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HELMINTHS INFECTING THE CARVALHO'S ESCUERZO
ODONTOPHRYNUS CARVALHOI FROM THE BRAZILIAN STATE OF CEARÁHELMINTOS QUE INFECTAN EL ESCUERZO DE CARVALHO
ODONTOPHRYNUS CARVALHOI DEL ESTADO BRASILEÑO DE CEARÁHELMINTOS INFECTANDO O SAPINHO CARVALHO *ODONTOPHRYNUS*
CARVALHOI NO ESTADO BRASILEIRO DO CEARÁTatiana Feitosa Quirino^{1*}; Dalilange Batista-Oliveira²; Matheus Calixto Saldanha³ &
Robson Waldemar Ávila^{1,2}

- 1 Graduate Course in Systematics, Use, and Conservation of Biodiversity, Department of Biology, Pici Campus, Federal University of Ceará, Fortaleza-CE Zip Code 60440-900, Brazil.
- 2 Graduate Course of Ecology and Natural Resources, Department of Biology, Pici Campus, Federal University of Ceará, Fortaleza - CE Zip Code 60440-900, Brazil.
- 3 Graduate Course Biology, Department of Biology, Pici Campus, Federal University of Ceará, Fortaleza-CE Zip Code 60440-900, Brazil.

* Corresponding author: tata_tatifeitosa@hotmail.com

Tatiana Feitosa Quirino: <https://orcid.org/0000-0001-8184-8705>Dalilange Batista-Oliveira: <https://orcid.org/0000-0002-4140-6643>Matheus Calixto Saldanha: <https://orcid.org/0009-0005-8884-7015>Robson Waldemar-Ávila: <https://orcid.org/0000-0003-3641-8321>

ABSTRACT

The family Odontophrynidae comprises 40 anuran species widely distributed in South America, ranging from Brazil to Argentina and Paraguay. *Odontophrynus carvalhoi* Savage and Cei, 1965, a medium-sized species with terrestrial habits and explosive reproduction, is one of the representatives of this family. Despite its extensive distribution, data regarding its natural history are limited, with published information primarily focused on activity patterns, diet, and defensive behavior. In this study, we present data on the composition and infection patterns of endoparasites associated with *O. carvalhoi* in a relictual forest in the Brazilian state of Ceará. The parasite community comprised 11 species, with *Aplectana hylambatis* (76.67%) and *Oswaldocruzia mazzai* (79.49%) being the most prevalent. Additionally, we report new occurrences of parasite species for *O. carvalhoi*, contributing to the understanding of the parasitic fauna

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in the Neotropical region. These findings underscore the importance of parasitological research for a comprehensive understanding of the ecology and biology of this amphibian species.

Keywords: Parasites – Amphibian – Odontophrynidae – Relictual forest

RESUMEN

La familia Odontophrynidae comprende 40 especies de anuros ampliamente distribuidas en América del Sur, desde Brasil hasta Argentina y Paraguay. *Odontophrynus carvalhoi* Savage & Cei, 1965, especie de tamaño mediano, hábitos terrestres y reproducción explosiva, es uno de los representantes de esta familia. A pesar de su extensa distribución, los datos sobre su historia natural son limitados, y la información publicada se centra principalmente en patrones de actividad, dieta y comportamiento defensivo. En este estudio, presentamos datos sobre la composición y patrones de infección de endoparásitos asociados con *O. carvalhoi* en un bosque relicto en el estado brasileño de Ceará. La comunidad de parásitos estuvo compuesta por 11 especies, siendo *Aplectana hylambatis* (76,67%) y *Oswaldocruzia mazzai* (79,49%) las más prevalentes. Además, reportamos nuevas ocurrencias de especies de parásitos para *O. carvalhoi*, contribuyendo al conocimiento de la fauna parasitaria en la región Neotropical. Estos hallazgos subrayan la importancia de la investigación parasitológica para una comprensión integral de la ecología y biología de esta especie de anfibio.

