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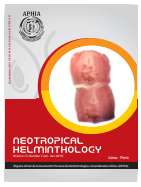

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

HELMINTHS OF *CHIASMOCLEIS ALBOPUNCTATA* (BOETTGER, 1885) (ANURA: MICROHYLIDAE) AND *DENDROPSOPHUS NANUS* (BOULENGER, 1889) (ANURA: HYLIDAE) IN CERRADO, SOUTHEASTERN BRAZIL

HELMINTOS DE *CHIASMOCLEIS ALBOPUNCTATA* (BOETTGER, 1885) (ANURA: MICROHYLIDAE) E *DENDROPSOPHUS NANUS* (BOULENGER, 1889) (ANURA: HYLIDAE) EM UMA ÁREA DE CERRADO, CENTRO-OESTE DO BRASIL

HELMINTOS DE *CHIASMOCLEIS ALBOPUNCTATA* (BOETTGER, 1885) (ANURA: MICROHYLIDAE) Y *DENDROPSOPHUS NANUS* (BOULENGER, 1889) (ANURA: HYLIDAE) EN CERRADO, SURESTE DEL BRASIL

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ABSTRACT

Brazil is the country with the largest amphibian richness in the world with about 1,136 described species, of which 1,093 are anurans. However, related studies on the knowledge of the associated helminth fauna are scarce, representing only 8% of anurans. This study aimed to identify the parasite communities, and their parasitic parameters in two amphibian species: *Chiasmocleis albopunctata* (Boettger, 1885) and *Dendropsophus nanus* (Boulenger, 1889) found in eastern Mato Grosso do Sul State. The hosts were collected through pitfall traps with drift fences (PTDF) and the Active Search method at the breeding sites, euthanized, and the helminths removed and identified. A total of 172 amphibians were collected: 70 individuals from *C. albopunctata* (Boettger, 1885) and 102 individuals from *D. nanus* (Boulenger, 1889). Of this total, 84 were parasitised by at least one taxon. The total number of helminths found was 675. Here we report ten helminth taxa, including two new records for the *D. nanus* host species, and five records for *C. albopunctata* host species. Thus, this study represents an important contribution to the knowledge of parasite and host diversity, since the amount of existing studies is insufficient and not representative.

Keywords: Anurans – Diversity Conservation – Helminthology – Parasitology – Wild Animals

RESUMEN

Brasil es el país con la mayor riqueza de anfibios del mundo con alrededor de 1.136 especies descritas, de las cuales 1.093 son anuros. Sin embargo, los estudios relacionados sobre el conocimiento de la fauna de helmintos asociados son escasos, y representan solo el 8% de los anuros. Este estudio tuvo como objetivo identificar las comunidades de parásitos y sus parámetros parasitarios en dos especies de anfibios: *Chiasmocleis albopunctata* (Boettger, 1885) y *Dendropsophus nanus* (Boulenger, 1889) que se encuentran en el este del estado de Mato Grosso do Sul. Los hospedadores fueron recolectados a través de trampas de caídas con cercas de deriva (PTDF) y por el método de búsqueda activa en los sitios de reproducción, sacrificados, y los helmintos fueron removidos e identificados. Se recolectaron un total de 172 anfibios: 70 individuos de *C. albopunctata* (Boettger, 1885) y 102 individuos de *D. nanus* (Boulenger, 1889). De este total, 84 fueron parasitados por al menos un taxón. El número total de helmintos encontrados fue de 675. Aquí informamos diez taxones de helmintos, incluidos dos nuevos registros para la especie hospedadora *D. nanus* y cinco registros para la especie hospedadora *C. albopunctata*. Por lo tanto, este estudio representa una contribución importante al conocimiento de la diversidad de parásitos y huéspedes, ya que la cantidad de estudios es insuficiente y no representativa.

Palabras clave: Anuros – Conservación de la Diversidad – Helmintología –Parasitología – Animales salvajes

INTRODUCTION

Brazil is the country with the largest amphibian diversity in the world with about 1,136 species described, of which 1,093 are anurans (Martins-Sobrinho *et al*., 2017; Segalla *et al*., 2019). However, related studies on the knowledge of the associated helminth fauna are scarce, representing only 8% of anurans (Campião *et al*., 2014, 2016; Graça *et al*., 2017). Given that Brazil has a rich helminth fauna, with about 164 taxa (57% of the rates found for South America) (Campião *et al*., 2014; Graça *et al*., 2017), faunal surveys are necessary since the vast majority of anurans have not had their helminth fauna described (Graça *et al*., 2017). Faunal surveys are urgently needed because the number of newly discovered organisms is disappearing faster than new organisms are described (Greene & Losos, 1988; Dobson *et al*., 2008; Muniz-Pereira *et al*., 2009). This is especially true in areas such as the Cerrado, which has a very large diversity of species, mainly endemic, but has a high degree of degradation recorded, being constantly impacted by anthropic activities which causes extinction of biodiversity, invasion of exotic species, soil erosion, and fragmentation of habitats (Klink & Machado, 2005; Myers *et al.*, 2000).

