

Description of *Rhabdias breviensis* n. sp. (Rhabditoidea: Rhabdiasidae) in two Neotropical frog species

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Abstract Nematodes of the genus *Rhabdias* Stiles & Hassall, 1905 (Rhabditoidea: Rhabdiasidae) have a dioecious free-living stage and a hermaphroditic stage that parasitises the lungs of amphibians and reptiles. Approximately 94 species of *Rhabdias* have been described. Because the similar morphological characteristics such as the labial structures, the location of the vulva and the shape of the tail of *Rhabdias* spp. hinder their identification, molecular biology techniques and scanning electron microscopy have been employed to diagnose species of this genus. This study describes *Rhabdias breviensis* n. sp., parasitic in the lungs of two Neotropical frog species *Leptodactylus petersii* Steindachner and *Leptodactylus macrosternum* Miranda-Ribeiro. The description of this species integrates classical taxonomy, scanning electron microscopy and

a molecular analysis of the mitochondrial COI gene. The new species differs from all other *Rhabdias* species parasitic in Neotropical hosts in certain morphometric parameters, the position of the vulva, the host group and the cephalic characters.

Introduction

The genus *Rhabdias* includes approximately 94 species distributed worldwide (Kuzmin & Tkach, 2012). The life-cycle of these nematodes consists of a dioecious free-living stage and a hermaphroditic stage that parasitises the lungs of amphibians and reptiles (Vicente et al., 1991; Anderson, 2000). Several researchers, including Kuzmin et al. (2003), Tkach et al. (2006), Kuzmin et al. (2007), Martínez-Salazar (2008) and Martínez-Salazar et al. (2009), have suggested that the similarity in the characters used to identify *Rhabdias* individuals at the species level leads to confusion and misidentification. Therefore, complementary approaches using molecular biology techniques, scanning electron microscopy, biogeographical analysis and identification of the host species are necessary to correctly identify species of this genus.

The application of molecular biology techniques to taxonomy, systematics and phylogeny has resulted in the reassessment of many species of *Rhabdias* and the reassignment of some previously described species (Lupchinski Jr. et al., 2006; Kuzmin et al., 2007). Taxonomic and phylogenetic studies of this type

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frequently use the nuclear ribosomal markers 5.8S, 28S and 18S rDNA and the two internal transcribed spacers (ITS1 and ITS2) in addition to mitochondrial markers such as cytochrome *b* (*cyt b*), cytochrome *c* oxidase subunit I (COI) and 12S rRNA genes (Dorris et al., 2002; Dare et al., 2008; Dubey & Shine, 2008; Lhermitte-Vallarino et al., 2009).

In Brazil, species of *Rhabdias* have been recorded in anurans belonging to the following families: Bufonidae Gray, Ranidae Rafinesque, Leptodactylidae Werner, Hylidae Rafinesque, and Microhylidae Günther (see Kung & Wu, 1945). Only one species has been reported in the latter family i.e. *Rhabdias globocephala* Kung & Wu, 1945 from *Microhyla ornata* Duméril & Bibron (see Kung & Wu, 1945).

The species of the leptodactylid genus *Leptodactylus* Fitzinger are distributed throughout South America, Southern North America and the West Indies (Frost, 2013). According to Vicente et al. (1991), the following species of *Leptodactylus* act as hosts for nematodes of the genus *Rhabdias* in South America: *Leptodactylus chaquensis* Cei (*Rhabdias* sp.), *Leptodactylus labyrinthicus* Spix (*Rhabdias fuelleborni* Travassos, 1926), *Leptodactylus syphax* Bokermann (*Rhabdias* sp.), *Leptodactylus podicipinus* Cope (*Rhabdias* sp.), *Leptodactylus melanonotus* Hallowell (*Rhabdias elegans* Gutierrez, 1945), and *Leptodactylus ocellatus* Linnaeus (*Rhabdias* sp.).

Among the species of *Leptodactylus* only *L. petersii* Steindachner and *L. macrosternum* Miranda-Ribeiro inhabit the Amazon Basin and have been reported to host parasitic helminths. *Leptodactylus macrosternum*, whose synonyms include *L. ocellatus* and *Leptodactylus latrans* Steffen (see Heyer et al., 2012), is the only host of *Rhabdias* spp. that has been reported in this region (Vicente et al., 1991; Goldberg et al., 2009).

