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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

HELMINTHS INFECTING THE BOM JARDIM TOAD *RHINELLA DAPSILIS* FROMAN ATLANTIC FOREST ENCLAVE AT NORTHEASTERN BRAZIL

HELMINTOS INFECTANDO EL SAPO BOM JARDIM *RHINELLA DAPSILIS* DE UN ENCLAVE DE LA MATA ATLÁNTICA

HELMINTOS INFECTANDO O SAPO BOM JARDIM *RHINELLA DAPSILIS* DE UM ENCLAVE DA MATA ATLÂNTICA NORDESTE DO BRASIL

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Running Head: Helminths infecting *Rhinella dapsilis*

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ABSTRACT

*Rhinella dapsilis (*Myers and Carvalho, 1945) is widespread in Amazonia, from Colombia to Brazil. Despite its huge distribution, information on basic biological aspects is scarce, even more regarding helminths parasites. Herein, we present data on helminth infecting *R. dapsilis* from an Atlantic Forest enclave from Northeastern Brazil. Ten taxa were found, being nine nematodes and one acanthocephalan, Cosmocercidae and *Rhabdias* sp. was the most prevalent taxa. We reported six new host records and expanded the knowledge about parasitism in Neotropical anurans.

Keywords: Amphibian – Bufonidae – highland marshes – Parasites

RESUMEN

*Rhinella dapsilis (*Myers and Carvalho, 1945) es ampliamente distribuida desde Colombia hasta Brasil. Sin embargo, a pesar de su amplia distribución, las informaciones al respecto de los aspectos básicos de su biología son escasos, especialmente en cuanto a los parásitos helmínticos. Presentamos datos de infecciones por helmintos en *R. dapsilis* de una parte de la Mata Atlántica en el Nordeste brasileño. Diez taxones fueron encontrados, entre ellos, nueve nematodos y uno acantocéfalo. Cosmocercidae y *Rhabdias* sp. siendo los más frecuentes. Presentamos seis nuevos registros, expandiendo el conocimiento sobre el parasitismo para los anuros Neotropicales.

Palabras-clave: Anfibios – Bufonidae – Pantano de altitud – Parásitos

RESUMO

*Rhinella dapsilis (*Myers and Carvalho, 1945) é amplamente distribuída na Amazônia, desde a Colômbia até o Brasil. Apesar da ampla distribuição, informações sobre aspectos básicos de sua biologia são escassas, especialmente quanto a parasitas helmínticos. Aqui apresentamos dados de helmintos infectando *R. dapsilis* de um enclave de Mata Atlântica no nordeste brasileiro. Dez táxons foram encontrados, sendo 9 nematódeos e um acantocéfalo. Cosmocercidae e *Rhabdias* sp. foram os táxons mais prevalentes. Apresentamos seis novos registros e expandimos o conhecimento acerca de parasitismo para anuros neotropicais.

Palavras-chave: Anfíbios – Bufonidae – Brejos de altitude – Parasitas

INTRODUCTION

Rhinella dapsilis (Myers and Carvalho, 1945), is a leaf-litter toad widely distributed in Amazonia, occurring in Colombia, Peru, Ecuador and Brazil (Ávila *et al*., 2018; Frost, 2024). In Brazil, *R. dapsilis* occurs in Amazonia at Acre, Amapá, Amazonas and Pará states, also being recorded in transition zones at Cerrado (Goiás, Maranhão and Tocantins states) and Atlantic Forest (Pernambuco and Alagoas states), including in a rainforest enclave inside Caatinga domain in Ceará state (Fouquet *et al*., 2024). As many of the species within the *R. margaritifera* group, *R. dapsilis* have serious taxonomic problems, with differentiation between *R. margaritifera* being extremely difficult (Caramaschi & Pombal, 2006; Vaz-Silva *et al*., 2015; Ávila *et al*., 2018; Pereyra *et al*., 2021; Fouquet *et al*., 2024). Despite this huge distribution, very little is known about its basic biological aspects, with habitat use, diet and defensive behavior already

published (Brito *et al*., 2013).

To date, 25 helminths are known to infect *R. dapsilis* (most of the records under the name *R. margaritifera*) from Peru, Ecuador and Brazil (Campião *et al*., 2014; Willkens *et al*., 2016; Chero *et al*., 2023). Those records, however, are restricted to Amazonia, without studies from Atlantic Forest populations. This lack of knowledge difficult the understanding of host-parasite relationships (Campião *et al*., 2009; Santos & Amato, 2010; Santos & Borges-Martins, 2013). Herein, we examined the helminths infecting *R. dapsilis* from an Atlantic Forest enclave in Northeastern Brazil.