Anurans are animals widely diverse in their ways of life and can spend their lives close to bodies of water, as well as use them only in the larval stages and breeding periods. They can even live in environments with low water availability. Anurans' way of life has a great influence on their parasitic composition, just as parasites are major determinants of this host group. However, amphibian helminths constitute a poorly sampled group in taxonomic survey studies (Anjos, 2011; Campião *et al*., 2014).

Knowledge of the associated helminth fauna is important in understanding the ecological interactions between the various species of a community, understanding the ecosystem functions (McCallum & Dobson, 1995; Poulin & Morand, 2000, 2004), biogeography (Rohde, 2005), and environmental quality assessment of ecosystems (Thomas *et al*., 2005). Projects related to environmental conservation and recovery require knowledge of ecology and the systematics of organisms and ecosystems (Scott *et al*., 1987). Given these assumptions, knowledge of wildlife

fauna, associated parasites, and ecological interactions is an important tool for conservation, recovery, and rational use of the environment (McCallum & Dobson, 1995; Poulin & Morand, 2000; Santos, 2003; Thomas *et al*., 2005, 2009).
This study aimed to reduce the gap in the helminthological knowledge of anurans by identifying parasite communities and their parasitic parameters in two amphibian species *Chiasmocleis albopunctata* (Boettger, 1885) and *Dendropsophus nanus* (Boulenger, 1889) in an area of Cerrado Biome, found in eastern Mato Grosso do Sul state in the Midwest region of Brazil.

MATERIAL AND METHODS

Study Area
The collections were performed in an area of the Gallery Forest adjacent to the Stream of Véstia, at UNESP Research and Extension Teaching Farm (FEPE), Ilha Solteira Campus (20 ° 21'48" S, 051 ° 24'17" W), Selvíria Municipality, Mato Grosso do Sul State, Brazil.

Host Collection
The hosts were collected through six sets of Pitfall Traps with Drift Fence (PTDF) (Corn, 1993), consisting of a Y-shaped fence with a centre bucket and a bucket at each "Y" vertex, usually in places near anuran breeding sites. We also used the searching in breeding sites method described by Scott & Woodward (1994), which consists of systematically walking around and through the given collection site and searching for the animals of interest (Halliday, 2006). In this work, the active search collections were always performed by an average of three people for three consecutive hours (from 20:00 to 23:00 hours). The collections took place monthly between September 2013 and November 2015 (SisBio 36667-2; CEUA no. 06/2014).

At UNESP's Ecology Laboratory of Parasitism (Lecop), the hosts were euthanised with sodium thiopental solution. All internal organs, the cellomatic cavity, and hind limb musculature were evaluated for the presence of helminths.

The hosts were deposited at the Museum of Zoology "Adão José Cardoso" State University of Campinas (ZUEC).

Helminths Collection
Helminths were collected, fixed, and processed following commonly employed methodologies (Amato *et al*., 1991). The nematodes were clarified with Aman's lactophenol (Andrade, 2000), and species identification was based on articles and classification keys (Vicente *et al*., 1990; Gibbons, 2010; Anderson *et al*., 2009). Trematodes were submitted to the hydrochloric carmine staining technique (Amato *et al*., 1991), diaphanized with clove oil, and identified according to Fernandes & Kohn (2014). Parasitic parameters such as Prevalence (P) and Mean Infection Intensity (IMI) were calculated according to Bush *et al.* (1997), with the aid of the SigmaEstat 3.1 program.

The morphometric, morphological, and photomicrograph data of the helminths were obtained using a computerised LAS V4 image analysis system (Leica Application Suite) adapted to the DM 2500-Leica microscopes with phase interferential contrast system.

The parasites were deposited in the Helminthological Collection of the Department of Parasitology, Institute of Biosciences, Paulista State University (UNESP) (CHIBB 8798 - 8818), Botucatu, State of São Paulo, Brazil.

Ethic aspects
All international, national, and institutional guidelines applicable to the care and use of animals were followed.