This study describes a new species of the genus *Rhabdias* parasitising two leptodactylid frog species (*L. petersii* and *L. macrosternum*) in Breves Municipality, Marajó Island (eastern Amazon). The description combines light and scanning electron microscopy observations with molecular data for the COI gene.

Materials and methods

Eleven specimens of *L. petersii* and three specimens of *L. macrosternum* were collected in January 2011 in

peridomiciliary areas at Breves Municipality, Marajó Island, Pará State, eastern Amazon, Brazil (1°40'55"S, 50°28'48"W).

Morphological data

The nematodes found in frog lungs were fixed in AFA (2% glacial acetic acid, 3% formaldehyde and 95% ethanol) at 60°C, dehydrated and cleared in an increasing ethanol/glycerine series, and examined under an Olympus BX41 microscope equipped with a drawing tube (with no zoom adjustment) to obtain drawings for subsequent morphometric analysis. All measurements are in micrometres unless otherwise stated and are expressed as the range followed by the mean and standard deviation in parentheses. After fixation in AFA, specimens from both host species were postfixed in 1% osmium tetroxide, dehydrated in acetone up to the carbon dioxide (CO₂) critical point and coated with gold for subsequent analysis by scanning electron microscopy (LEO 1450 and LEO 1430 instruments).

Molecular data

Nematode DNA was obtained by phenol/chloroform extraction following the standard procedures of Sambrook et al. (1989). A fragment of the mitochondrial COI gene of each sample was amplified by polymerase chain reaction (PCR) in a total reaction volume of 25 µL containing 10 ng of genomic DNA, 1.0 mM of each dNTP, 3.0 mM of MgCl₂, 1 unit of Taq DNA polymerase and 5 µM of each primer [HCO (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3')] (Folmer et al., 1994). The amplification profile consisted of 30 cycles comprising 1 min at 95°C, 1 min at 55°C and 1 min at 72°C. These cycles were preceded by an initial 2-min step at 95°C to homogenise the temperature of the block and followed by a final 5-min step at 72°C to polymerise any remaining molecules from which the polymerase may have dissociated before the fragment was fully synthesised.

Amplification products were cloned into the pGEM-T Vector (Promega®), inserted into electro-competent *Escherichia coli* TOP10 (Invitrogen®) and sequenced using an automated DNA analyser [model ABI 3130, Applied Biosystems] in combination with the BigDye Terminator V3.1 kit according to the manufacturer's instructions. To confirm the observed mutations, each sample was sequenced with both primers, forward and reverse. The nucleotide