MATERIAL AND METHODS

Specimens of *R. dapsilis* were collected at the Guaramiranga municipality (4° 15′ 46′ S, 38° 55′ 58′ O), located at the Baturité massif, a rainforest enclave within Caatinga Domain, Brazil. The climate is classified as tropical sub-hot humid and tropical hot humid, with 1,737.5 mm of rainfall per year and average temperatures of 24º to 26º. The rainy season occurs from January to May and the vegetation is characterized by evergreen and seasonal evergreen forest, classified as a remnant of the Atlantic Forest (IPECE, 2017; Silvera *et al*., 2020).

Specimens of *R. dapsilis* were collected from 2019 to 2024 through active searches or pitfall traps with drift fences and deposited at Coleção Herpetológica da Universidade Federal do Ceará (CHUFC-A 9975, 12286-89, 12664-68, 12670, 12283-85, 12290-93). Eighteen individuals were collected, euthanized with a lethal injection of lidocaine (collecting permit SISBIO 29613-1).

The snout-vent length (SVL) of the specimens were measured with a digital caliper, necropsied through a midventral incision and all organs and body cavity were dissected for the search of parasitic helminths. The helminths were collected

and preserved according to Amato *et al*. (1991) and Andrade (2000). For identification, the nematodes were clarified in lactic acid, while acanthocephalans were stained according to the hydrochloric carmine protocols and then clarified with eugenol on temporary slides (Amato *et al*., 1991). Helminth identification was performed following Yamaguti (1961), Sprent (1978), Vicente *et al*. (1991), Anderson (2000) and Gibbons (2010).

Slides were analyzed under a microscope with a computerized image analysis system. Voucher helminths were deposited at Coleção Parasitológica of the Universidade Federal do Ceará. Parasitological descriptors (prevalence, mean intensity of infection and abundance) were calculated according to the specifications of Bush *et al*. (1997).

Ethic aspects: This study was approved by the Animal Ethics Committee of the Universidade Federal do Ceará (CEUA-UFC, process # CEUA 6314010321).

RESULTS

A total of 770 helminths were found infecting *R. dapsilis*, comprising 10 taxa. The helminths belonged to the phyllum Nematoda (767 individuals from 9 taxa) and Acanthocephala (3 individuals from 1 taxa). The parasites showed an overall prevalence of 100%, mean overall infection intensity of 45.33 ± 6.40 and mean overall abundance of 45.33 ± 6.40. The highest helminth abundance was Cosmocercidae, followed by *Rhabdias* sp. and *Oxysomatium petrolinensis* (Félix-Nascimento 2020) (Table 1). Most endoparasites found to *R. dapsilis* were located on the lungs (n= 324), followed by the large intestine (n= 295). By contrast, the body cavity and bladder were less infected with a total of 4 and 8 endoparasites, respectively. Six taxa were reported for the first time infecting *R. dapsilis* (Table 1).

DISCUSSION

Most of the parasites found here belong to the family Cosmocercidae. Like many studies (Lins *et al*., 2017; Oliveira *et al*., 2019), Cosmocercidae gen. sp. is not identified due to difficulties in determining morphological characters or the absence of males, in which reproductive organs are essential for species determination. The species of the family, including the identified species here (*Aplectana membranosa* (Schneider 1866), *Cosmocerca parva* (Travassos, 1925) and *Oxysomatium petrolinensis*) have a direct life cycle, and their hosts become infected by ingesting infective larvae or actively penetrating larvae present in the soil (Anderson, 2000). Of the species found here, only *C. parva* have been already reported for *R. dapsilis* (as *R. margaritifera*; Campião *et al*., 2014). *A. membranosa* have been reported infecting other six bufonids (Campião *et al*., 2014) and *O. petrolinensis* were recently described infecting *Leptodactylus macrosternum* (Miranda-Ribeiro, 1926). Other cosmocercids reported infecting *R. dapsilis* (= *R. margaritifera*) are *Aplectana hylambatis* (Baylis, 1927), *Cosmocerca podicipinus (*Bursey et al., 2001), and *Cosmocerca* sp. and *Raillietnema ibañezi* (Cordova, 1998) (Campião *et al*., 2014; Chero *et al*., 2023).

Nematodes of the genus *Rhabdias* are lung parasites of amphibians and reptiles, which directly infects its hosts by active penetration into the skin (Langford & Janovy, 2009; Kuzmin *et al*., 2022). Identification is very difficult due to the high morphological similarity, and Müller *et al*. (2018) strongly suggests the use of molecular data for species recognition. This is the first record of *Rhabdias* sp. infecting *R. dapsilis*, although *R. sphaerocephala* have been already reported (Chero *et al*., 2023).

