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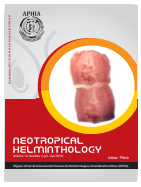

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

NEMATODES PARASITES OF *RHINELLA JIMI* (STEVAUX, 2002) (ANURA: BUFONIDAE) IN AREAS OF CAATINGA, NORTHEASTERN BRAZIL

NEMÁTODOS PARÁSITOS DE *RHINELLA JIMI* (STEVAUX, 2002) (ANURA: BUFONIDAE) EN ÁREAS DE CAATINGA, NORDESTE DE BRASIL

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ABSTRACT

Amphibians harbor a wide variety of parasites, acting as paratenic, intermediary and definitive hosts. Actually, just 8% of Brazilian amphibians have been investigated regarding associated helminths. Herein, we analyzed 20 specimens of the toad *Rhinella jimi* Stevaux, 2002 from Ceará and Rio Grande do Norte states. Seven taxa of nematodes were found infecting *R. jimi* (*Oswaldocruzia lopesi* Travassos, 1938, *Raillietnema spectans* Gomes, 1964, *Physaloptera* sp., *Parapharyngodon* sp., *Rhabdias* sp., Cosmocercidae, and Unidentified Nematoda larvae). All nematode species are new records for *R. jimi*. These results expand the Nematode records for *R. jimi* as well as increase knowledge of the diversity of parasites in anurans in South America.

Keywords: Helminth – Neotropical – *Oswaldocruzia lopesi* – *Rhabdias* – toads – Semiarid

RESUMEN

Los anfibios hospedan una amplia variedad de parásitos, sea de forma temporal (hospedero paraténico), intermediaria o definitiva. Solamente un 8% de los anfibios en Brasil han sido investigados con relación a sus helmintos asociados, por lo que en este trabajo analizamos 20 especímenes de *Rhinella jimi* Stevaux, 2002 colectados en los estados de Ceará y Rio Grande do Norte, en la región Nordeste de Brasil. Siete taxones de nematodos se encontraron infectando individuos de *R. jimi* (*Oswaldocruzia lopesi* Travassos, 1938, *Raillietnema spectans* Gomes, 1964, *Physaloptera* sp., *Parapharyngodon* sp., *Rhabdias* sp., Cosmocercidae, y una larva no identificada). Todas las especies encontradas representan nuevos registros de parásitos para *R. jimi,* resultado que amplía los registros de nematodos para esta especie, así como aumentan el conocimiento de la diversidad de parásitos en anuros para América del Sur.

Palabras clave: Helmintos – Neotropical – *Oswaldocruzia lopesi* – *Rhabdias* – Sapos – semiárido

INTRODUCTION

Amphibians harbor a wide variety of parasites, acting as paratenic, intermediary and definitive hosts (Santos *et al.,* 2013; Campião *et al.,* 2014; Teles *et al.,* 2018). Environmental characteristics, different patterns of life cycles and reproductive strategies are the main factors influencing helminth infection in amphibians (Aho, 1990; Campião *et al.,* 2012; 2014; Toledo *et al.,* 2017; Sena *et al.,* 2018).

Brazil has the highest diversity of amphibians in the world, especially in anurans with 1040 species (Segalla *et al.,* 2016). Most studies on helminths infecting amphibians in South America are from Brazilian species (Santos *et al.,* 2013; Campião *et al.,* 2014; Martins-Sobrinho *et al.,* 2017; Teles *et al.,* 2018). However, only 8% of Brazilian amphibians have been studied so far regarding helminth associated (Santos *et al.,* 2013; Campião *et al.,* 2014; Teles *et al.,* 2018).

Rhinella jimi (Stevaux, 2002) is a widespread bufonid inhabiting Caatinga areas from Northeastern Brazil (Stevaux, 2002; Loebernan & Roberto & Loebmann, 2016, Segalla *et al.,* 2016). The species is also found frequently in rural and urban areas, being an opportunistic feeder of insects and small vertebrates (Moreira & Barreto, 1996; Stevaux, 2002), and status of conservation least concern (LC) according to the IUCN (2019). According to Campião *et al.* (2014) and Müller *et al.* (2018), only six species of helminths have been reported for *R. jimi*: the trematode *Gorgoderina rochalimae* Dobin Jr., 1957 and the nematodes *Aplectana membranosa* Schneider, 1866; Miranda, 1924, *Oswaldocruzia subauricularis* Rudolphi, 1819; *Rhabdias fuelleborni* Travassos, 1926, *Rhabdias sphaerocephala* Goodey, 1924 and *Rhabdias pseudosphaerocephala* Kuzmin *et al.,* 2007. Herein, we present data on nematodes infection in the toad *Rhinella jimi* from areas of Caatinga of two Brazilian states.