RESULTS

A total of 172 amphibians, 70 *C. albopunctata* (Boettger, 1885) individuals and 102 *D. nanus* (Boulenger, 1889) individuals were collected. Of these, 84 were parasitised by at least one taxon (P = 48.8%). The total number of helminths found was 675, with a mean infection intensity of 5.8 ± 1.4 (1-154).

In the host species *C. albopunctata* (Boettger, 1885), 214 helminths were found, of which 207

were nematodes, and seven were trematodes. The overall prevalence was 62.8%, and the IMI was 2.8 ± 0.3 (1-14). The nematodes were the most prevalent helminths (97.7%) with an IMI of 2.8 ± 0.3 (1-14), while trematodes had a prevalence of 2.3% and a total of seven individuals collected in a single host.

In the host species *D. nanus* (Boulenger, 1889), 461 helminths were found, of which 452 were trematodes, five were acanthocephalans, and four were nematodes. The total prevalence of helminths was 39.2%, and the IMI was 11 ± 3.7 (1-154). The prevalence of trematode infection in this host species was 90% with an IMI of 12.2 ± 4.2 (1-154); for acanthocephalans, the prevalence was 5% with an IMI of 2.5 ± 1.5 (1-4), and for nematodes the prevalence was 7.5% with an IMI of 1.3 ± 0.3 (1-2). The taxa found in each host species, infection sites, their prevalence (P) and mean infection intensity (IMI), and abundance are described in Table 1.

Table 1. Parasitic parameters of nematodes and trematodes associated with anurans *Chiasmocleis albopunctata* (Boettger, 1885) and *Dendropsophus nanus* (Boulenger, 1889). ID = Small Intestine; Cav = Cavity; GI = Large Intestine; P = Overall Prevalence; IMI = Mean Intensity of Infection; SI = Site of Infection; E = stomach.

Chiasmocleis albopunctata (Boettger, 1885)			
Helminths	P (%)	IMI	SI
Cosmocercidae	60.5	3.2 ± 0.46 (1-12)	ID, IG, Cav
Aplectana sp.	2.3	3	ID
Cosmocerca parva	67.4	2.5 ± 0.4 (1-14)	ID, IG, Cav
Mesocoelium monas	2.3	7	ID
Larva of Nematoda	2.3	1	IG
P (%)	58.6		
IMI	5.2 ± 2.7		
Dendropsophus nanus (Boulenger, 1889)			
Helminths	P (%)	IMI	SI
Cosmocercidae	7.5	1.3 ± 0.33 (1-2)	ID, IG
Lophosicyadiplostomum sp.	85	13.1 ± 4.5 (1-154)	Rim
Cyst of Trematoda digenea	2.5	1	Rim
Metacercaria unidentified	2.5	3	E
Neohaematoloechus neivai	2.5	2	Pulmão
Echinorhynchidae	5	2.5 ± 1.5 (1-4)	E, ID
P(%)	41.2		
IMI	11.5 ± 24.5		

Table 2. List of taxa found for each host species, highlighting what has been reported in the literature and what is being reported for the first time.

	Dendropsophus nanus (Boulenger, 1889)
Family Cosmocercidae	Campião *et al.* (2014, 2016)
Lophosicyadiplostomum sp.	Hamann & González (2009)
Cyst of Trematoda Digenea	Unidentified
Metacercaria unidentified	Unidentified
Neohaematoloechus neivai	First report
Family Echinorhynchidae	First report
	Chiasmocleis albopunctata (Boettger, 1885)
Family Cosmocercidae	First report
Aplectana sp.	First report
Cosmocerca parva	First report
Mesocoelium monas	First report
Larva of Nematoda	First report

DISCUSSION

We report here ten helminth rates and seven new records, in addition to the host species *C. albopunctata* (Boettger, 1885), which had not been studied so far (Table 2).

The anuran *D. nanus* (Boulenger, 1889) is found from Suriname and French Guiana, to Uruguay, Argentina, Paraguay and Bolivia, and in Brazil it occurs from the Northeast to the South (Frost, 2019). The registration of two new helminth taxa for this species demonstrates the lack of studies in this area.