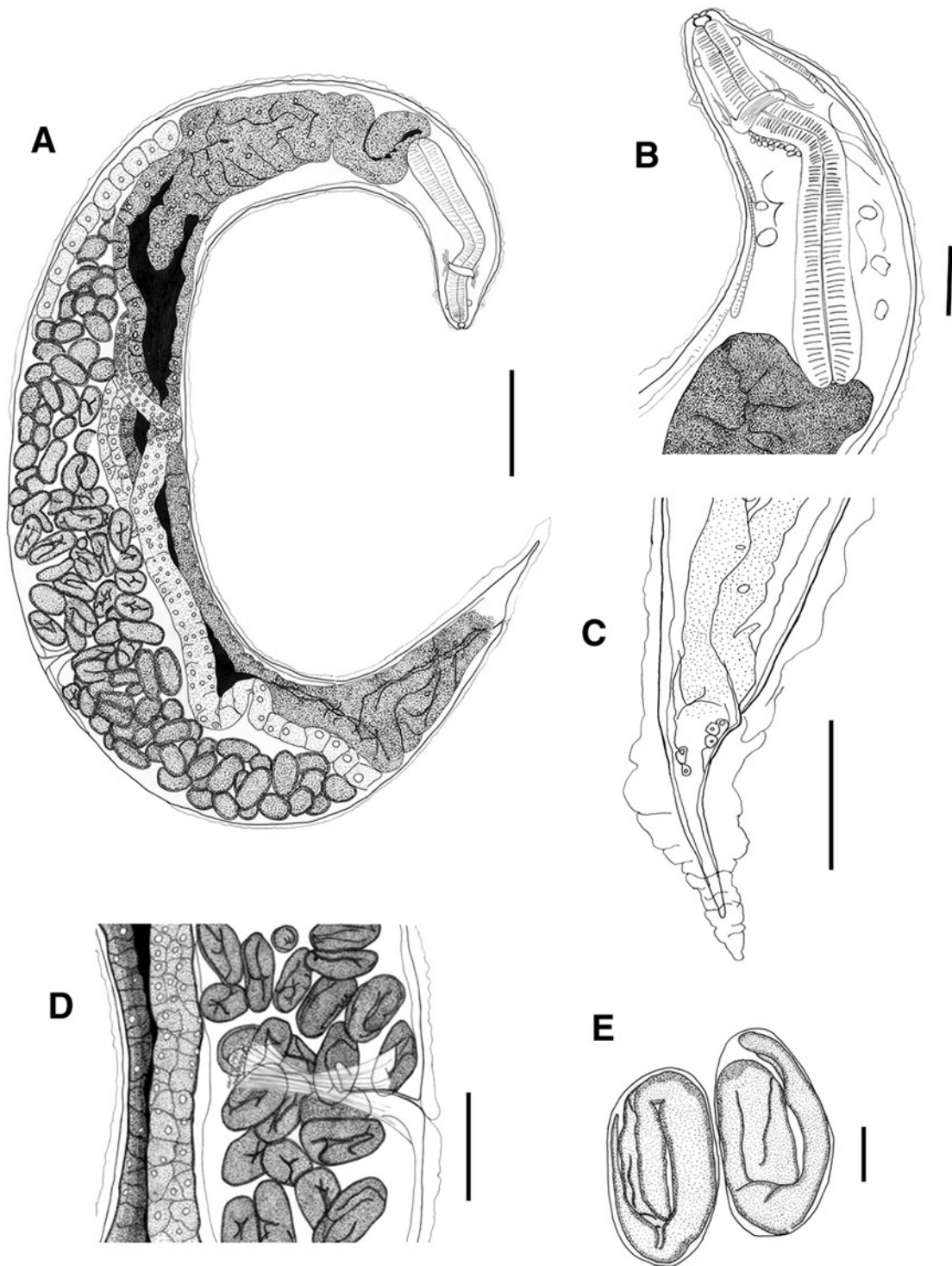


Fig. 1 *Rhabdias brevisensis* n. sp. A, overall view of the body. B, anterior third of the body, showing the goblet-shaped buccal capsule and the nerve-ring located in the anterior third of the claviform oesophagus; C, posterior third of the body, showing the conical shape of the tail; D, lateral view of the post-equatorial vulva; E, embryonated eggs with thin shells. Scale-bars: A, C, D, 200 μ m; B, 50 μ m; E, 20 μ m

sequences were edited and aligned using the BioEdit software (Hall, 2007).

Order Rhabditida Chitwood, 1933

Family Rhabdiasidae Railliet, 1916

Rhabdias breviensis n. sp.

Type-host: *Leptodactylus petersii* Steindachner.

Other host: *Leptodactylus macrosternum* Miranda-Ribeiro.

Type-locality: Breves Municipality, Marajó Island, Pará State, Brazil (1°40'55"S, 50°28'48"W).

Site of infection: Lungs.

Type-specimens: Holotype MPEG 00010, paratype MPEG 00011 and paratype MPEG 00012, deposited in the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará State, Brazil.

Prevalence: In *L. petersii*: 63.64%; in *L. macrosternum*: 100%.

Mean abundance: In *L. petersii*: 30 (range 20–40); in *L. macrosternum*: 36 (range 30–45).

Molecular data: Three sequences of the mitochondrial COI gene (706 nt) were obtained, two from specimens from *L. petersii* and one from *L. macrosternum*. All three sequences shared a single haplotype (GenBank accession number KC512382). No termination codons or indels were observed.

Etymology: The species is named after the type-locality, Breves Municipality.

Description (Figs. 1, 2)

[Based on the holotype and nineteen paratypes (Fig. 1A–E)]. Anterior third of hermaphroditic female truncated, posterior extremity curved dorsally (Fig. 1A–B). Total body length 2.63–3.63 (3.14 ± 0.34) mm; width near vulva region 370–543 (423 ± 59); width at lower oesophageal dilation 145–187 (160 ± 12).

Buccal capsule goblet-shaped (Fig. 1A–B), 4–9 (6 ± 1) × 7–13 (11 ± 2). Oesophagus claviform (Fig. 1B), 238–410 (355 ± 44) × 45–54 (51 ± 2) (width measured in the ganglia region).