Palabras clave: Parásitos – Anfibios – Odontophrynidae – Bosque relicto

RESUMO

A família Odontophrynidae compreende 40 espécies de anfíbios amplamente distribuídas na América do Sul, abrangendo desde o Brasil até a Argentina e o Paraguai. *Odontophrynus carvalhoi* Savage e Cei, 1965, uma espécie de porte médio com hábitos terrestres e reprodução explosiva, é uma das representantes dessa família. Apesar de sua distribuição extensiva, dados sobre sua história natural são limitados, com informações publicadas concentradas principalmente em padrões de atividade, dieta e comportamento defensivo. Neste estudo, apresentamos dados sobre a composição e padrões de infecção de endoparasitas associados a *O. carvalhoi* em uma floresta relictual no estado brasileiro do Ceará. A comunidade de parasitas incluiu 11 espécies, sendo *Aplectana hylambatis* (76,67%) e *Oswaldocruzia mazzai* (79,49%) as mais prevalentes. Adicionalmente, relatamos novas ocorrências de espécies de parasitas para *O. carvalhoi*, contribuindo para a compreensão da fauna parasitária na região Neotropical. Essas descobertas destacam a importância da pesquisa parasitológica para uma compreensão abrangente da ecologia e biologia dessa espécie de anfíbio.

Palavras-chave: Helminthos – Anfíbio – Odontophrynidae – Floresta relictual.

INTRODUCTION

The family Odontophrynidae is composed by 40 anuran species widely distributed in South America, from Brazil to Argentina and Paraguay (Segalla *et al.*, 2019; Mângia *et al.*, 2020; Frost, 2021). The genus *Odontophrynus* Reinhardt & Lütken, 1862, currently have 12 species (Frost *et al.*, 2017) belonging to three phenetic groups (Savage & Cei, 1965; Caramaschi, 1996; Caramaschi & Napoli, 2012): *O. americanus*, *O. cultripes* and *O. occidentalis*. Besides, *O. salvatori* actually is not allocated to any group (Amaro *et al.*, 2009).

Odontophrynus carvalhoi, belongs to *O. cultripes* group, was described by Savage & Cei, 1965, from a single specimen collected in Poção municipality, state of Pernambuco, Brazil. Its distribution encompasses the Jequitinhonha River valley, in Minas Gerais state, to northeastern Goiás state and Ceará state in the north, in phytogeographies of Atlantic Forest, Cerrado and Caatinga Biomes (Juncá, 2006; Lisboa *et al.*, 2010; Caramaschi & Napoli, 2012; Dias *et al.*, 2014; Santos *et al.*, 2017).

It is a medium sized species, with terrestrial habits and explosive reproduction (Lynch, 1971; Freitas & Silva, 2004; Caramaschi & Napoli, 2012). Tadpoles are laid in small streams inside forested habitats (Santos *et al.*, 2017). Despite its huge distribution, data on natural history of *O. carvalhoi* are scant, with published information about activity patterns and diet (Brito *et al.*, 2012) and defensive behavior (Bezerra *et al.*, 2010; Borges-Nojosa *et al.*, 2016). Until now, there is no information regarding endoparasites infecting *O. carvalhoi*, and several studies pointed out the importance of knowledge of parasites given the role of these organisms in ecosystem regulation. In particular, the diversity of helminths associated with amphibians is considered rich and diversified, although it is a hidden diversity and therefore undersampled (Poulin, 2014; Campião *et al.*, 2014).

Herein, we present data on composition and infection patterns of endoparasites associated with *O. carvalhoi* from a relictual forest in the Brazilian state of Ceará.

MATERIAL AND METHODS

This study was carried out in the municipality of Guaramiranga, located at the Baturité massif, northern Ceará state, Brazil. Its mountainous relief, dissected forming small valleys, covered by rainforest vegetation, is classified as a remnant of the Atlantic Forest. Altitude.

The annual rainfall has an average of 1737.5 mm/year, with a rainy season from January to May. Its average temperature ranges from 24° to 26°C (Vale & Soares, 2006; IPECE, 2017).

Field work was taken in three sampling points: Guaramiranga farm (04°15'54,91" S; 38°56'00,24" W), Álvaro farm (04°17'17,29" S; 38°57'00,05" W), Riacho Fundo farm (04°15'42,86" S; 38°55'07,57" W), Parque das Trilhas (04°16'13,63" S; 38°56'19,10" W), Vale das Nuvens (4°16'05,10" S; 38°54'54,96" W) and Pernambuco farm (04°12'19,73" S; 38° 57'37,70" W). We used specimens collected from 1994 to 2022, that are deposited in the Herpetological Collection of the Universidade Federal do Ceará (CHUFC-A 2.918, 3.738-39, 3.740-41, 3.769-73, 3.799, 3.864, 3.875-76, 9.961-70, 10.275-81, 10.283-10.288, 10.999), and Herpetological Collection of the Universidade Regional do Cariri (URCA-H 16.041), Ceará state, Brazil.

