separated from remaining body by distinct constriction at level of anterior part of oesophagus (Figs. 3A,B, 4B). Posterior to constriction, body abruptly widening to 80 (65–80), then gradually widening posteriorly.

Six very small lips present on apical surface (Fig. 3D). Submedian lips elongated, overhanging edge of oral opening. Lateral lips reduced, located at distance from oral opening. Each lip with prominent internal labial papilla on inner edge. External labial papillae not observed. Amph-



**Fig. 3.** *Rhabdias stenocephala* sp. n. from *Leptodactylus pentadactylus* (Laurenti), line drawings. **A** – anterior part of body, lateral view (holotype); **B** – anterior end, lateral view (holotype); **C** – posterior part of body, lateral view (holotype); **D** – anterior end of body, *en face* view; **E** – optical section through vestibulum; **F** – optical section through anterior part of buccal capsule; **G** – optical section through posterior part of buccal capsule.

dial openings indistinct. Vestibulum short, cuticularised, with rhomboid lumen in apical view (Fig. 3E). Buccal capsule doliiform, widest at mid-depth, with narrowing lumen (Fig. 3B). Buccal capsule walls separated into longer anterior part and ring-shaped posterior part. Wall of anterior part transparent; in apical view, outer surface of wall circular, inner surface irregularly folded (Fig. 3F). Posterior part denser, with circular thickening seen in apical view (Fig. 3G), connected to cuticularised oesophageal lumen. Depth of buccal capsule 9 (8–11), including both anterior and posterior parts, maximum width 17 (15–18); depth to width ratio 0.5 (0.5–0.7).

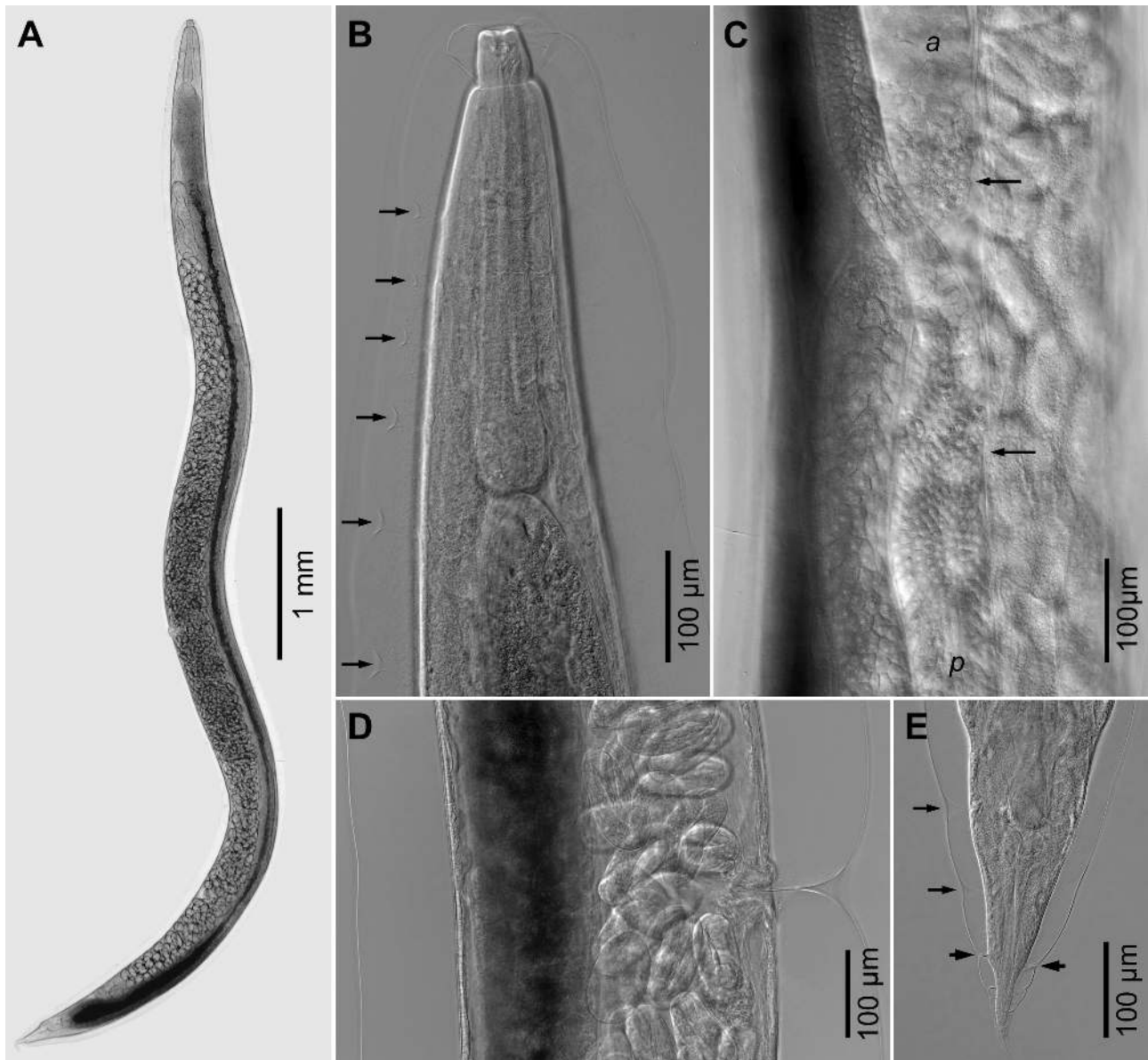
Oesophagus 410 (385–442) long [5.3% (5.0–6.1%) of body length], generally club-shaped (Fig. 3A), its anterior end at level of buccal capsule mid-length (Fig. 3B). Slight dilatation present in anterior part of oesophagus, at posterior part of its anterior third. Width of oesophagus at anterior end 31 (30–34), maximum width of anterior dilatation 45 (40–47). Posterior to dilatation, oesophagus 41 (34–41) wide, then gradually widening to oval-shaped posterior bulb, 71 (60–74) wide. Nerve-ring surrounding oesophagus posterior to its anterior dilatation, at 202 (192–225) from anterior end, this distance corresponding to 49% (47–53%) of oesophagus length. Excretory pore observed in most specimens (Fig. 3A), at 245 (245–273) from anterior end. Excretory duct narrow, straight, poorly visible. Excretory glands absent.

