ISSN Versión impresa 2218-6425 ISSN Versión Electrónica 1995-1043

ORIGINAL ARTICLE /ARTÍCULO ORIGINAL

FIRST REPORT OF *RHABDIAS* (NEMATODA: RHABDIASIDAE) IN *HYPSIBOAS RANICEPS,* CERRADO, BRAZIL

PRIMER REGISTRO DE RHABDIAS (NEMATODA: RHABDIASIDAE) EN *HYPSIBOAS RANICEPS*, CERRADO, BRASIL

Franciele Cristina de Souza[1]*, Ottilie Carolina Forster[1], Murilo de Souza Queiroz[1] & Luciano Alves dos Anjos[1].

[1] Post-Graduate Program in Biological-Zoological Sciences, Universidade Estadual Paulista "Júlio de Mesquita Filho"- UNESP. Ecology and Parasitism Laboratory, Department of Biology and Zoology. Passeio Monção, 226. CEP 15385000. Ilha Solteira – SP, Brasil.
*Corresponding author: francielexingu@gmail.com

Neotropical Helminthology, 2016, 10(2), jul-dic: 295-300.

ABSTRACT

A new record of *Rhabdias* parasitizing *Hypsiboas raniceps* Stiles & Hassal 1905 in a Cerrado area is presented in this work. The genus is commonly found parasitizing the lungs of amphibians and reptiles worldwide. Seven *H. raniceps* specimens were analyzed, where two were parasitized with nematodes of the genus *Rhabdias* in the lungs. A total of six nematodes were found, and identified based on their structural morphologies, and on their measurements. This note presents the first record of *Rhabdias* in *H. raniceps* given that the species is a new *Rhabdias* host.

Keywords: Cerrado – *Hypsiboas raniceps* – *Rhabdias* – South America

RESUMO

Um novo registro de *Rhabdias* parasitando *Hypsiboas raniceps* Stiles & Hassal 1905 em uma área de Cerrado é apresentado neste trabalho. O gênero é comumente encontrado parasitando pulmões de anfíbios e reptéis no mundo todo. Foram analisados sete espécimes de *H. raniceps*, onde dois estavam parasitados com nematódeos do gênero *Rhabdias* nos pulmões. Um total de seis nematódeos foram encontrados, e identificados com base na morfologia das estruturas, e nas medidas realizadas nos espécimes. Esta nota apresenta o primeiro registro de *Rhabdias* em *H. raniceps*, sendo assim a espécie um novo hospedeiro de *Rhabdias*.

Palavras chaves: America do sul - Cerrado - *Hypsiboas raniceps* - *Rhabdias*

INTRODUCTION

The *Rhabdias* Stiles & Hassal 1905, is a worldwide widely distributed genus, whose hosts include several species of amphibians and reptiles (Baker, 1987; Bursey *et al.*, 2003; González & Hamann, 2006). They are typical lung parasites, considered specific and rarely parasitize more than one host (Tkach *et al.,* 2006). Until now 100 nematode species of the *Rhabdias* genus have been registered worldwide (Kuzmin & Tkach, 2014), in the neotropical region, 15 species parasitize amphibians: *R. alabialis* Kuzmin *et al.*, 2007, *R. androgyna* Kloss, 1971, *R. breviensis* Nascimento *et al.*, 2013, *R. elegans* Gutiérrez, 1945, *R. fuelleborni* Travassos, 1926, *R. hermafrodita* Kloss, 1971, *R. kuzmini* Martínez-Salazar & León-Règagnon, 2007, *R. manantlanensis* Martínez-Salazar, 2008, *R. mucronata* Schuurmans-Stekhoven, 1952, *R. paraensis* Santos *et al.*, 2011, *R. peninsularis* Martínez-Salazar *et al.*, 2013, *R. pseudosphaerocephala* Kuzmin *et al.*, 2007, *R. savagei* Bursey & Goldberg, 2005, *R. tobagoensis* Moravec & Kaiser, 1995, and *R. truncata* Schuurmans-Stekhoven, 1952 (Travassos, 1926; Gutierrez, 1945; Schuurmans-Stekhoven, 1952; Kloss, 1971; Moravec & Kaiser, 1995; Bursey & Goldberg, 2005; Kuzmin *et al.*, 2007; Martínez-Salazar & León-Règagnon, 2007; Martínez-Salazar, 2008; Santos *et al*., 2011; Martínez-Salazar *et al*., 2013; Nascimento *et al.*, 2013; Kuzmin *et al*., 2015).