MATERIAL AND METHODS

We analyzed a total of 20 specimens of *R. jimi*, being 5 adult males (mean snout-vent length (SVL) = 108.34 ± 38.92), 2 adult females (mean SVL = 99.59 ± 41.30) and 13 juveniles (mean SVL = 66.60 ± 15.65). Specimens were collected manually from 2006 to 2014, in eight municipalities on Ceará state: Aiuaba (n=2) (06° 36′ S, 40° 07′ W), Acopiara (n=1) (6° 5' S, 39° 27' W), Caucaia (n=1) (3° 44' S, 38° 39' W), Barbalha (n=2) (7° 18′ S, 39° 18′ W), Farias Brito (n=1) (06º 55' S 39º 33' W), Santana do Cariri (n=1) (7° 11' S, 39° 44' W), Nova Olinda (n=5) (7° 34' S, 39° 4' W), and Brejo Santo (n=1) (7° 29' S, 38° 58' W); Additionally, six specimens from the municipality of João Câmara (05° 33′ S, 35° 56′ W) in the state of Rio Grande do Norte were also examined. Caatinga covers these areas with a semiarid climate (IDEMA, 2008; IPECE, 2016).

After the capture, specimens were euthanized with lethal injection of sodium Thiopental (Thiopentax®), and have their SVL measured with a digital caliper (precision 0.01 mm). Sex was determined by visual inspection of gonads. Specimens were then fixed in formalin 10% (Franco & Salomão, 2002) and deposited in the Coleção Herpetológica da Universidade Regional do Cariri (URCA–H 471–476, 1737, 3024, 3164, 3413, 4192, 4193, 4875, 7956, 8399, 8400–8403, 8928). Toads were necropsied with a midventral incision and all organs and coelomic cavity were searched for helminths. These parasites found were preserved in 70% ethyl alcohol.

Nematodes found were mounted in temporary slides (Yamaguti, 1961), cleared in lactophenol and analyzed under microscope with computerized image analysis system (Carl Zeiss Microimaging GmbH, Gottingen, Germany). Thereafter, the helminths were deposited in the Coleção Parasitológica da Universidade Regional do Cariri (URCA-P).

Ethic aspects: The collecting methods were defined and authorized by the regulatory the ethics committee of Universidade Regional do Cariri (CEUA/URCA, process No. 00260/2016.1).

RESULTS

Of the 20 toads examined, 13 (65%) were infected

by at least one species of Nematode, and 191 nematodes specimens were recovered.
Seven taxa of nematodes were found infecting *R. jimi*: *Oswaldocruzia lopesi* Travassos, 1938, *Raillietnema spectans* Gomes, 1964, *Physaloptera sp., Parapharyngodon sp., Rhabdias sp.*, unidentified Cosmocercidae Travassos, 1925, and encysted larvae of unidentified nematodes. All represent are new records for *R. jimi* (Table 1).

Table 1. Site of infection of the Nematodes community associated with *Rhinella jimi* in areas of Caatinga, Northeastern Brazil.

Nematoda	Site of infection
Oswaldocruzia lopesi	Stomach, Small intestines
Physaloptera sp.	Stomach
Cosmocercidae	Large intestines, Small intestines
Parapharyngodon sp.	Large intestines, Small intestines, Stomach
Encysted larvae (unidentified nematodes)	Small intestines
Raillietnema spectans	Large intestines
Rhabdias sp.	Lungs

DISCUSSION

Nematoda phylum was the only one recorded infecting the toad *R. jimi* in the present study, likewise other parasitological studies with species of the genus *Rhinella* (e.g. Santos, *et al.,* 2013; Teles *et al.,* 2018). The exclusive infection by nematodes may be related to host life cycle and foraging strategies (Yoder & Coggins 2007; Santos *et al.,* 2013). In anurans, the occurrence of nematodes may be associated with the time spent in water or on land (Aho, 1990; Anderson, 2000; Yoder & Coggins 2007; Santos *et al.,* 2013). In terrestrial habitats most nematodes infect anurans by skin penetration or egg ingestion, which may explain the infection in individuals of the genus *Rhinella* (Aho, 1990; Anderson, 2000; Bolek & Coggins 2000, Santos *et al.,* 2013*;* Teles *et al.,* 2018).

All nematodes found in the study present are monoxenous, except *Physaloptera* sp. Species of the genus *Physaloptera* have already been recorded infecting the stomach of mammals, fish, reptiles and amphibians, including anurans of the Bufonidae family, where this parasite is commonly recorded in larval stage (Anderson, 2000; Ávila & Silva, 2010; Santos *et al.,* 2013; Aguiar *et al.,* 2014; Campião *et al.,* 2014; Teles *et al.,* 2018). In the present study, we recorded an adult specimen, however, due to the preservation conditions, it was not possible to identify to species level. Infection by parasites of this genus may occur by ingestion of parasitized arthropods (Anderson, 2000; Campião *et al.,* 2014, 2015).

