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Helminth parasites of *Leptodactylus podicipinus* (Anura: Leptodactylidae) from south-eastern Pantanal, State of Mato Grosso do Sul, Brazil

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Abstract

Forty-three specimens of *Leptodactylus podicipinus* (Anura: Leptodactylidae) were collected in the south-eastern Pantanal, municipality of Corumbá, State of Mato Grosso do Sul, Brazil in February and July 2007, and examined for endoparasites. Forty (93%) specimens were infected with at least one helminth species. The predominant parasites were nematodes (*Aplectana* sp., *Cosmocerca podicipinus*, *Oswaldocruzia lopesi*, *Physalopteroides venancioi*, *Rhabdias* sp.), but the trematode *Catadiscus propinquus* also showed high prevalence. The trematodes *Infidum infidum* and *Travtrema stenocotyle* were also found, but in only one specimen. Adult frogs showed higher parasite diversity than subadults. *Leptodactylus podicipinus* was preferentially infected by direct life-cycle parasites and was reported as a new host record for seven helminth species.

Introduction

The global decline of amphibians (Stuart *et al.*, 2004) and the recording of malformations (Blaustein & Johnson, 2003) have received considerable attention, since members of this group are considered bioindicators of environmental quality. This justifies the intense monitoring of amphibian populations (Esch & Fernández, 1993; Daszak *et al.*, 2003). Parasites play a central role in ecosystems, affecting the ecology and evolution of their hosts, host population growth, regulation and community biodiversity (Brooks *et al.*, 2001). Evaluating the patterns of abundance of the parasites within the host population and studying their cycles of transmission are considered central points in the study of the host–parasite interaction in wild animals (Bonsall, 2002).

Several studies with parasite communities of anurans have been conducted, but the vast majority are with species from temperate latitudes (Bolek & Coggins, 2000, 2001, 2003; Paredes-Calderón *et al.*, 2004; Luque *et al.*, 2005). In Brazil, for the Pantanal region, studies with anurans are rare and fragmentary, limited to species listings and some preliminary comments on reproductive biology (Lutz, 1946; Prado *et al.*, 2000; Gordo & Campos, 2003).

Among the anurans found in the southern Pantanal, one of the most abundant species of frog is *Leptodactylus podicipinus* (Cope, 1862) which can be found in Paraguay, Argentina, Bolivia and south-western Brazil. This anuran can be found in forests and on the margins of permanent and temporary ponds (Prado *et al.*, 2000). The only record of endoparasites infecting *L. podicipinus* is from Baker & Vaucher (1984), describing the species *Cosmocerca podicipinus* Baker & Vaucher, 1984 in specimens collected in Paraguay. In Brazil, despite being fairly abundant in the Pantanal floodplain, there are no records of parasitism in this species.

This study reports the helminth fauna of *L. podicipinus* from the south-eastern Pantanal, municipality of Corumbá, Mato Grosso do Sul State, Brazil.

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Materials and methods

The study was carried out with specimens collected from the riparian forest of the Miranda River (19°34′37″S, 57°00′42″W) at Base de Estudos do Pantanal (BEP), municipality of Corumbá, State of Mato Grosso do Sul, Brazil.

The Miranda River is flooded from January to April and the phytophysiognomy consists of *cerrado* (savanna-like vegetation), semideciduous and gallery forests, and grassland fields. The average monthly temperature ranges between 23 and 26°C. The region is characterized by a rainy summer season from October to April and dry winter from May to September with average annual rainfall of about 1100 mm (Silva & Abdon, 1998).

The anurans were collected in February and July 2007, including wet and dry seasons, respectively. All specimens were deposited in the Coleção Zoológica de Referência do Campus de Campo Grande, Seção Herpetologia (CEUCH), of the Universidade Federal de Mato Grossodo Sul, Brazil (accession numbers: CEUCH 3821–3825, 3828–3835, 3843–3851, 4100–4120, 4128). The frogs were hand-captured and then transported to the laboratory where they were euthanatized with sodium thiopental solution. The snout–vent length (SVL) was recorded using digital calipers to the nearest 0.01 mm and body weight by dynamometer to the nearest 0.1 g. Subsequently, the lungs, digestive tract, kidney, bladder, muscle, skin, spleen and liver were examined for helminth parasites. Helminths were fixed in alcohol–formaldehyde–acetic acid and preserved in 70% GL ethyl alcohol. For identification, trematodes were stained with carmine and nematodes cleared with lactophenol. Morphometric analysis of helminths was performed in a computerized system for image analysis (Qwin Lite 3.1, Leica Microsystems, Wetzlar, Germany).