Kuzmin *et al.* (2016) has just described two new *Rhabdias* species: *Rhabdias galactonoti and Rhabdias stenocephala,* found parasitizing the lung of *Leptodactylus pentadactylus* and *L. paraensis* in the state of Pará, Brazil.

Hypsiboas raniceps Cope, 1862 is a Neotropical Hylidae commonly found in Cerrado and in Caatinga (Guimarães & Bastos, 2003). The specie is considered stable in the endangered animals list extinction of UCN, sometimes found in the international pet trade but at levels that do not currently constitute a major threat (La Marca *et al.,* 2004), It occurs in the central and northeastern regions of Brazil, with *H. raniceps* also inhabiting the Brazilian Amazon Basin, southern Colombia, north of French Guyana, Bolivia, Paraguay and northeast Argentina (Frost, 2009). The aim of the analysis is to report of *Rhabdias* (Nematoda: Rhabdiasidae) in *H. raniceps,* Cerrado, Brasil.

MATERIALS AND METHOD

During a field work in search of Helminthological studies, 7 individual *Hypsiboas raniceps* were collected, in December 2015 in a marsh area in the field of Teaching, Research and Extension - FEPE/UNESP-Universidade Estadual Paulista "Júlio de Mesquita Filho located in the city of Selvíria, in the state of Mato Grosso do Sul, under the SISBio 36667-2 license, the 06/2014/CEUA Ethics Committee Protocol and Animal Use. For specimen capture, a visual search method (VS) was carried out in the species habitat during the species activity period. Next, the amphibians were acclimated in 2 liter plastic bags and transported alive to the Ecology and Parasitism Laboratory, of the Ilha Solteira Campus – UNESP. The specimen was sacrificed by a lethal dose of anesthetic Benzocaine (according to Resolution No. 714/2002), weighed, measured and necropsied and their lungs removed for examination under the stereomicroscope.

The nematodes found were collected with thin brushes and stylets, and transferred to a Petri dish with saline solution of 0.65%, to carry out individual cleaning. Once clean, they were fixed in 70% alcohol heated to approximately 65-70 ºC, for muscle stretch. Slides were

mounted using Lactophenol of Amann enabling the visualization of parts of taxonomic importance of the group necessary for identification. Then, the specimen was transferred to vials containing 70% alcohol, where they remained preserved (Amato *et al.,* 1991). Later, LEICA DM 2500 photomicrographic microscopes from the Ecology and Parasitism Laboratory – LECOP, UNESP, of the Ilha Solteira Campus, were used. All the helminths found will be deposited in the Helminthological Collection of the Institute of Biosciences (CHIBB), of the State Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, Botucatu campus. In the analysis carried out, all the helminths found in other organs, are part of a study to be published later.

RESULTS

The analyzed amphibians presented a mean width of 7.84±0,60 cm and mean weight of 10.24±1,97. Of the seven analyzed individuals, only two were parasitized with *Rhabdias* sp. totaling six nematode adults. The parasitism in *H. raniceps*, presented a prevalence of 28.57%, mean abundance of 0.85, mean intensity of 3 (according to Bush *et al.,* 1997, Serra-Freire, 2002). The measurements were carried out based on all the specimen found. Based on the specimen structural morphologies analyzed, these were identified as belonging to the Rhabdiasidae family, of the *Rhabdias* genus.

Description of the *Rhabdias* Stiles & Hassal, 1905 genus (Fig. 1).