Existing parasitological studies revealed 11 parasitic helminth taxa of *D. nanus* (Boulenger, 1889), these being *Centrorhyncus* sp., Unidentified Acanthocephala, *Contracaecum* sp., *Cosmocerca podicipinus* Baker & Vaucher, 1984, Cosmocercoidea gen. sp., *Cylindrotaenia* sp., *Creptotrema* sp., Digenea gen. sp., Plagiorchiata gen. sp, Diplostomidae gen. sp., and *Brevimulticaecum* sp. (Hamann & Kehr, 1998; González & Hamann, 2011; Aguiar *et al*., 2014; Campião *et al*., 2014, 2016; Graça *et al*., 2017).

The anuran *D. nanus* (Boulenger, 1889) has an aquatic larval phase, remains in the vegetation near the water as an adult, and uses the aquatic environment in reproduction (Uetanabaro *et al*., 2008). Proximity to water during the anuran life cycle is highly related to the high prevalence of trematode parasites (McAlpine, 1997; Muzzall *et al*., 2001; Bolek & Coggins, 2001, 2003; Paredes-Calderón *et al*., 2004; Hamann *et al*., 2006ab; Schaefer *et al*., 2006; Todd, 2007; Toledo *et al*., 2018), which explains the helminth fauna described for *D. nanus* (Boulenger, 1889) in this work. Thus, the use of water bodies during the life cycle of *D. nanus* (Boulenger, 1889) contributed to determining the parasite community of this anuran.

The Cosmocercidae family comprises of several nematode species and have been reported in several South American countries parasitising the intestine, rectum, stomach, and lungs of their hosts. Unidentified individuals of the family have been reported in *Dermatonotus muelleri* (Boettger, 1885), *Physalaemus nattereri* (Steindachner, 1863) (=*Eupemphix nattereri*), *Boana prasina* (Burmeister, 1856) (=*Hypsiboas prasinus*), *Leptodactylus latrans* (Steffen, 1815), *Leptodactylus pentadactylus* (Laurenti, 1768),

Leptodactylus podicipinus (Cope, 1862), *Pethecopus azureus* (Cope, 1862) (=*Phyllomedusa azurea*), *Rhinella crucifer* (Wied-Neuwied, 1821), *Rhinella icterica* (Spix, 1824) and *Rhinella diptycha* (Cope, 1862)(=*Rhinella schneideri*) (Campião *et al*., 2014). In the present study, for the species *D. nanus* (Boulenger, 1889), individuals of this family were found in the small and large intestines of the anurans. Acanthocephalans, belonging to the family Echinorhynchidae, have been reported in Paraguay and Brazil parasitising the intestine and body cavity of the hosts, being some species from this family *Acanthocephalus acutispinus* Machado Filho, 1968, *Acanthocephalus caspanensis* Fernandez and Ibarra, 1989, *Acanthocephalus correalimai* Machado Filho, 1970, *Acanthocephalus ula* Lent and Santos, 1989 and *Pseudoacanthocephalus lutzi* (Hamann, 1891) (Campião *et al*., 2014). However, in this study, individuals of this family were found in the small intestine and the stomach of the anurans. Trematoda *Lophosicyadiplostomum* is an anuran parasite in its larval phase and has been found in Argentina and Brazil parasitising the kidneys of these hosts. Individuals of the genus have been reported in *Scinax nasicus* (Cope, 1862), *Lysapsus limellum* Cope, 1862 (=*Pseudis limellum*), and *D. nanus* (Boulenger, 1889) (=*Hyla nana*) (Hamann & González, 2009; Campião *et al*., 2014). In the present study, this species was found parasitising the kidneys. The trematode *Neohaematoloechus neivai* (Travassos & Artigas, 1927), have been found in Brazil and Venezuela parasitising the lungs of their hosts (Campião *et al*., 2014; Fernandes & Kohn, 2014), having already been reported in *Leptodactylus latrans* (Steffen, 1815), *Leptodactylus labyrinthicus* (Spix, 1824), *Leptodactylus pentadactylus* (Laurenti, 1768), *Pseudis paradoxa* (Linnaeus, 1758), and *Lithobates palmipes* (Spix, 1824) (=*Rana palmipes*).

In this study, with the registration of the trematode *Neohaematoloecus neivai* (Travassos & Artigas, 1927) and acanthocephalans of the family Echinorhynchidae, we increased the number of helminth taxa of *D. nanus* (Boulenger, 1889) to 13.

The frog *C. albopunctata* (Boettger, 1885) is found in Bolivia, Argentina, Paraguay, and the Midwest and Southeast Regions of Brazil (Frost, 2019). Although their distribution covers four different countries, the associated helminth fauna of this anuran has never been studied.