Thirty-nine individuals of *O. carvalhoi* were collected by hand through active and auditory searches (Bernarde, 2012). The specimens were placed in separate containers and euthanized by a lethal injection of lidocaine (CFMV, 2013), fixed with 10% formalin according to Calleffo (2002). Morphometric measurements of hosts were taken with a digital caliper Mitutoyo® (precision 0.01 mm).

The specimens were necropsied with a midventral incision and all organs and coelomic cavity were searched for helminths. Parasites found were preserved in 70% ethanol, fixed according Amato *et al.* (1991) and Andrade (2000), and mounted in temporary slides for identification. For identification, we follow Yamaguti (1961), Sprent (1978), Vicente *et al.* (1991), Anderson (2000) and Gibbons (2010), as well recent descriptions. Slides were analyzed under microscope with 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).

To evaluate the impact of host size on infection intensity, we employed linear regression. Differences in the prevalence and intensity of infection between genders were assessed using the chi-square method. The analyses were conducted on the R platform, "Commander R" package (R CORE TEAM, 2022, version 4.2.0) (R Foundation, 2017).

Ethic aspects: This study was approved by the Ethics Committee on Animal Use of the Federal University of Ceará (CEUA-UFC) under the protocol 6314010321.

RESULTS

We examined 39 specimens of *O. carvalhoi*, being 16 males and 23 females, which are infected with at least one endoparasite species (overall prevalence 84,97%). We found 5.681 helminths, with mean intensity of infection 153.54 ± 32.38 . Parasite community was composed by 11 species: *Aplectana hylambatis* (Baylis, 1927), *Oswaldocruzia mazzai* Travassos, 1935, *Cosmocerca brasiliense* Travassos,

1925, *Gorgoderina parvicava* Travassos, 1922, *Physaloptera* sp., *Raillietnema* sp., *Rhabdias* sp., *Strongyloides* sp., *Parapharyngodon* sp., *Oxyascaris* sp. and *Ochoterenella* sp., besides Cosmocercidae larvae. The most prevalent taxa were *A. hylambatis* (76.67%) and *O. mazzai* (79.49%), being the first one with the higher abundance (102.49) and mean intensity of infection (121.12). *G. parvicava*, *Strongyloides* sp. and *Parapharyngodon* sp. have the lower prevalence (2.56%) (Table 1).

Table 1. Hosts, number of helminths (NH), mean abundance (MA), mean intensity of infection (MII) with standard error (SE), intensity of infection amplitude (IIA) and infection site (IS) of helminths associated with *Odontophrynus carvalhoi*.

Helminth	NH	MA±SE	MII±SE	IIA	IS
<i>Aplectana hylambatis</i>	3692	102.49±24.57	121.12±27.87	1-725	B/E/IG/ID
<i>Oswaldocruzia mazzai</i>	618	79.49±15.85	19.94±9.94	1-312	E/IG/ID
<i>Cosmocerca brasiliense</i>	11	0.28±0.2	5.5±0.5	5-6	ID
<i>Gorgoderina parvicava</i>	5	0.13±0.13	5	5	B
<i>Physaloptera</i> sp.	219	5.62±1.92	15.64±4.28	1-52	E/IG/ID
<i>Oxyascaris</i> sp.	2	0.05±0.04	1	1	IG/ID
<i>Raillietnema</i> sp.	273	7.0±6.4	91.0±79.25	1-249	B/ID
<i>Rhabdias</i> sp.	139	3.56±0.83	6.04±1.15	1-21	P/F/E
<i>Strongyloides</i> sp.	2	0.05±0.05	2	2	ID
<i>Parapharyngodon</i> sp.	1	0.03±0.03	1	1	IG
<i>Ochoterenella</i> sp.	414	10.62±5.34	18.82±9.17	1-205	CAV/B/F/C/ID

Mean richness was 3.41 ± 0.23 helminths/hosts, and the higher richness (S=6) was found in a single host. There was an influence of host size on mean intensity of infection ($r=0.48$; $p<0.05$; $n=39$). As for the influence of size on the average intensity of infection between the sexes, we can observe that were significant for males and females ($r=0.56$; $p<0.05$; $n=16$, $r=0.47$; $p<0.05$; $n=23$, respectively).

DISCUSSION

Helminths community associated with amphibians are characterized by generalist species (Aho, 1990). Low host specificity, combined with the general lack of studies with Neotropical amphibian species, are often the main causes of new host records (Campião *et al.*, 2015; Oliveira *et al.*, 2019). In Brazil, species of Odontophrynidae still need further studies to understand what are the host specificities and patterns of infection and abundance (Campião *et al.*, 2014).