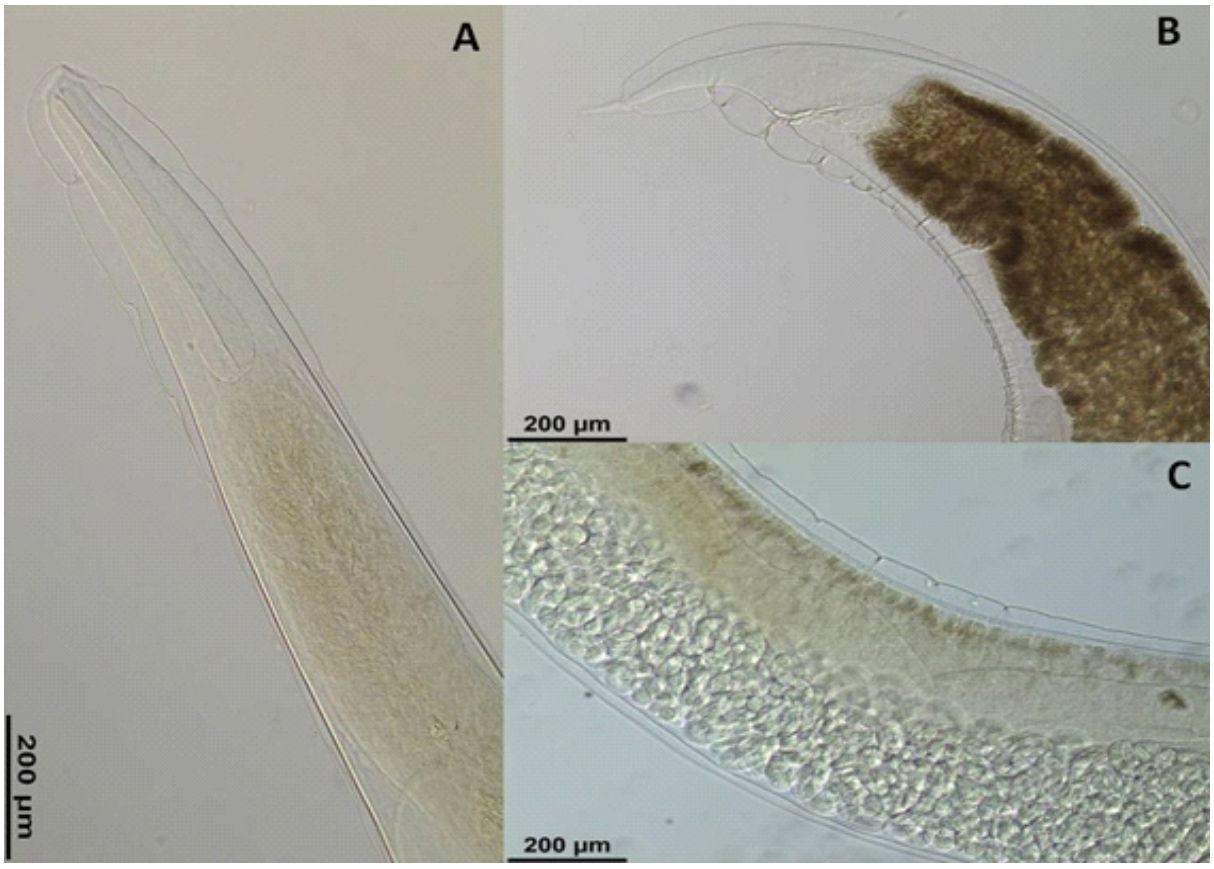

Figure 1. A-C: A- portion anterior, B- portion posterior and tail C- eggs.

Parasitic generation with much larger life-free specimens; mouths surrounded by six very small lips, sometimes with lateral wings which are wider anteriorly and posteriorly. Small cup-shaped oral capsule; small, cylindrical esophagus, ending in a later dilation; conical tail; vulva near the middle of the body; didelfa, anfidelfa, oviporous; thin-shell eggs containing a morula or underdeveloped larva.

Family: Rhabdiasidae Railliet, 1915
Genus: *Rhabdias* Stiles & Hassall 1905
Host: *Hypsiboas raniceps* (Cope, 1862)
Site infection: Pulmão
Locality: Selvíria, Mato Grosso do Sul, Brasil

Description (based on 6 specimen): Body size 11,099-4,567; body width 0,565-0,443; length of esophagus 0,582-0,546; width of esophagus 0,067-0,051; nervous ring at anterior far-end 0,173-0,149; egg length 0,111-0,103; egg width 0,060-0,054; posterior far-end anus 0,471-0,207.

DISCUSSION

Data about *H. raniceps* helminth are scarce, and the following parasitic species are recorded in literature as follows: *Aplectana hylambatis* Gonzales & Hamann, 2006, *Cosmocerca parva* Gonzales & Hamann 2011, *Oswaldocruzia proencai* Lent *et al.*, 1946; Masi & Maciel, 1974, *Glypthelmins vitelliniphilum* Travassos *et al*, 1969, and *G. vitellinophilum* Travassos *et al*, 1969 and *Ophiotaenia* sp. La Rue, 1911 in Paraguay (La Rue, 1911; Lent *et al.,* 1946; Lent & Freitas, 1948; Dobbin, 1958; Travassos *et al*, 1969; Masi & Maciel, 1974; Chambrier *et al*, 2006; Gonzales & Hamann, 2006, 2011).

Queiroz (2015) found in the same place, *Rhabdias* parasitizing *Leptodactylus chaquensis, L. mystacinus* and *L. podicipinus* individuals, which are completely terrestrial animals, while *H. raniceps* is an arboreal animal (Hamann *et al.*, 2006). Therefore, field observations are necessary to understand the mode of infection of these parasites in the lungs. During the literature review, no information about *Rhabdias* sp. in *H. raniceps* was found in South America. This implies that this amphibian is a new *Rhabdias* host, therefore reinforcing the importance and need of studies related to the hosts and their parasites.

