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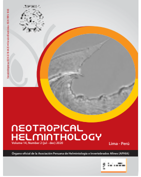

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

HELMINTHS ASSOCIATED WITH 15 SPECIES OF ANURANS FROM THE IBIAPABA PLATEAU, NORTHEASTERN BRAZIL

HELMINTOS ASOCIADOS CON 15 ESPECIES DE ANUROS DE LA MESETA DE IBIAPABA, EN EL NORESTE DE BRASIL

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ABSTRACT

The diversity of helminths in host groups with a high diversity of species like anurans are still underrepresented, especially in sites with severe environmental conditions such as arid and semiarid regions. This knowledge is needed to understand the role of parasites at different levels of ecological organization. This study aimed to evaluate the parasite composition a taxocenosis of anuran species, describe the richness and diversity of helminths at the component and infracommunity levels, and evaluate the influence of body size on the abundance and diversity of parasites. The anuran hosts were collected at the Environmental Protection Area (EPA) Bica do Ipu in Brazilian semi-arid. The collected hosts were euthanized, necropsied, and examined for helminth parasites. Helminths were identified and the statistical tests were performed. A total of 15 host species composed this study and 1,216 helminths were collected with mean abundance (MA) of 12.9 ± 3.38 and mean intensity of infection (MII) of 25.84 ± 6.44. The mean richness of helminth was 2.3 ± 0.53 (range = 6) and helminth diversity (H´) was 1.36. Helminths infecting the studied amphibian hosts comprised 13 *taxa*: two cestode species, one acanthocephalan, one trematode, and nine nematodes. The present study contributes to the knowledge of the helminths infecting amphibians from Brazilian Caatinga, as well as the understanding of the diversity patterns of parasitic infracommunities associated with amphibians.

Keywords: Amphibians – Inventory – Endoparasites – Nematodes – Mountain swamp – Semi-arid

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RESUMEN

La diversidad de los helmintos en los grupos de huéspedes con gran diversidad de especies como los anfibios anuros siguen estando subrepresentados, especialmente en sitios con condiciones ambientales severas como las regiones áridas y semiáridas. Este conocimiento es necesario para comprender el papel de los parásitos en los diferentes niveles de la organización ecológica. Con el fin de evaluar la composición del parásito una taxocenosis de las especies de anuros, describir la riqueza y diversidad de los helmintos a nivel de componentes y de la infracomunidad; además de evaluar la influencia del tamaño del cuerpo en la abundancia y diversidad de los parásitos, se realizó este estudio. Los anuros huéspedes se recogieron en la Zona de Protección Ambiental (EPA) Bica do Ipu en el semiárido brasileño. Los huéspedes recolectados fueron sometidos a eutanasia y a una necropsia en busca de parásitos. En secuencia, se identificaron los helmintos y se realizaron las pruebas estadísticas. Un total de 15 especies de huéspedes compusieron este estudio y se recogieron 1.216 helmintos con abundancia media (MA) de 12,9 ± 3,38 y intensidad media de la infección (MII) de 25,84 ± 6,44. La riqueza media de helmintos fue de 2,3 ± 0,53 (rango = 6), y la diversidad de helmintos (H) fue de 1,36. La comunidad de componentes de helmintos que infectan a los anfibios huéspedes comprende 13 taxones: dos especies de cestodos, un acantocéfalo, un trematodo y nueve nematodos. El presente estudio contribuye al conocimiento de los helmintos que infectan a los anfibios de la Caatinga brasileña, así como a la comprensión de los patrones de diversidad de las infracomunidades parasitarias asociadas a los anfibios.

Palabras clave: Anfibios – Inventario – Endoparásitos – Nematodos – Pantano de montaña – Semiárido

INTRODUCTION

Documenting parasite diversity and host relationships are needed to understand the role of parasites at different levels of ecological organization (Wood & Johnson, 2015). Parasites can affect host immunity and population dynamics, thus having effects on community composition and trophic interactions also acting on population control (Bittencourt & Rocha, 2003; Hudson, 2005). Although play essential roles in ecological processes, parasites remain taxonomically neglected and the actual number of species in different groups cannot yet be determined (Windsor, 1998; Poulin & Morand, 2004).