The species *C. albopunctata* (Boettger, 1885) is considered terrestrial and uses the aquatic environment only in the larval phase and during breeding periods (Uetanabaro *et al*., 2008; Giaretta *et al*., 2008). Contact with soil may favour nematode infection (Barton, 1999; Bolek & Coggins, 2000, 2003; Iannacone, 2003; Luque *et al*., 2005; Sena *et al*., 2018; Toledo *et al*., 2018), which explains this marked prevalence of these helminths for this host species. Despite this prevalence of nematodes, the richness of parasite species for this host was low with only three species identified.

For the species *C. albopunctata* (Boettger, 1885), nematodes of the family Cosmocercidae were found parasitising the body cavity of the hosts. For the *Cosmocerca parva* Travassos, 1925 helminth, there are studies indicating its presence in Argentina, Brazil, Colombia, Paraguay, Guyana, and Peru (Campião *et al*., 2014). These parasites are found mainly in the intestine and rectum of their hosts, and have been reported in several species of various genera such as *Ameerega, Colostethus*, *Dendropsophus*, *Edalorhina*, *Elachistocleis*, *Hamptophryne*, *Hypsoboas*, *Hylodes*, *Leptodactylus*, *Odontophrynus*, *Oreobates*, *Phyllomedusa*, *Physalaemus, Pristimantis*, *Rhaeboophion*, *Scella*, *Procella*, *Rhinella*, *Proceratophrys, Scarthyla,* and *Scinax* (Campião *et al*., 2014). In this study, we found *Cosmocerca parva* Travassos, 1925 in the body cavity of the hosts. Individuals of the genus *Aplectana* have been documented in several South American countries parasitising the stomach, intestine and rectum of their hosts. Unidentified individuals of the genus have been found parasitising *Ceratophrys Cranwelli* Barrio, 1980, *Dendropsophus microps* (Peters, 1872), *Dermatonotus muelleri* (Boettger, 1885), *Haddadus binotatus* (Spix, 1824), *Boana albopunctata* (Spix, 1824) (=*Hypsiboas albopunctata*), *Boana pardalis* (Spix, 1824) (=*Hypsiboas pardalis*), *Leptodactylus bufonius* Boulenger, 1894, *Leptodactylus chaquensis* Cei, 1950, *Leptodactylus elenae* Heyer, 1978, *Leptodactylus fuscus* (Schneider, 1799), *Adenomera marmorata* Steindachner, 1867

(=*Leptodactylus marmoratus*), *Leptodactylus mystacinus* (Burmeister, 1861), *Leptodactylus podicipinus* (Cope, 1862), *Leptodactylus syphax* Bokermann, 1969, *Physalaemus signifier* (Girard, 1853), *Rhinella granulosa* (Spix, 1824), *Rhinella icterica* (Spix, 1824), *Rhinella marina* (Linnaeus, 1758), *Scinax acuminatus* (Cope, 1862), *Thoropa miliaris* (Spix, 1824), and *Trachycephalus mesophaeus* (Hensel, 1867) (Campião *et al*., 2014). In this study, *Aplectana* sp. was found in the small intestine of the host. The *Mesocoelium monas* (Rudolphi, 1819) Trematoda, according to Campião *et al.* (2014) and Fernandes & Kohn (2014), have been found in Argentina, Brazil, Colombia, Paraguay, Peru and Venezuela parasitising the small intestine of their hosts, and have been reported in *Siphonops annulatus* (Mikan, 1822), *Leptodactylus fuscus* (Schneider, 1799), *Leptodactylus mystaceus* (Burmeister, 1861), *Leptodactylus mystacinus* (Burmeister, 1861), *Leptodactylus latrans* (Steffen, 1815), *Leptodactylus pentadactylus* (Laurenti, 1768), *Rhinella arenarum* (Hensel, 1867), *Rhinella crucifer* (Wied-Neuwied, 1821), *Rhinella icterica* (Spix, 1824), *Rhinella marina* (Linnaeus, 1758), *Rhinella diptycha* (Cope, 1862) (=*Rhinella schneideri*), *Incilius nebulifer* (Girard, 1854) and *Scinax nasicus* (Cope, 1862). In the present study it was also found in the small intestine of the host.

All taxa found in *C. albopunctata* (Boettger, 1885) have never been reported for this host species, as this is the first study using this approach. Here we highlight the five taxa record for this host species. Additional studies with populations from other locations may show different patterns from those found here.

This study represents an important contribution to the knowledge of the diversity of parasites and hosts since the number of studies is insufficient and not representative. It also helps to complement information for ecosystem studies with a view to facilitating conservation projects.

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