ACKNOWLEDGEMENTS

We thank the Ecology and Parasitism Laboratory team – UNESP for their contribution to this field work.

REFERÊNCIAS BIBLIOGRÁFICAS

Amato, JFR, Boeger, WA & Amato, SBC. 1991. *Protocolos para laboratório-Coleta e processamento de parasitos de pescado*. Imprensa Universitária: Rio de Janeiro.

Baker, 1987. *Synopsis of the nematoda parasitic in amphibians and reptiles*. Memorial University of Newfoundland. Occasional papers in Biology, vol. 11. pp 325.

Bursey, C, Goldberg, RS & Telford, SRJr. 2003. Rhabdias anolis *sp. nov.(Nematoda: Rhabdiasidae) from the lizard,* Anolis frenatus *(Sauria: Polychrotidae), from Panama*. Journal Parasitology, vol. 89, pp. 113-117.

Bursey, CR, & Goldberg, SR. 2005. *New species of* Oswaldocruzia *(Nematoda: Molineoidae), new species of* Rhabdias *(Nematoda: Rhabdiasidae), and other helminths in* Rana *cf.* forreri *(Anura: Ranidae) from Costa Rica*. Journal

Parasitology, vol. 91, pp. 600–605.

Bush, AO, Lafferty, KD, Lotz, JM & Shostak, AW. 1997. *Parasitology meets ecology on its own terms: Margolis et al.* revisited. Journal of Parasitology, vol. 83, pp. 575–583.

Chambrier, A, Coquille, SC & Brooks, DR. 2006. Ophiotaenia bonneti *sp. n. (Eucestoda: Proteocephalidea), a parasite of* Rana vaillanti *(Anura: Ranidae) in Costa Rica*. Folia Parasitologica vol. 53, pp. 125-133.

Dobbin, Jr JE. 1958. Glypthelmins vitellinophilum *sp. n., parasito de* Hyla raniceps *Cope*. Memórias do Instituto Oswaldo Cruz, vol. 56, pp. 153-157.

Frost, D. 2009. *Amphibian Species of the World: an Online Reference.* Version 5.2 (1998-2009). Available in: http://research.amnh.org/herpetology/amphibia/index.php. Acess in 25 June, 2016.

Gonzalez, CE. & Hamann, MI. 2006. *Nematodes parásitos de* Chaunus granulosus major *(Müller & Hellmich, 1936) (Anura: Bufonidae) en Corrientes, Argentina.* Cuadernos de Herpetología, vol. 20, pp. 43–49.

Gonzalez, C.E. & Hamann, M.I. 2011. Cosmocercid Nematodes of three species of frogs (Anura: Hylidae) from Corrientes, Argentina. Comparative Parasitology, vol. 78, pp. 212–216.

Guimarães, LD. & RP. Bastos. 2003. Vocalizações e interações acústicas em Hyla raniceps (Anura, Hylidae) durante a atividade reprodutiva. Iheringia, vol. 93, pp. 149-158.

Gutiérrez, RO. 1945. *Contribución al conocimiento de los nemátodos parásitos de anfibios argentinos.* Thesis. Museo de La Plata, Universidad Nacional de La Plata, La Plata, Argentina.

Hamann, MI. Kehr, AI, CE & González. 2006. *Species affinity and infracommunity ordination of helminths of* Leptodactylus chaquensis *(Anura: Leptodactylidae) in two contrasting environments from Northeastern Argentina*. Journal of Parasitology, vol. 92, pp. 1171–1179.

Kloss, GR. 1971. *Alguns* Rhabdias *de Bufo no Brasil*. Papéis Avulsos de Zoologia. São Paulo, vol. 24, pp. 1–52.

Kuzmin, Y. Tkach, VV & Brooks, DR. 2007. *Two new species of* Rhabdias *(Nematoda: Rhabdiasidae) from the Marine toad, Bufo marinus (L.) (Lissamphibia: Anura: Bufonidae), in Central America*. Journal of Parasitology, vol. 93, pp.159-165.

Kuzmin, Y & Tkach VV. 2014. *Rhabdias*. World Wide Web electronic publication. Available in http://izan.kiev.ua/ppages/rhabdias. Acess in 29 of July 2016.

Kuzmin, Y, Preez LH & Junker K. 2015. *Some nematodes of the genus* Rhabdias *Stiles et Hassall, 1905 (Nematoda: Rhabdiasidae) parasitising amphibians in French Guiana*. Folia Parasitologica, 62: 031.