Nerve-ring (Fig. 1B) located at 41–84 (61 ± 10) from anterior extremity. Vulva post-equatorial (Fig. 1C), 1,870–2,348 (1,988 ± 478) from the anterior extremity of the nematode [65–71 (63)% of body length]; tail short, 139–191 (160 ± 14).

Hermaphroditic females amphidelphic, with larvae found near the vulvar opening and eggs distant from this aperture (Fig. 1D–E). Embryonated eggs measure on average 81 ± 4 × 43 ± 0.3.

Scanning electron microscopy data

Scanning electron microscopy observations provided additional morphological details for the new species from the lungs of *L. petersii* and *L. macrosternum*. The body is covered by a delicate inflation of the cuticle extending from the oral aperture to the end of the tail (Fig. 2A–D). Two pairs of submedian cephalic papillae and one pair of lateral cephalic papillae are present at the anterior extremity, in addition to two lateral amphids. The oral aperture is circular and surrounded by four submedian and two lateral lips (Fig. 2A–B). The submedian cephalic papillae are spherical in shape and located on the internal portion of the submedian lips, whereas the lateral cephalic papillae are conical in shape and located on the internal portion of the lateral lips (Fig. 2A–B). The larvae present in the uterus are covered by thin shells (Fig. 2E–F).

Discussion

Rhabdias breviensis n. sp. is a parasite of *L. petersii* and *L. macrosternum* which inhabit the same niche, the peridomiciliary area. This shared niche likely favours the transmission cycle and the adaptation of this nematode to both species of *Leptodactylus*. The concomitant parasitism of both hosts was inferred from the morphological data and confirmed by the molecular data i.e. the COI gene sequences of the lung parasites from *L. macrosternum* and *L. petersii* were identical thus confirming the parasitism by *R. breviensis* n. sp. in these distinct hosts. This finding is important because it corroborates the statements of Martínez-Salazar (2008) and Martínez-Salazar et al. (2009) that *Rhabdias* spp. usually do not parasitise hosts belonging to different families and are selective with regard to the genus of the host. Similarly, Tkach et al. (2006) observed that *Rhabdias bakeri* Walton, 1929 and *Rhabdias ranae* Walton, 1929 parasitised both *Rana sylvatica* LeConte and *Lithobates pipiens* Schreber (= *Rana pipiens*); this observation was later confirmed by Dare et al. (2008) through experimental infections and molecular biology studies.