The voucher parasite specimens were deposited in the Coleção Helmintológica (CHIBB) of the Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista – UNESP, municipality of Botucatu, State of São Paulo, Brazil. The species identifications were based on Travassos *et al.* (1969), Yamaguti (1961, 1971), Vicente *et al.* (1991), Vrcibradic *et al.* (2000) and González & Hamann (2004).

Prevalence (number of hosts infected with one or more individuals of a particular parasite species), mean intensity (average intensity of a particular species of parasite among the infected members of a particular host species) and mean abundance (total number of individuals of a particular parasite species in a sample of a particular host divided by the total number of hosts of that species examined) were analysed according to Bush *et al.* (1997). The hosts were classified as young and adults according to the reproductive stage and SVL. According to Prado *et al.* (2000), *L. podicipinus* shows sexual dimorphism. We considered: young males (SVL <26 mm) and females (SVL <30 mm); and adult males (SVL >26 mm) and females (SVL >30 mm). These values were adopted based on the smallest male and female which were already reproductively mature. The Shannon Index was used to calculate diversity for the two age groups (young and adults) and Student's *t*-test was used to examine whether there were significant differences between them, as recommended by Magurran (1988). The tests were calculated by the software Divers and Divert (Ecolog/Ecosoft).

Results

A total of 43 specimens of *L. podicipinus* were collected, and 40 (93%) were infected by at least one helminth species. Each infected anuran had from one to five endoparasite species. We found a total of 310 helminthes distributed in eight species. The greatest richness of species was found among nematodes (five species), followed by trematodes (three species). Helminths were found in the lungs (*Rhabdias* sp. Stiles and Hassall, 1905 and *Cosmocerca podicipinus*), stomach (*Physalopteroides venancioi* Lent, Freitas and Proença, 1946 and *Oswaldocruzia lopesi* Freitas and Lent, 1938), small intestine (*C. podicipinus*) and large intestine (*C. podicipinus*, *Aplectana* sp. Railliet and Henry, 1916, *O. lopesi*, *Catadiscus propinquus* Freitas and Dobbin, 1956, *Infidum infidum* (Faria, 1910) Travassos, 1916 and *Travtrema stenocotyle* (Cohn, 1902) Goodman, 1951) (table 1).

Adult frogs ($n = 26$) showed higher diversity of parasites than the subadults ($n = 17$), both in males ($H'_{young} = 0.62$, $H'_{adult} = 1.16$, $t = 3.47$, $P = 0.013$) and females ($H'_{young} = 0.72$, $H'_{adult} = 1.33$, $t = 5.86$, $P < 0.001$). A higher richness of parasites in frogs collected in the rainy season was also observed (fig. 1), but in this season a higher number of adults ($n = 21$) was collected in comparison to the dry season ($n = 5$).

Discussion

The helminth fauna of *L. podicipinus* from the Miranda River region, municipality of Corumbá, Mato Grosso do Sul State, Brazil was evaluated in the present study. Eight helminth species were found and *C. podicipinus* and *C. propinquus* were the most prevalent. Moreover, *L. podicipinus* is a new host recorded for *Aplectana* sp., *C. propinquus*, *I. infidum*, *O. lopesi*, *P. venancioi*, *Rhabdias* sp., and *T. stenocotyle*. These data contribute to the knowledge of anuran helminth fauna and geographical distribution of these helminth species.