The interaction host-parasite is an outcome of their coevolutionary history, diet, body size, sex, infection site, geographic distribution, behavior, host species, climatic characteristics, and host phylogeny (Muzzall *et al*., 2001; Araujo-Filho *et al*., 2017)——. Anurans can act as definitive, intermediate, and paratenic hosts of a wide variety of helminths (Campião *et al*., 2009; Santos & Amato, 2010; Campião *et al*., 2014). Body size is a determining factor for the composition of the anuran parasite community, due to the greater amount of food eaten and added extra and intracorporeal surface, increasing the possibilities of oral and skin infection (Santos & Amato, 2010; Campião *et al*., 2016b; Hamann *et al*., 2009).

Amphibian's biology makes it an excellent model for evaluating patterns in the structure of helminth communities (Aho, 1990). They occupy a variety of habitats, have different patterns of life cycle, different reproductive strategies, and occupy various positions in food webs (Koprivnikar *et al*., 2012). Studies on amphibian parasites have increased in recent years (e.g. Campião *et al*., 2016a; Oliveira *et al*., 2019). However, information about the parasites of some amphibian groups is still scarce, and most of restricted to taxonomic descriptions or records of occurrence (Pinhão *et al*., 2009).

Brazil has the most diverse anuran fauna, with 1,137 species (Segalla *et al*., 2019). Nevertheless, there are helminth reports for only 185 (7%) species. About 164 helminth species are currently known for Brazilian amphibians, totaling 57% of the taxa described for South America. However, studies describing the ecological aspects of parasites associated with Brazilian amphibian taxocenosis remain underrepresented in the literature (Toledo *et al*., 2017; da Graça *et al*., 2017). Data scarcity is even more pronounced in semi-arid regions where amphibians are strongly influenced by unpredictable rainfall. Severe environmental conditions in arid and semiarid regions may limit species diversity by selecting clades tolerant to such conditions while shaping their ecology, natural history, and behavior (Garda *et al*., 2017).

Therefore, the objectives of this study were: (1) evaluate the parasite composition of 15 anuran species; (2) describe the richness and diversity of helminths at the component and infracommunity levels; (3) to evaluate the influence of body size on the abundance, diversity, and richness in anurans of Caatinga, Brazil.

MATERIAL AND METHODS

Anurans were sampled from the Environmental Protection Area (EPA) Bica do Ipu (4°19'10" S, 40°43'04" W), Ipu municipality, Ceará state, Brazil (Fig. 1). The EPA Bica do Ipu, is located at the slopes of the Ibiapaba plateau with an extension of 3,500 ha approximately. The Ibiapaba plateau is a highland marsh (known as brejo de altitude) composed by a mosaic of phytophysiognomies (Santos & Souza, 2012).

Amphibians (n = 92 specimens), comprising representatives of five families: Bufonidae: *Rhinella jimi* (Stevaux, 2002) (n = 1), *Rhinella granulosa* (Spix, 1824) (n = 11); Hylidae: *Corythomantis greeningi* Boulenger, 1896 (n = 1), *Scinax x-signatus* (Spix, 1824) (n = 4); Phyllomedusidae: *Pithecopus nordestinus* (Caramaschi, 2006) (n = 4); Leptodactylidae: *Leptodactylus fuscus* (Schneider, 1799) (n = 4), *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 (n = 4), *Leptodactylus mystaceus* (Spix, 1824) (n = 1), *Leptodactylus vastus* Lutz, 1930 (n = 6), *Physalaemus albifrons* (Spix, 1824) (n = 21), *Physalaemus cicada* Bokermann, 1966 (n = 9), *Physalaemus cuvieri* Fitzinger, 1826 (n = 3), *Pleurodema diplolister* (Peters, 1870) (n = 10), *Pseudopaludicola mystacalis* (Cope, 1887) (n = 12); Odontophrynidae: *Proceratophrys cristiceps* (Müller, 1883) (n = 1) were captured by hand during visual surveys from 7 to 15 April 2014. Specimens were euthanized with intraperitoneal injection of Propofol (CFMV, 2013), after the snout–vent length (SVL) of each specimen was recorded. During necropsy, hosts were sexed and the organs like gastrointestinal tract, lungs, liver, and kidneys were separated and surveyed for helminths under stereomicroscope. Voucher hosts were fixed with 10% formalin, conserved with 70% ethanol and deposited at the Herpetological Collection of the Universidade Regional do Cariri (URCA 9127-9132, 9134-9143, 9145-9148, 9152-9185, 9187-9202, 9107-9211, 9226-9229, 9232-9239, 9241-9245), municipality of Crato, Ceará state, Brazil.