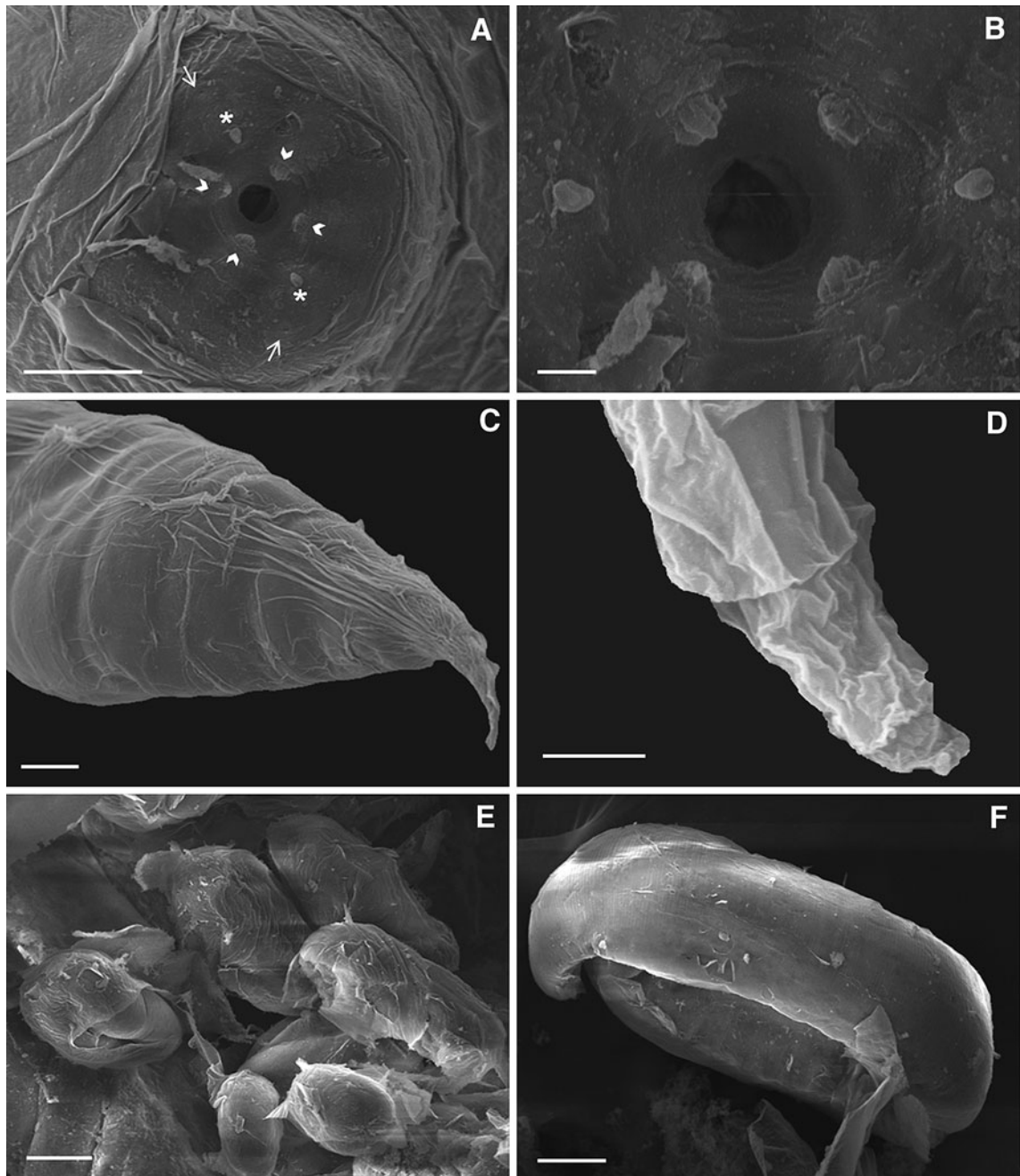


Fig. 2 Scanning electron micrographs of *Rhabdias brevisensis* n. sp. A, frontal view of the anterior extremity, showing the six lips surrounding the circular oral aperture, the submedian cephalic papillae (arrowheads), the lateral cephalic papillae (asterisks) and two lateral amphids (arrows); B, detail of the anterior extremity, showing the submedian and lateral cephalic papillae; C, frontal-lateral view of the posterior third of the body, showing the conical tail; D, extremity of the conical tail, showing the inflation of the cuticle covering this region; E, embryonated eggs, showing the thin egg-shells covering the larvae; F, detail of the larvae without their egg-shells. Scale-bars: A, F, 10 μ m; B, 2 μ m; C, 30 μ m; D, 5 μ m; E, 20 μ m

A wide variety of *Rhabdias* spp. parasitise amphibians and reptiles, but only four have been reported from Neotropical frogs: *Rhabdias mucronata*

Schuermans-Stekhoven, 1952 from *Leptodactylus bolivianus* Boulenger, *R. truncata* Schuurmans-Stekhoven, 1952 from *Telmatobius schreiteri* Vellard, *R.*

tobagoensis Moravec & Kaiser, 1995 from *Pristimantis charlottesvillensis* Kaiser, Dwyer, Feichtinger & Schmid, and *R. savagei* Bursey & Goldberg, 2005 from *Lithobates forreri* Boulenger (see Moravec & Kaiser, 1995; Bursey & Goldberg, 2005; Kuzmin & Tkach, 2012).

Rhabdias breviensis n. sp. differs from *R. savagei* in having six lips around the oral aperture vs four lips (see Bursey & Goldberg, 2005). Additionally, the new species exhibits reduced morphometric dimensions compared with *R. savagei* and *R. tobagoensis*. Despite these morphometric differences, *R. breviensis* n. sp. resembles *R. tobagoensis* in the number of lips surrounding the oral aperture. These two species differ in the location of the vulva, which is post-equatorial in *R. breviensis* n. sp. and equatorial in *R. tobagoensis* (see Moravec & Kaiser, 1995).

No data are available in the literature for the hermaphroditic forms *R. mucronata* and *R. truncata* parasitic in amphibians, however only juvenile forms were described by Schuurmans-Stekhoven (1952); this hinders a comparison with *R. breviensis* n. sp.