Species of *Aplectana* are usually found infecting the large intestine of reptiles and amphibians, have a direct life cycle and actively infect their hosts (Travassos, 1931; Anderson, 2000; Campião *et al.*, 2014; Lins *et al.*, 2017). This genus has been already reported in four odontophrynids: *Proceratophrys tupinamba* Prado and Pombal, 2008 and *P. boiei* (Wied-Neuwied, 1824) infected by *A. delirae* Fabio, 1971 (Boquimpani-Freitas *et al.*, 2001; Klaion *et al.*, 2011), and *P. cristiceps* (Silva *et al.*, 2019; Sampaio *et al.*, 2020) and *Odontophrynus americanus* Duméril & Bibron, 1841, infected by *A. membranosa* (Lent & Freitas, 1948).

Aplectana hylambatis is a generalist species that parasitizes a wide range of hosts. In the Neotropical region it has been observed in different countries and families of anurans such as Bufonidae, Leptodactylidae, Ceratophryidae e Microhylidae from Peru (Burse *et al.*, 2001); Bufonidae, Mycrohylidae, Leiuperidae, Leptodactylidae and Hylidae from Paraguay (Masi Pallares & Maciel, 1974; Baker & Vaucher, 1986); Bufonidae from Uruguay (Lent & Freitas, 1948); Bufonidae, Leptodactylidae and Leiuperidae from Argentina (Gutiérrez, 1945; Sueldo & Ramírez, 1976;

Ramírez *et al.*, 1979; Baker, 1980; González & Hamann, 2006, 2010). In Brazil, it was reported infecting Hylidae, Microhylidae and Leptodactylidae at São Paulo and Mato Grosso do Sul states (Campião *et al.*, 2016; Aguiar *et al.*, 2021).

Cosmocerca Diensing, 1861, is a genus that pursues a wide geographic distribution and is commonly found as parasites of amphibians (Navarro *et al.*, 1988). *Cosmocerca brasiliense* can infect amphibian hosts by ingestion of an infective larvae (L3) or penetration through the skin (Goldberg *et al.*, 2002a). After infection, larvae migrate to the host intestine, reaching sexual maturity. This species has been recorded parasitizing many hosts in South America, such as Ecuador (Dyer & Altig, 1976; Mcallister *et al.*, 2010a), Guiana (Mcallister *et al.*, 2010b) and Peru (Burse *et al.*, 2001). In Brazil, it was reported by Travassos (1925); Vicente *et al.* (1991); Boquimpani-Freitas *et al.* (2001); Martins & De Fabio (2005); Goldberg *et al.* (2007); Santos *et al.* (2013), (2016); Aguiar *et al.* (2014); Klaion (2011); Campião *et al.* (2014); Oliveira *et al.* (2022).

Oswaldocruzia mazzai infect several anurans (e.g., Campião *et al.*, 2014; Teles *et al.*, 2015; Alcantara *et al.*, 2018; Oliveira *et al.*, 2019), but we reported for the first time the infection of this nematode in the genus *Odontophrynus*. The great host diversity recorded for *O. mazzai* is related to the direct life cycle and the simple mode of transmission that can occur by ingestion of eggs or larval penetration of the host's skin (Anderson, 2000).

The genus *Gorgoderina* Looss, 1902, includes 57 species, all of them described as parasites of the bladder of anurans and salamanders. In Brazil, seven species of this genus are known. *G. parvicava* is well distributed in Neotropical region and has already been reported as parasites of several anurans in Brazil, such as *Leptodactylus chaquensis*, Cei, 1950, *L. labyrinthicus*, (Spix, 1824), *L. latrans* (= *L. ocellatus*) (Steffen, 1815), *L. pentadactylus*, (Laurenti, 1768), *Rhinella crucifer*, (Wied-Neuwied, 1821), *R. diptycha*, (Cope, 1862), *R. ictérica*, (Spix, 1824), *R. marina*, (Linnaeus, 1758), *Pseudis paradoxa* (Linnaeus, 1758), and *Pristimantis relictus* Roberto *et al.*, 2022, (Alcantara *et al.*, 2022; Oliveira *et al.*, 2022).

Nematodes of the genus *Physaloptera* have a worldwide distribution and have been recorded in several terrestrial vertebrates, including felines (Ogassawara, 1986), rodents (Tung *et al.*, 2009), lizards (Da Silva *et al.*, 2008, Cabral *et al.*, 2018) and anurans (Da Graça *et al.*, 2017). In amphibians, this parasite is usually found in the larval stage, making it difficult to identify at the species

level, and it is suggestive that these amphibians are not definitive hosts. Although there is not enough data on their life cycle, nematodes of this genus are known to utilize insects during their intermediate phase (Anderson, 2000). Additionally, the acquisition of *Physaloptera* by anuran hosts occurs through the ingestion of infected insects, mainly Orthoptera (Klaion *et al.*, 2011).