Helminths were fixed in hot alcohol and preserved in 70% ethyl alcohol. For identification, the nematodes were clarified in lactic acid, and cestodes, trematodes, and acanthocephalans were stained with hydrochloric carmine and cleared with creosote. Thereafter, the helminths were mounted in temporary slides and examined under the light microscope ZEISS Axio Imager M2. Species identification followed Travassos *et al.* (1969), Vicente *et al.* (1991), and recent bibliographies. The voucher species were deposited at the Herpetological Collection of Universidade Regional do Cariri.

Parasitological descriptors follow Bush *et al.* (1997): prevalence, intensity, and abundance of infection were calculated, followed by their respective standard errors. All values are expressed as the mean ± standard error (SE). Parasites' abundance data were tested for normality by the Kolmogorov-Smirnov test. To calculate the wealth and diversity of the community we use the species richness (= total number of helminth species), Shannon index (H′) (Zar, 2010). The Berger–Parker index of dominance (d) was used to determine the most dominant species (Magurran, 2004). Spearman's rank test (rs) was used to assess the relationship between the host body size and parasitological descriptors. Statistical analyses were performed using BioEstat 5.0 (Ayres *et al*., 2007) and software R platform, version 2.15.0 (RC team, 2017).

Ethic aspects

Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO) for collection permits (ICMBio number 32758-1). To Comitê de Ética em Pesquisa of the Universidade Regional do Cariri, permits 00026/2015.

RESULTS

Of the 92 analyzed hosts, 44 (47.8%) were parasitized with at least one helminth species. Only three analyzed host species presented no infection with helminth parasites (*C. greening, R. jimi*, and *S. x-signatus*). A total of 1,190 helminths from 13 taxa was recovered from 12 hosts of five anuran families (Table 1), with mean abundance of 12.9 ± 3.38 and mean intensity of infection of 25.84 ± 6.44. Helminth diversity (H`) was 1.36. The mean helminth richness was 2.3 ± 0.53 (max. = 6) species per infected host.

Schrankiana sp. and *Raillietnema spectans* Gomes, 1964 were the most abundant taxa (d = 0.39, d = 0.36 respectively). *Oswaldocruzia mazzai* Travassos, 1935 and *R. spectans* were the helminth taxa that infected the greatest number of host species (8 and 9, respectively). The nematodes *Aplectana membranosa* Schneider, 1866, *Ochoterenella* sp., *Parapharyngodon* sp., and *Physaloptera* sp. larvae were found only in anuran species. The digenean *Gorgoderina parvicava* Travassos, 1922 was found only in *L. vastus*. *Cylindrotaenia americana* Jewell, 1916 (Cestoda), and *Oligacanthorhynchus* sp. (Acanthocephala) were found only in *P. diplolister* (Table 1).

The highest intensity of parasites was found in the rufous frog *L. fuscus* (n = 446). *Pleurodema diplolister* and *R. granulosa* were the hosts exhibiting the highest helminth richness (n = 6). The diversity of the helminths tended to be greatest in *P. cicada* (H' = 1.15) followed by *L. vastus* (H'= 1.03) (Table 2).

The overall host body size correlated positively with richness (rs = 0.542, p < 0.001) and abundance of parasites (rs = 0.578, p < 0.001). Considering the analysis of each anuran host species, it was also observed a significant positive correlation with abundance and parasite richness for *P. albifrons* (abundance/length: rs = 0.489, p = 0.02; richness/length: rs = 0.475, p = 0.02) and *P. diplolister* (abundance/length: rs = 0.882, p < 0.001; richness/length: rs = 0.740, p = 0.01). However, for *L. vastus*, the relation of abundance and length was negatively correlated (rs = -0.941, p = 0.01) (Table 3).