Intestine narrow at its junction with oesophagus, abruptly widening posteriorly (Figs 3A, 4A). One or two coelomocytes visible posterior to oesophago-intestinal junction on ventral side of body. Rectum funnel-shaped, prominently cuticularised (Fig. 3C).

Genital system amphidelphic, typical of genus. Vulva postequatorial, located at 4.1 mm (3.5–4.4 mm) from anterior end [53% (51–55%) of body length]. Lips of vulva slightly salient (Fig. 4D). Vagina short, transverse, cuticularised. Uteri joined, sac-like, thin-walled, elongated, filled with numerous (>100) eggs. Most eggs containing fully-developed embryos. Egg size 112–132 × 58–72 (N = 10, measured in uteri of holotype). Both syngonia U-bent in opposite directions at level of seminal receptacles. Anterior bend located at 1063 (814–1250) from anterior end, posterior bend at 623 (574–846) from posterior end. In bent areas, several additional loops usually present, more often in anterior syngonium. Testis zone observed in at least one of syngonia (Fig. 4C). Proximal ends of syngonia narrow, rounded, overlapping vulva.

Tail conical, gradually tapering posteriorly, somewhat dilated at level of phasmids (Fig. 3C). Slight elevation of body wall usually present on ventral side posterior to anus. Tail length 301 (266–339), or 3.9% (3.3–4.2%) of body length. Cuticular inflation not reaching tail tip. Phasmids located at about mid-length of tail (Fig. 4E), at 147 (120–173) from tail end.





**Fig. 4.** *Rhabdias stenocephala* sp. n. from *Leptodactylus pentadactylus* (Laurenti), photomicrographs (paratypes). **A** – general view; **B** – anterior part of body, dorsolateral view showing lateral pores (arrows); **C** – mid-region of body, dorsolateral view showing anterior (*a*) and posterior (*p*) syngonia with testis zones (arrows); **D** – region of vulva, lateral view; **E** – posterior end, dorsal view showing phasmids (arrowheads) and lateral pores (arrows).

**Variability.** Body length and related metrical characters in specimens from *L. paraensis* were somewhat smaller than in specimens from *L. pentadactylus*, though the ranges of each metrical character overlapped (Table 2). The dimensions of the buccal capsule, anterior constricted part of the body and distance from phasmids to tail end were almost identical in the two samples. Qualitative characters in specimens from the two host species were found not to be different.

Immature specimens of *R. stenocephala* were not recovered in the present study.

**Type specimens:** Holotype (MPEG.NEM 000064) and 10 paratypes (MPEG.NEM 000065) are deposited in the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil.

**Type locality:** Caxiuanã National Forest (Floresta Nacional de Caxiuanã), Melgaço municipality, Pará, Brazil;

1°47'32"S; 51°26'03"E.

**Type host:** *Leptodactylus pentadactylus* (Laurenti) (Amphibia: Anura: Leptodactylidae).

**Other host:** *L. paraensis* (Heyer) (Amphibia: Anura: Leptodactylidae).

**Localisation in host:** Lungs.

**Prevalence and intensity of infection:** *L. pentadactylus* – prevalence 25% (1 of 4), intensity 11; *L. paraensis* – prevalence 45% (5 of 11), intensity 3.2 (1–5).