When compared with the Neotropical species of *Rhabdias* that parasitise different host groups, *R. breviensis* n. sp. exhibits the same number of lips surrounding the oral aperture as *Rhabdias anolis* Bursey, Goldberg & Telford, 2003, *R. vellardi* Pereira, 1928, *R. lamothei* Martínez-Salazar & León-Règagnon, 2006, *R. nicaraguensis* Bursey, Goldberg & Vitt, 2007 and *Rhabdias fuelleborni* Travassos, 1926 (see Bursey et al., 2003, 2007; Martínez-Salazar & León-Règagnon, 2007). However, *R. breviensis* n. sp. differs markedly from these species in its morphology, morphometric dimensions and host group. Furthermore, *R. anolis*, *R. nicaraguensis*, *R. vellardi* and *R. lamothei* are parasites of reptiles (*Anolis frenatus* Cope, *Anolis capito* Peters, *Philodryas schottii* Girard, and *Leptodeira maculata* Hallowell, respectively) and *R. fuelleborni* parasitises toads and frogs (*Rhinella marina* Linnaeus, *R. schneideri* Werner, *R. arenarum* Hense, *R. icterica* Spix and *Thoropa miliaris* Spix) (Bursey et al., 2003; Martínez-Salazar & León-Règagnon, 2007; Kuzmin & Tkach, 2012).

The post-equatorial position of the vulva suggests that *R. breviensis* n. sp. is taxonomically closer to five Neotropical species, i.e. *Rhabdias androgynia* Kloss, 1971, *R. elegans* Gutierrez, 1945, *R. hermaphrodita* Kloss, 1971, *R. leonae* Martínez-Salazar, 2006, and *R. paraensis* Santos, Melo, Nascimento, Nascimento,

Giese & Furtado, 2011. However, the new species differs from these taxa in its host group and cephalic characteristics. In addition, the cuticle of *R. breviensis* n. sp. lacks pores or canaliculi connecting these pores to the hypodermal cells, characteristics observed by Santos et al. (2011) in *R. paraensis*. Thus, *R. breviensis* n. sp. differs from all other *Rhabdias* spp. parasitising Neotropical hosts in terms of its morphometric dimensions, the position of the vulva, the host group and certain cephalic characteristics. This is the first report of a species of *Rhabdias* from *L. petersii*.

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References

- Anderson, R. C. (2000). *Nematode parasites of vertebrates: Their development and transmission* (2nd ed.). Wallingford, Oxon, UK: CAB International.
- Bursey, C. R., & Goldberg, S. R. (2005). New species of *Oswaldocruzia* (Nematoda: Molineoidea), new species of *Rhabdias* (Nematoda: Rhabdiasidae), and other helminths in *Rana* cf. *forreri* (Anura: Ranidae) from Costa Rica. *Journal of Parasitology*, 91, 600–605.
- Bursey, C. R., Goldberg, S. R., & Telford Jr., S. R. (2003). *Rhabdias anolis* n. sp. (Nematoda: Rhabdiasidae) from the lizard, *Anolis frenatus* (Sauria: Polychrotidae), from Panama. *Journal of Parasitology*, 89, 113–117.
- Bursey, C. R., Goldberg, S. R., & Vitt, L. J. (2007). New species of *Rhabdias* (Nematoda: Rhabdiasidae) and other