DISCUSSION

The richness found in the present study is similar to other studies dealing with the helminth community associated with anurans (Aguiar *et al*., 2014; Campião *et al*., 2014; 2016a; da Graça *et al*., 2017; Müller *et al*., 2018). However, it may be underestimated because of unidentified species, since the occurrence of cryptic species has already been recorded for the region, as in Rhabdiasidae (Müller *et al*., 2018). Also, larger numbers of nematode species seem to be a general pattern for South American amphibians (Campião *et al*., 2014).

Monoxenous nematodes, such as *R. spectans* and *O. mazzai*, infected more host species. According to Anderson (2000), parasites with direct life cycles have low specificity and simple mode of transmission that can occur through egg ingestion or larval penetration through the host skin. Moreover, *R. spectans* and *O. mazzai* have been recorded infecting several Brazilian anurans, including *P. diplolister, Dermatonotus muelleri* (Boettger, 1885), *Leptodactylus latrans* (Steffen, 1815), *Rhinella crucifer* (Wied Neuwied, 1821), *Rhinella icterica* (Spix, 1824), *L. fuscus*, *L. mystaceus, Physalaemus albifrons, P. cicada*, and *P. cuvieri* (Campião *et al*., 2014; Teles *et al*., 2015; Alcantara *et al*., 2018; Oliveira *et al*., 2019).

Cylindrotaenia americana is also a monoxenous parasite infecting the intestinal mucosa (Stumpf, 1981). Actually, it has been reported in North and South America, Asia, and Europe in hosts of the families Bufonidae, Ranidae, Hylidae, Brachycephalidae, and Dendrobatidae (Goldberg & Bursey, 2008). In Brazil, there are records of this cestode species infecting *R. icterica*, *Rhinella fernandezae* (Gallardo, 1957), *Ischnocnema guentheri* (Steindachner, 1864), *Hypsiboas prasinus* (Burmeister, 1856) (Campião *et al*., 2014), *Ischnocnema parva* (Girard, 1853), *Hylodes phyllodes* Heyer & Cocroft, 1986 (Aguiar *et al*., 2014), and *P. cicada* (Oliveira *et al*., 2019). *Pleurodema diplolister* is a new host recorded for *C. americana.*

Acanthocephalans of the genus *Oligacanthorhynchus* are heteroxenous and usually have mammals as final hosts (Gallas &

Table 1. Helminths recorded in the 12 host species collected in the Environmental Protection Area (EPA) Bica do Ipu, Ceará, Brazi. Dominance (d), prevalence (%), mean abundance (MA) ± standard deviation (SE), range and number of parasites/host species are presented. *Lfus = Leptodactylus fuscus; Lmac = L. macrosternum; Lmys = L. mystaceus; Lvas = L. vastus; Phal = Physalaemus albifrons; Phci = P. cicada; Phcu = P. cuvieri; Pinor = Pithecopus nordestinus; Pldi = Pleurodema diplolister; Prca = Proceratophrys caramaschii; Psmy = Pseudopaludicola mystacalis; Rhgr = Rhinella granulosa.* The number in parenthesis is the number of studied hosts.

Parasite taxa	*d*	%	MA ± SE	Range	Number of parasites/host species											
					Lfus (4)	*Lmac* (4)	*Lmys* (1)	*Lvas* (6)	*Phal* (21)	*Phci* (9)	*Phcu* (3)	*Pinor* (4)	*Pldi* (10)	*Prca* (1)	*Psmy* (12)	*Rhgr* (11)
Acantocephala																
Oligacanthorhynchus sp.	0.003	1.1	0.01 ± 0.01	4									4			
Cestoda																
Cylindrotaenia americana	0.003	1.1	0.01 ± 0.01	4									4			
Unidentified				10												10
Digenea																
Gorgoderina parvicava	0.0008	1.1	0.01 ± 0.01	10				10								
Nematoda																
Aplectana membranosa	0.0008	1.1	0.01 ± 0.01	1					1							
Falcaustra mascula	0.04	4.35	0.58 ± 0.36	1-31	49								1			
Ochoterenella sp.	0.0008	1.1	0.01 ± 0.01	1	1											
Oswaldocruzia mazzai	0.18	18.5	2.34 ± 1.19	1-104	104	60		16	20	1			12	1		1
Parapharyngodon sp.	0.0008	1.1	0.01 ± 0.01	1												1
Physaloptera sp. larvae	0.0008	1.1	0.01 ± 0.01	1											1	
Raillietnema spectans	0.36	19.6	4.59 ± 2.16	1-180	20		56	4	9	4	3	2	218			109
Rhabdias sp.	0.007	3.3	0.09 ± 0.07	1-7		1				1						7