**Etymology:** The name '*stenocephala*' is given to the species due to its characteristically narrowed anterior end.

**Remarks.** *Rhabdias stenocephala* is assigned to the genus *Rhabdias* due to the following morphological characters: inflated body cuticle, small buccal capsule, amphidelphic genital system with short transverse vagina and

**Table 2.** Metrical characters of *Rhabdias stenocephala* sp. n. from *Leptodactylus* spp. All measurements are in micrometres unless otherwise indicated.

Characters	11 specimens* from <i>L. pentadactylus</i> , mean (min.–max.)	15 specimens from <i>L. paraensis</i> , mean (min.–max.)	Entire sample, 26 specimens, mean ± SD
Body length (mm)	7.7 (6.9–8.1)	6.9 (4.5–7.9)	7.2 ± 0.8
Body width at vulva	406 (325–439)	348 (284–396)	373 ± 43
Body width at oesophago-intestinal junction	175 (164–188)	163 (142–182)	168 ± 11
Length of anterior constricted part	48 (39–58)	49 (38–55)	48 ± 5
Maximum width of anterior constricted part	55 (48–61)	51 (46–60)	53 ± 4
Body width posterior to constriction	75 (65–80)	72 (61–88)	73 ± 7
Buccal capsule maximum width	17 (15–18)	17 (16–18)	17 ± 1
Buccal capsule depth	9.5 (8.0–11.0)	9.5 (9.0–10.0)	9.5 ± 0.6
Buccal capsule depth/width ratio	0.6 (0.5–0.7)	0.6 (0.5–0.6)	0.6 ± 0.04
Oesophagus length	414 (385–442)	405 (354–453)	409 ± 23
Oesophagus length in % of body length	5.4 (5.0–6.1)	6.0 (5.4–7.8)	5.7 ± 0.7
Width of oesophagus anterior end	31 (30–34)	30 (26–35)	31 ± 2
Width of oesophagus anterior dilatation	44 (40–47)	42 (36–47)	43 ± 3
Width of oesophagus posterior to dilatation	39 (34–41)	37 (30–42)	38 ± 3
Width of oesophageal bulb	69 (60–74)	66 (65–83)	68 ± 7
Distance from anterior end to nerve-ring	208 (192–225)	194 (168–214)	200 ± 13
Distance from anterior end to vulva (mm)	4.1 (3.5–4.4)	3.7 (2.4–4.1)	3.9 ± 0.4
Distance from anterior end to vulva in % of body length	53 (51–55)	54 (48–59)	54 ± 2
Distance from anterior end to anterior bend of genital system	1 010 (814–1 250)	1 036 (600–1 387)	1 025 ± 191
Distance from posterior bend of genital system to tail end	685 (574–846)	622 (491–794)	649 ± 100
Tail length	299 (266–339)	283 (234–345)	290 ± 28
Tail length in % of body length	3.9 (3.3–4.2)	4.1 (3.3–5.7)	4.1 ± 0.6
Distance from phasmids to tail end	145 (120–173)	145 (112–177)	145 ± 17

\* – type series.

joined uteri; and due to its lung parasitism in amphibian hosts.

The new species differs from all known *Rhabdias* spp. parasitising Neotropical anurans, as well as from any other species of the genus, in the specific shape of its anterior end: narrow anterior part separated from the remaining body by a distinct constriction. With regard to other morphological characters, *R. stenocephala* is similar to several mid-sized (body 4–8 mm long) Neotropical species possessing distinct lips and parasitising anuran hosts: *R. manantlanensis*, *R. peninsularis*, *R. savagei*, *R. tobagoensis* and *R. galactonoti* sp.n.

*Rhabdias stenocephala* differs from *R. manantlanensis* by a smaller buccal capsule, which is 19–27 µm wide in the latter species (Martínez-Salazar 2008); additionally, *R. manantlanensis* has a mostly pre-equatorial vulva and shorter tail, representing 1.5–3.3% of body length (Martínez-Salazar 2008) vs 3.3–5.7% in *R. stenocephala*.

In contrast to *R. stenocephala*, the cuticular inflation in *R. peninsularis* is prominent mostly in the anterior and posterior parts but does not reach the tip of the tail. Furthermore, *R. peninsularis* has a short vestibulum and a differently structured buccal capsule: almost spherical, thick-walled, with wide, not cylindrical lumen (Martínez-Salazar et al. 2013). Eggs in *R. stenocephala* are somewhat larger than in *R. peninsularis*: 112–132 µm × 58–72 µm vs 85–104 µm × 42–54 µm. The tail in *R. peninsularis* is short (139–213 µm long), the tail tip is covered by the inflated cuticle (Martínez-Salazar et al. 2013), whereas in *R. stenocephala* the tail is longer (234–345 µm long) and the cuticular swelling does not reach the tail tip.