- helminths from *Norops capito* (Sauria: Polychrotidae) from Nicaragua. *Journal of Parasitology*, 93, 129–131.
- Dare, O. K., Nadler, S. A., & Forbes, M. R. (2008). Nematode lungworms of two species of anuran amphibians: Evidence for co-adaptation. *International Journal for Parasitology*, 38, 1,729–1,736.
- Dorris, M., Viney, M. E., & Blaxter, M. L. (2002). Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. *International Journal for Parasitology*, 32, 1,507–1,517.
- Dubey, S., & Shine, R. (2008). Origin of the parasites of an invading species, the Australian cane toad (*Bufo marinus*): Are the lungworms Australian or American? *Molecular Ecology*, 17, 4,418–4,424.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 5, 294–299.
- Frost, D. R. (2013). Amphibian Species of the World: an Online Reference. Version 5.6 (9 January 2013). Electronic Database accessible at <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, New York, USA.
- Goldberg, S. R., Burse, C. R., Caldwell, J. P., & Shepard, D. B. (2009). Gastrointestinal helminths of six sympatric species of *Leptodactylus* from Tocantins State, Brazil. *Comparative Parasitology*, 76, 258–266.
- Hall, B. G. (2007). *Phylogenetic Trees Made Easy: A How-to Manual* (3rd ed.). Sunderland, MA: Sinauer Associates.
- Heyer, R., Langone, J., La Marca, E., Azevedo-Ramos, C., Di Tada, I., Baldo, D., et al. (2012). *Leptodactylus latrans*. In: *IUCN 2012. IUCN red list of threatened species. Version 2012.2*. www.iucnredlist.org. Accessed 11 November 2012.
- Kung, C. C., & Wu, H. W. (1945). Parasitic nematodes of amphibians from Pehpei Szechwan, China. *Sinensia*, 16, 73–83.
- Kuzmin, Y., & Tkach, V. V. (2002–2012). *Rhabdias*. World Wide Web electronic publication. Accessed 4 February 2012.
- Kuzmin, Y., Tkach, V. V., & Brooks, D. R. (2007). Two new species of *Rhabdias* (Nematoda: Rhabdiasidae) from the marine toad, *Bufo marinus* (L.) (Lissamphibia: Anura: Bufonidae), in Central America. *Journal of Parasitology*, 93, 159–165.
- Kuzmin, Y., Tkach, V. V., & Snyder, S. D. (2003). The nematode genus *Rhabdias* (Nematoda: Rhabdiasidae) from amphibians and reptiles of the Nearctic. *Comparative Parasitology*, 70, 101–114.
- Lhermitte-Vallarino, N., Barbuto, M., Junker, K., Boistel, R., Ineich, I., Wanji, S., et al. (2009). *Rhabdias rhampholeonis* n. sp. and *Rhabdias mariauxi* n. sp. (Nematoda, Rhabdiasoidea), first lung worms from leaf chameleons: Description, molecular evidence and notes on biology. *Parasitology International*, 58, 375–383.
- Lupchinski Jr., E., Vargas, L., Ribeiro, R. P., Moreira, H. L. M., Valentim, M., & Povh, J. A. (2006). A importância da utilização da técnica RAPD para a identificação de dactilogirídeos em Tilápias do Nilo (*Oreochromis niloticus*). *Arquivos de Ciências Veterinárias e Zoologia*, 9, 49–57.
- Martínez-Salazar, E. A. (2008). A new rhabdiasid species from *Craugastor occidentalis* (Anura: Brachycephalidae) from Sierra de Manantlán, Jalisco, Mexico. *Revista Mexicana de Biodiversidad*, 79, 81–89.
- Martínez-Salazar, E. A., & León-Règagnon, V. (2007). New species of *Rhabdias* (Nematoda: Rhabdiasidae) from *Bufo occidentalis* (Anura: Bufonidae) from Sierra Madre del Sur, Mexico. *Journal of Parasitology*, 93, 1,171–1,177.
- Martínez-Salazar, E. A., León, G. P. P., & Olea, G. P. (2009). First record of the genus *Rhabdias* (Nematoda: Rhabdiasidae), endoparasite from *Scinax staufferi* (Anura: Hylidae) in Mexico. *Revista Mexicana de Biodiversidad*, 80, 861–865.
- Moravec, F., & Kaiser, H. (1995). Helminth parasites from West Indian frogs, with descriptions of two new species. *Caribbean Journal of Science*, 31, 252–268.
- Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: A laboratory manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Santos, J. N., Melo, F. T. V., Nascimento, L. C. S., Nascimento, D. E. B., Giese, E. G., & Furtado, A. P. (2011). *Rhabdias paraensis* sp. nov.: A parasite of the lung of *Rhinella marina* (Amphibia: Bufonidae) from Brazilian Amazonia. *Memórias do Instituto Oswaldo Cruz*, 106, 433–440.
- Schuurmans-Stekhoven, J. H. S. (1952). Nematodos parasitos de anfibios, pájaros y mamíferos de la República Argentina. *Acta Zoologica Lilloana*, 10, 315–400.
- Tkach, V. V., Kuzmin, Y., & Pulis, E. E. (2006). A new species of *Rhabdias* from lungs of the wood frog, *Rana sylvatica*, in North America: The last sibling of *Rhabdias ranae*? *Journal of Parasitology*, 92, 631–636.
- Vicente, J. L., Rodrigues, H. O., Gomes, D. C., & Pinto, R. M. (1991). Nematoides do Brasil. 2ª parte: Nematoides de anfibios. *Revista Brasileira de Zoologia*, 7, 549–561.