Table 2. Ecological indices of parasite communities from species of anuran hosts collected in the Environmental Protection Area (EPA) Bica do Ipu, Ceará, Brazi. *Lfus = Leptodactylus fuscus; Lmac = L. macrosternum; Lmys = L. mystaceus; Lvas = L. vastus; Phal = Physalaemus albifrons; Phci = P. cicada; Phcu = P. cuvieri; Pinor = Pithecopus nordestinus; Pldi = Pleurodema diplolister; Prca = Proceratophrys caramaschii; Psmy = Pseudopaludicola mystacalis; Rhgr = Rhinella granulosa.* The number in parenthesis is the number of studied hosts.

Component community	*Lfus* (4)	*Lmac* (4)	*Lmys* (1)	*Lvas* (6)	*Phal* (21)	*Phci* (9)	*Phcu* (3)	*Pinor* (4)	*Pldi* (10)	*Prca* (1)	*Psmy* (12)	*Rhgr* (11)
Richness	4	2	1	4	4	4	2	1	6	1	1	6
Diversity (H′)	0.72	0.08	0	1.03	0.98	1.15	0.69	0	0.46	0	0	0.98
Dominance (d)	0.70	0.90	-	0.60	0.60	0.50	-	1	0.90	-	-	0.50
Dominant species	S	Om	-	Fm	Om	Rs	-	Rs	Rs	-	-	S

S = *Schrankiana* sp.; Om = *Oswaldocruzia mazzai*; Fm = *Falcaustra mascula;* Rs = *Raillietnema spectans.*

Table 3. Spearman correlation (r_s) between richness/abundance and the body size of anuran hosts collected in Ibiapaba plateau, Northeastern Brazil. N – number of studied hosts. *values statistically significative ($p < 0.05$).

Hosts	N	Richness		Abundance	
		rs	p	rs	p
Physalaemus albifrons	21	0.47	0.029*	0.48	0.02*
Physalaemus cicada	9	0.54	0.11	0.63	0.05
Pleurodema diplolister	10	0.74	0.010*	0.88	< 0.001*
Leptodactylus fuscus	4	0.25	0.75	0.20	0.91
Rhinella granulosa	11	-0,06	0.83	-0.51	0.11
Leptodactylus macrosternum	4	-0.25	0.75	-1.00	0.08
Pseudopaludicola mystacalis	12	0.50	0.10	0.50	0.10
Leptodactylus vastus	6	-0.75	0.10	-0.94	0.01*
Total hosts	84	0,54	< 0.001*	0.57	< 0.001*

Silvera, 2012; Richardson *et al*., 2014). Eventually, some vertebrates can act as paratenic hosts (Yamaguti, 1963; Goldberg & Bursey, 2004), in this case, the parasites can encyst until they reach the proper host (Baker, 2007). In South America, *Oligacanthorhynchus* sp. was recorded infecting *Odontophrynus americanus* (Duméril & Bibron, 1841) in Paraguay (Campião *et al*., 2014). Thus *P. diplolister* is a new host record for *Oligacanthorhynchus* sp.