Unidentified nematodes of the Cosmocercidae family are frequently recorded infecting reptiles and amphibians (Avila & Silva, 2010; Campião *et al.,* 2014) and have been found to parasite anurans of the genus *Rhinella* (Santos *et al.,* 2013; Campião *et al.,* 2014; Toledo *et al.,* 2017; Teles *et al.,* 2018). These parasites can cause infection when ingested or penetrated through the skin of the host (Anderson, 2000). Encysted larvae of unidentified nematodes were also recorded in the present study. The occurrence suggests that *R. jimi* may be acting as intermediate or paratenic hosts of these parasites (Anderson, 2000).

Rhabdias Stiles & Hassal, 1905, are common pulmonary parasites of amphibians and reptiles (Anderson, 2000; Ávila & Silva, 2010; Campião *et al.,* 2014). Infection in anurans may occur by direct penetration into the skin of the host, however the life cycle of these parasites is alternated between a free and parasitic life stage, but only females act as parasites, infecting the lungs of their hosts (Anderson, 2000). In South America there are more

than 19 *Rhabdias* species reported parasitizing amphibians (Kuzmin *et al.,* 2016., Muller *et al.,* 2018), including *R. jimi*, infected by *R. fuelleborni*, *R. sphaerocephala* and *R. pseudosphaerocephala* (Vicente *et al.,* 1991; Campião *et al.,* 2014; Kuzmin *et al.,* 2016; Muller *et al.,* 2018).

The molineid *Oswaldocruzia lopesi* usually infects the stomachs of lizards and amphibians (Ávila & Silva; Campião *et al.,* 2014). In toads of the genus *Rhinella*, *O. lopesi* have already been reported intecting *R. icterica, R. margaritifera* and *R. marina* (Campião *et al.,* 2014). The nematode *Raillietnema spectans* is a common anuran parasite (Vicente *et al.,* 1991; Campião *et al.,* 2014; Alcântara *et al.,* 2018; Teles *et al.,* 2018). In Brazil, it has been reported infecting *Leptodactylus latrans*, *Pleurodema diplolister*, *Dermatonotus muelleri*, *R. crucifer, R. icterica, R. diptycha, R. granulosa,* and *R. jimi* (Vicente, *et al.,* 1991; Campião *et al.,* 2014; Teles, *et al.,* 2015; Alcântara *et al.,* 2018; Teles *et al.,* 2018; present study).

Infection by *Parapharyngodon* spp*.,* can occur by coprophagy or through ingestion of infected larvae (Anderson, 2000). Despite being considered typical parasites of lizards, many studies report their occurrence in anurans (e.g Ramallo *et al.,* 2002; Bursey *et al.,* 2013; Araújo-Filho *et al.,* 2015; Alcântara *et al.,* 2018). To date it has been recorded infecting species of the families Eleutherodactylidae, Hylidae and Microhylidae and Bufonidae (Campião *et al.,* 2014; Araújo-Filho *et al.,* 2015; Alcântara *et al.,* 2018), including *R. marina*, *R. icterica* and *R. jimi* (Luque *et al.,* 2005; Bursey *et al.,* 2013, present study).

Rhinella jimi was infected by seven nematode taxa, all new host records. Previous studies with Anurans of genus *Rhinella* in South America recorded helminth richness for *R. fernandezae, R. major, R. icterica* greater than 10 species (Luque *et al.,* 2005; Santos & Amato *et al.,* 2010; Hamann *et al.,* 2013; Santos *et al.,* 2013; Hamann & González, 2015; Toledo *et al.,* 2017). However, individuals of *Rhinella* genus recorded so far regarding occurrence of helminths, a large (*R. jimi*; *R. marina*, *R. diptycha* (=*R. schneideri*), *R. spinulosa, R. bergi, R. granulosa, R. limensis, R. poeppigii*) has richness less than 9 (Galicia-Guerrero *et al.*, 20022; Chero *et al.,* 2015; 2016; Espínola-Novelo *et al.,* 2017; Toledo *et al.,* 2017; Teles *et al.,* 2018, present study). Compared to other South American bufonids, *R. jimi* presented a relatively high parasite richness (e.g. Santos & Amato, 2010; Toledo *et al.,* 2017; Teles *at al.,* 2018). The species richness recorded in *R. jimi* may be related to the environmental conditions that may favor infection, as well as the low nematode specificity (Aho, 1990; Bolek & Coggins 2000; Campião *et al.,* 2012; Sena *et al.,* 2018).

In conclusion *R. jimi* presented seven new nematode registries, demonstrating that the parasites richness associated with Bufonidae Family, especially genus *Rhinella*, is more diverse than one currently documented. This study contributed to increase inventory of helminths for Anurans in South America.

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