*Rhabdias stenocephala* is somewhat larger than *R. savagei*, which is 4.2–5.3 mm long according to Bursey and Goldberg (2005); the latter species possesses a larger buccal capsule, 18–24 µm wide, and narrower oesophageal bulb, 43–52 µm wide. The excretory pore in *R. savagei* is situated at the level of the oesophageal bulb, 336–415 µm from the anterior end, whereas in *R. stenocephala* the excretory pore is located just posterior to the mid-length of the oesophagus, 214–273 µm from the anterior end.

*Rhabdias stenocephala* differs from *R. tobagoensis* by having a narrower buccal capsule (15–18 µm vs 18–21 µm wide) and small, less conspicuous lips; lips are distinct in the latter species (Moravec and Kaiser 1995).

Smaller lips also separate *R. stenocephala* from *Rhabdias galactonoti*; in addition, the latter species has a mostly pre-equatorial vulva and a narrower body at the level of the vulva, 189–270 µm vs 284–439 µm in *R. stenocephala* (Tables 1, 2).

## DISCUSSION

*Rhabdias galactonoti* sp. n. and *R. stenocephala* sp. n. are the 16<sup>th</sup> and 17<sup>th</sup> nominal species of *Rhabdias* known from amphibian hosts in the Neotropical Realm. However, *R. mucronata* and *R. truncata* were inadequately described based on juveniles only and are considered *species inquirendae* (Bursey et al. 2003, Martínez-Salazar et al. 2009, 2013). Therefore, the number of valid species of the genus parasitising amphibians of the Neotropics is still 15, including the two new species described in the present paper.



In the Neotropics, *Rhabdias* spp. parasitise amphibian hosts from various families. Eight species (*R. alabialis*, *R. androgyna*, *R. elegans*, *R. fuelleborni*, *R. hermaphrodita*, *R. kuzmini*, *R. paraensis*, *R. pseudosphaerocephala*) are known exclusively or mostly from Bufonidae, others are from hosts belonging to Leptodactylidae (*R. breviensis*), Craugastoridae (*R. manantlanensis*, *R. tobagoensis*), Hyliidae (*R. peninsularis*), Ranidae, Craugastoridae and Leptodactylidae (*R. savagei*) (Bursey and Brooks 2010; Kuzmin et al. 2015). *Rhabdias galactonoti* is the first species of the genus described from Dendrobatidae; *R. stenocephala* is the second species specific to Leptodactylidae. Dendrobatid and leptodactylid frogs are diurnal, showing complex behaviour patterns; some species display parental care and diverse reproductive strategies (Heyer 1969, Duellman 1992, Brust 1993, Caldwell 1997). Interestingly, *A. galactonotus* is a generally terrestrial species, in contrast to other mostly arboreal Dendrobatidae (Duellman 1992). This feature might have facilitated its association with a specific *Rhabdias* parasite.

The morphological differences documented in the two samples of *R. galactonoti* from spatially separated populations of orange and blue colour morphs of the host (see Table 2) might demonstrate the speciation process caused by the isolation and/or specialisation in a particular host morph. However, the studied samples were too small to reveal statistically significant differences. In our opinion, further morphological studies combined with molecular investigations may clarify the intraspecific structure of the species or identify cryptic species within the *R. galactonoti* complex.

Studied samples of *R. stenocephala* from two hosts, *L. pentadactylus* and *L. paraensis*, appeared to be similar

both in metrical and qualitative characters. Populations of the two hosts occur in the same habitats of the study area, and the parasite is apparently shared by them. Presently it is not clear, whether one of the host species is more important for the persistence of the parasite population, since both host samples were rather small, 4 and 11 specimens, correspondingly.

The wide host range reported for some *Rhabdias* spp. in the Neotropics is presumed, in some cases, to be a result of incorrect species identification (Martínez-Salazar et al. 2009, Kuzmin et al. 2015). Thorough morphological studies and further characters revealed by them might improve species diagnostics and make identifications more reliable. Detailed examination of the buccal capsule may provide additional diagnostic characters for *Rhabdias* spp. (Lhermitte-Vallarino et al. 2010, Kuzmin et al. 2015). For example, in the presently described two *Rhabdias* species, the inner surface of the anterior part of the buccal capsule bears similar folds, which differentiates them from *R. paraensis* and *R. fuelleborni*, in both of which the inner surface of the anterior part of the buccal capsule is smooth (Kuzmin et al. 2015, Melo et al. 2016). In our opinion, detailed studies of the morphology of the anterior end are essential for further investigations of *Rhabdias* spp. in the Neotropical Realm as well as worldwide.

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