Nematodes of the genus *Physaloptera* have been recorded infecting the stomachs of mammals, fish, reptiles, and amphibians (Anderson, 2000; Macedo *et al*., 2023). They are usually found in larval stages parasitizing amphibians that act as intermediate or paratenic hosts (Anderson, 2000). This is the first record of larvae of *Physaloptera* sp. infecting *R. dapsilis*, but *Physaloptera retusa* was already reported (Campião *et al*., 2014).

Nematodes of the genus *Strongyloides* can either infect their hosts direct or indirectly (Graham *et al*., 2023). Infection occurs through skin penetration or ingestion of infected

158 prey (Mati & Melo, 2014; Hallinger *et al*., 2020). Other bufonids have already been
159 infected by *Strongyloides* spp. (Campião *et al*., 2014). This is the first record of
160 *Strongyloides* sp. infecting *R. dapsilis*.
161 *Oswaldocruzia* spp. also infects its hosts directly by ingestion of eggs or larval penetration
162 of the host's skin (Kirillova, 2020). Many amphibians have been reported as hosts of *O.*
163 *mazzai* (Travassos, 1935), including *R. dapsilis* (Campião *et al*., 2014). Besides *O.*
164 *mazzai*, other four species of *Oswaldocruzia* have been reported infecting *R. dapsilis*: *O.*
165 *albareti* (Slimane & Desset, 1996), *O. chambrieri* (Slimane & Desset, 1993), *O. lescurei*
166 *(*Slimane & Desset, 1996), *O. lopesi* (Gonçalves et al., 2002)*, O. proencai (González and*
167 *Hamann, 2008)*, *O. subauricularis* (Vicente et al., 1991) (Campião *et al*., 2014; Chero *et al*.,
168 2023).
169 The genus *Centrorhynchus* infects birds of the Falconiformes and Strigiformes orders as
170 definitive hosts and has invertebrates as intermediate hosts, with reptiles and amphibians
171 serving as paratenic hosts (Oliveira et al., 2024). Cystacanths of *Centrorhynchus* sp. are
172 found in several amphibians, including bufonids (Campião *et al*., 2014). Previously, only
173 unidentified cystacanths have been reported infecting *R. dapsilis* (Chero *et al*., 2023).
174 The genus *Foleyella* currently has three species infecting frogs in South America, *F.*
175 *convoluta* (Walton, 1935), *F. scalaris* (Walton, 1935) and *F. vellardi* (Travassos, 1929) that
176 have been recorded infecting frogs of the Hylidae, Leptodactylidae and Bufonidae in Brazil.
177 In our study, we found a single female specimen, which makes the identification difficult at
178 species level.
179 Despite the recent increase in studies dealing with helminth parasites of Neotropical
180 amphibians (Mascarenhas *et al*., 2021; Quirino *et al*., 2023), the majority of the species are
181 still unstudied (Campião *et al*., 2015). Even in widely distributed species with available
182 information of helminths, new records of parasites are often published (Campião *et al*., 2014;
183 2015). In our study, 60% of the helminths are new records for *R. dapsilis*, raising to 31
184 helminth taxa actually known to infect this toad. This highlights the need for more studies
185 dealing with helminths infecting amphibians, in order to expand the knowledge in the

Neotropical region.

Author contributions: CRediT (Contributor Roles Taxonomy)

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Formal Analysis: VHH, DBO, RWA

Funding acquisition: RWA

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346 **Table 1.** Parasitological indices of helminths in *Rhinella dapsilis* from Baturité Massif.
347 Development stage (DS), Larvae (L), Adult (A), Prevalence values (P%), number of
348 endoparasites (NE), mean intensity of infection and standard deviation (MII ± SD), mean
349 abundance (MA), site of infection (Site): BC=Body cavity; ST= stomach; LI= large
350 intestine; SI= small intestine, LU= lungs; UB= Urinary Bladder. * New record for *R.*
351 *dapsilis*.

Endoparasites	**DS**	**P%**	**NE**	**MII±SD**	**MA**	**Site**
Acanthocephala						
Centrorhynchus sp.*	L	11.1	3	-	-	BC
Nematoda						
*Aplectana membranosa**	A	11.1	9	-	-	SI/ LI
Cosmocerca parva	A	11.1	10	-	-	SI/ LI
Cosmocercidae gen. sp.	A	77.8	292	19.5±13.5	16.2±14.3	SI/ LI/UB
Foleyella sp.*	A	5.5	1	-	-	ST
Oswaldocruzia mazzai	A	16.7	51	17±12.3	2.8±7.8	LU
*Oxysomatium petrolinensis**	A	5.5	84	-	-	LI
Physaloptera sp.	L	61.1	38	2.6±2.2	1.6±2.1	ST/SI/ LI
Rhabdias sp.*	A	83.3	273	16.6±11.2	15.7±11.6	ST/ LU
Strongyloides sp.*	A	5.5	9	-	-	SI

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353