Kuzmin, Y, Melo FTV, Silva Filho HF, Santos, JN. 2016. Two new species of Rhabdias *Stiles et Hassall, 1905 (*Nematoda: Rhabdiasidae) from anuran amphibians in Pará, Brazil. Folia Parasitologica, 63: 015.

La Rue GR. 1911. A revision of the cestode family Proteocephalidae. Zoologischer Anzeiger, vol. 38. pp. 473–482.

La Marca, E, Azevedo-Ramos, C, Silvano, D, Scott, N, Aquino, L, & Faivovich, J. 2004. *Hypsiboas raniceps*. The IUCN Red List of Threatened Species. Available in http://www.iucnredlist.org/details/55622/0. Acess on 26 September 2016.

Lent, H., J. F. T. Freitas, and M. Proenca. 1946. *Alguns helmintos de batraquios colecionados no Paraguai*. Memorias do Instituto Oswaldo Cruz Rio de Janeiro, vol 44, pp. 195–214.

Lent, H & Freitas, JFT. 1948. *Una coleção de*

nematodeos, parasitos de vertebrados, do Museu de Historia Natural de Montevideo. Memórias do Instituto Oswaldo Cruz, vol. 46, pp. 1-71.

Masi P.R. & Maciel, S. 1974. *Helminthes en batracios del Paraguay (1°Parte), con descripción de una nueva especie* Aplectana pudenda *(Oxyuridae: Cosmocercinae*). Revista Paraguaya de Microbiología, vol. 9, pp. 55-60.

Martínez-Salazar, EA & León-Règagnon, V. 2007. *New species of* Rhabdias *(Nematoda: Rhabdiasidae) from* Bufo occidentalis *(Anura: Bufonidae) from Sierra Madre del Sur, México*. Journal of Parasitology, México, vol. 93, pp.1171-1177.

Martínez-Salazar, EA. 2008. *A new rhabdiasid species from* Craugastor occidentalis *(Anura: Brachycephalidae) from Sierra de Manantlán, Jalisco, Mexico*. Revista Mexicana Biodiversidad, vol. 79, pp. 81-89.

Martínez-Salazar, EA, Falcón-Ordaz J, González-Bernal E, Parra-Olea G & Pérez-Ponce De León G. 2013. *Helminth parasites of* Pseudacris hypochondriaca *(Anura: Hylidae) from Baja California, Mexico, with the description of two new species of nematodes*. Journal of Parasitology, vol. 99, pp. 1077–1085.

Moravec, F & Kaiser, H. 1995. *Helminth parasites from West Indian frogs, with descriptions of two new species. Caribbean*. Journal of Science, vol. 31, pp. 252-268.

Nascimento, LC, Gonçalves EC, Melo, FT, Giese, EG, Furtado AP & Santos, JN. 2013. *Description of* Rhabdias brevi*ensis n. sp. (Rhabditoidea: Rhabdiasidae) in two Neotropical frog species*. Systematic Parasitology, vol. 86, pp. 69–75.

Queiroz, MS. 2015. *Helmintofauna de anfíbios da família Leptodactylidae em três fisionomias de Cerrado*. Dissertação de mestrado em Ciências biológicas-Zoologia. Universidade Estadual Paulista Júlio de Mesquita Filho, Instituto de Biociências de Botucatu.

Santos, JN, Melo, FTV, Nascimento, LCS, Nascimento, DEB, Giese, EG & Furtado, AP. 2011. Rhabdias paraensis *sp. nov.: a parasite of the lungs of* Rhinella marina *(Amphibia: Bufonidae) from Brazilian Amazonia*. Memórias do Instituto Oswaldo Cruz, vol. 106. pp. 433–440.

Serra-Freire, NM. 2002. *Planejamento e análise de pesquisas parasitológicas*. Universidade Federal Fluminense. Niterói. 199p.

Schuurmans-Stekhoven, JHS.1952. *Nemátodos parásitos de anfibios, pájaros y mamíferos de la República Argentina*. Acta Zoologica Lilloana, vol. 10, pp. 315–400.

Tkach, VV, 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, vol. 92, pp. 631–636.

Travassos, L. 1926: Entwicklung des Rhabdias fülleborni n. sp. Archiv für schiffs- und tropen-hygiene, vol. 30, pp. 594–602.

Travassos, L, Freitas, T & Kohn, A. 1969. *Trematódeos do Brasil*. Memórias do Instituto Oswaldo Cruz, vol. 67, pp. 1-886.

Received August 1, 2016.
Accepted September 28, 2016.