Raillietnema sp. is known to have a direct life cycle and transmission that occurs via ingestion or penetration of larvae through the skin (Anderson, 2000). Studies have reported the presence of this parasite infecting *P. aridus* (Teles *et al.*, 2017), as well as infecting lizards. The species *R. spectans* (Burse *et al.*, 1998), are the most frequent in anurans (Vicente *et al.*, 1991; Teles *et al.*, 2015).

The specific identification of the *Rhabdias* found here was not possible, because there is high morphological similarity, which makes molecular data useful in species recognition (Müller *et al.*, 2018). Nematodes of this genus are lung parasites, commonly infecting amphibians and reptiles, by direct transmission and infection occurs by active penetration into the skin of hosts (Langford & Janovy, 2009; Kuzmin *et al.*, 2015).

Although the life cycle of *Strongyloides* is not known, it can be considered a direct or indirect cycle, the first being the most common (Santos *et al.*, 2010). Infection occurs on land through skin penetration or ingestion of infected prey (Mati & Melo, 2014; Sulieman *et al.*, 2015). Although there are several records of this nematode infecting amphibian species (Campião *et al.*, 2014; Sulieman *et al.*, 2015; Mascarenhas *et al.*, 2021), this is the second record of the genus *Strongyloides* acting as a parasite for species of the Odontophrynidae family and the first for *O. carvalhoi*.

Parapharyngodon Chatterji, 1933, is a genus of parasitic nematodes with monoxenic cycle (Anderson, 2000). These have already been registered in amphibian species (Pereira *et al.*, 2017), being commonly found in reptiles (Avila & Silva, 2010).

Ochoterenella is reported from South and Central America, with several species known only from females and larvae. The genus is remarkably diverse in bufonids such as *Rhinella marina* (Linnaeus, 1758) (Travassos, 1929; Caballero, 1944; Bain & Prod'Hon, 1974; Bain *et al.*, 1979; Esslinger 1986, 1987, 1988a, b, 1989), while only two of 15 species have been described for Leptodactylidae with few records for hylids (Lima *et al.*, 2012).

Oxyascaris genus was reported parasitizing the digestive system of anurans and reptiles from Paraguay, Argentina and Costa Rica. In Brazil, species of this genus were recorded in Bufonidae, Hylidae, Leptodactylidae, Phyllomedusidae and Cycloramphidae anuran families (Freitas, 1958; Baker & Vaucher, 1985; Luque *et al.*, 2005; Bursey & Goldberg, 2007; Lux-Hoppe *et al.*, 2008; González & Hamann, 2008; Goldberg & Bursey, 2008a; Goldberg & Bursey, 2008b; González, 2009; Bursey & Goldberg, 2010; Klaion *et al.*, 2011; Santos *et al.*, 2013; Campião *et al.*, 2014, 2016; De Sena *et al.*, 2018; Felix-Nascimento *et al.*, 2020). Despite being recorded only in Neotropical hosts, very little is known about its life cycle and how transmission occurs.

In recent years, there is an increase in studies of helminths associated with amphibians (Camião *et al.*, 2016a, b; Lins *et al.*, 2017; Teles *et al.*, 2017; Leivas *et al.*, 2018; Alcantara *et al.*, 2018; Oliveira *et al.* 2019; Mascarenhas *et al.*, 2021), with new host records being quite common. In this study, we present new records of parasite species for the species of *O. carvalhoi*, contributing to the knowledge of the parasitic fauna of the Neotropical region.

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Author contributions: CRediT (Contributor Roles Taxonomy)

TFQ = Tatiana Feitosa Quirino

DBO = Dalilanga Batista-Oliveira

MCS = Matheus Calixto Saldanha

RWA = Robson Waldemar Ávila

Conceptualization: TFQ, DBO, MCS, RWA

Data curation: TFQ, DBO, MCS, RWA

Formal Analysis: RWA

Funding acquisition: TFQ, DBO, RWA

Investigation: TFQ

Methodology: TFQ, DBO, MCS

Project administration: TFQ

Resources: TFQ, DBO, MCS, RWA

Software: TFQ, DBO, MCS, RWA

Supervision: RWA

Validation: TFQ, DBO, MCS, RWA

Visualization: TFQ, DBO, MCS, RWA

Writing – original draft: TFQ, DBO, MCS

Writing – review & editing: RWA

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