Currently, the genus *Rhabdias* is composed of 84 species (Kuzmin & Tkach, 2018), being 18 reported in Neotropical anurans (Kuzmin *et al*., 2016; Willkens *et al*., 2020). Müller *et al.* (2018) assessing the molecular diversity of *Rhabdias* in Brazil, reports the occurrence of cryptic species and three Amazonian strains infecting anurans in the northeast region: *Rhabdias breviensis* Nascimento *et al.* 2013 in *R. granulosa* from Piauí state, *Rhabdias pseudosphaerocephala* Kuzmin, Tkach & Brooks, 2007 in *Rhinella jimi* from Piauí and Ceará states, and *R.* cf. *stenocephala* in *Leptodactylus vastus* and *L. macrosternum. Rhabdias* spp. are pulmonary parasites of anurans and lizards (Baker, 1987; Teles *et al*., 2015; Campião *et al*., 2016a; Toledo *et al*., 2017; Teles *et al*., 2018). *Rhabdias* spp. are cryptic species, so only with classical taxonomy, without the help of molecular, it was not possible to precisely identify the species.

Variation in host specificity may determine the structure of helminth communities (Toledo *et al*.,

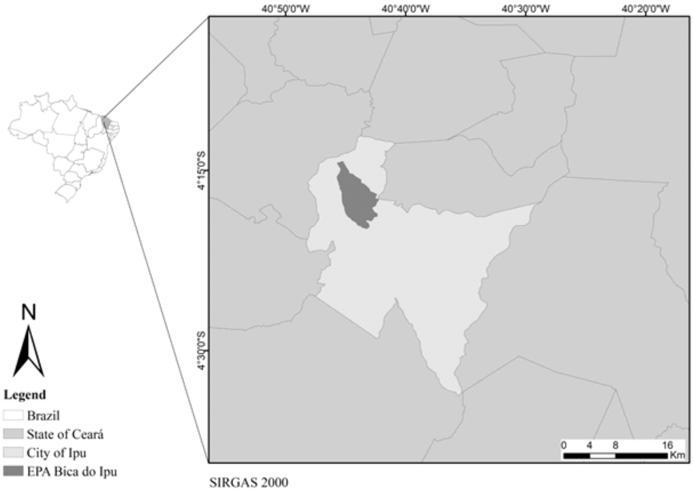

Figure 1. Map of the study site in the municipality of Ipu, Ceará state, northeastern Brazil.

2017). Herein, *R. spectans*, *O. mazzai*, *Schrankiana* sp., and *Rhabdias* sp. may be considered generalists and infected more than one host species. Thus, sympatric hosts, although phylogenetically distant may share some helminth *taxa*, as they are exposed to similar environmental conditions (Krasnov *et al*., 2012; Lima *et al*., 2012; Brito *et al*., 2014). *Schrankiana* sp. has been recorded in amphibians from the Hylidae and Leptodactylidae families (Goldberg *et al*., 2007; Campião *et al*., 2014; Müller *et al*., 2018; Oliveira *et al*., 2019). This is the first record for Bufonidae ®. *granulosa*).

The high pattern of infection, abundance, and prevalence was evident among individuals of *R. granulosa* and *L. fuscus.* In general, members of the Leptodactylidae are associated with terrestrial habitats being frequently found closer to water bodies, which may expose then to both aquatic and terrestrial parasites (Campião *et al*., 2016a).

Host body size can influence the establishment of parasite communities; usually larger individuals offer larger colonization areas providing adequate resources for parasite development and reproduction (Poulin, 2004; Campião *et al*., 2016b). Besides, some studies suggest that body size influence on species richness and abundance of parasitic helminths (Yoder & Coggins, 2007; Ibrahim, 2008; Hamann *et al*., 2012, 2013; Toledo *et al*., 2015, 2017). In the present study, the overall size and the size of some amphibian individuals were a determining factor in the richness and abundance of parasite species. According to Toledo *et al.* (2017), this fact may be related to the greater surface area available for colonization by parasites, as well as the greater intake and more diversified diet of larger frogs. However, an interesting case was observed for *L. vastus*, in which the abundance of parasites were negatively correlated with host size, contrasting to the pattern mentioned above (Poulin, 2004; Yoder & Coggins, 2007; Ibrahim, 2008; Hamann *et al*., 2012, 2013; Campião *et al*., 2016b; Toledo *et al*., 2015, 2017).

The present study contributes to the knowledge of the helminths infecting amphibians from Brazilian Caatinga, as well as the understanding of the diversity patterns of parasitic infracommunities associated with amphibians.

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