Cosmocerca podicipinus and *Rhabdias* sp. were the most prevalent nematode species. The former infects several anuran species and occurs in many countries in South America (Baker & Vaucher, 1984; González & Hamann, 2004; Goldberg *et al.*, 2007). The infection occurs by penetration of the infective larvae through the host's skin. Similarly, *Rhabdias* species have a direct life cycle, alternating between free-living and parasite stages, with the host infection also occurring by means of active skin penetration (Anderson, 2000). The genus *Aplectana* has a direct life-cycle and the infection occurs by ingestion of the larval stage (Goldberg *et al.*, 2007). These data suggest that for *L. podicipinus*, parasites with a direct life cycle and active mechanism of infection are more likely to find their hosts, which would explain the high prevalence of these nematodes. Similar results were found for other *Leptodactylus*

Table 1. Prevalence, intensity, mean abundance and site of infection of helminth parasites of *Leptodactylus podicipinus* from the riparian forest of the Miranda River, municipality of Corumbá, State of Mato Grosso do Sul, Brazil.

Helminth	Prevalence (%)	Mean intensity ± SD	Mean abundance ± SD	Site of infection			
				S	SI	LI	L
Nematoda							
Aplectana sp. CHIBB 2075, 2088*	4.6	1.5 ± 0.7	0.07 ± 0.5			X	
Cosmocerca podicipinus CHIBB 2078, 2083–4, 2101, 2108, 2123, 2172, 2184, 2187, 2190, 2193–4, 2204, 2206, 2209, 2212, 2216, 2220, 2222, 2225, 2541, 2543, 2545, 2547, 2550–1, 2554, 2557, 2559, 2560, 2564, 2566–8, 2571, 2574, 2577, 2581–2, 2585, 2590, 2595	62.7	3.55 ± 2.3	2.23 ± 14.5		X	X	X
Oswaldocruzia lopesi CHIBB 2192, 2216, 2221–2	4.6	1.5 ± 0.7	0.07 ± 0.5	X		X	
Physalopteroides venancioi CHIBB 2070, 2077, 2082, 2087, 2124, 2127, 2183, 2203, 2208, 2211, 2215, 2219	20	5.33 ± 11.1	1.11 ± 8.5	X			
Rhabdias sp. CHIBB 2071, 2076, 2085, 2089, 2102, 2129, 2185, 2217, 2223, 2565, 2573, 2579, 2587, 2596	18.6	1.87 ± 1.1	0.34 ± 2.3				X
Trematoda							
Catadiscus propinquus CHIBB 2079, 2086, 2103, 2126, 2126, 2173–4, 2186, 2188, 2191, 2195, 2205, 2213, 2218, 2224, 2226–7, 2534, 2536, 2540, 2542, 2544, 2546, 2548–9, 2552, 2555–6, 2558–9, 2562–3, 2569–70, 2572, 2575–6, 2578, 2580, 2583–4, 2586, 2588–9, 2591–4	58	5.72 ± 9.8	3.32 ± 22.1			X	
Infidum infidum CHIBB 2125	0.02	1	0.023			X	
Travtrema stenocotyle CHIBB 2103	0.02	1	0.023			X	

SD, standard deviation. Site of infection: S, stomach; LI, large intestine; SI, small intestine; L, lungs.
*Numbers in the first column are the accession numbers of voucher species in CHIBB – Coleção Helmintológica of the Departamento de Parasitologia, Instituto de Biociências, Universidade Estadual Paulista.

species in America (Bursey *et al.*, 2001; Goldberg & Bursey, 2002; Goldberg *et al.*, 2002a, b).

Although the *P. venancioi* life cycle is unknown, some studies indicate that phisalopterines generally require an intermediate host to complete their life cycle (Anderson, 2000). This nematode has often been reported in lizards (Fabio & Rolas, 1974; Vrcibradic *et al.*, 2000; Bursey *et al.*, 2005). However, there are only two reports on its occurrence in amphibians. Lent *et al.* (1946) found *P. venancioi* infecting *Rhinella schneideri* (Werner, 1894) from Paraguay. Bursey *et al.* (2001) reported the occurrence of this nematode species in several anurans, including some *Leptodactylus* species, but *L. podicipinus* is a new host recorded for *P. venancioi*.

Leptodactylus podicipinus is a generalist and opportunistic forager (Rodrigues *et al.*, 2004) and is strongly associated with terrestrial habitat, living in margins of permanent water bodies and ground litter of riparian and gallery forests. Recent studies show that terrestrial frogs are dominated by direct life cycle and generalist parasites (Bolek & Coggins, 2003). In fact, most of the helminths found in *L. podicipinus* are monoxenous and live in other species of frogs (Vicente *et al.*, 1991).

This study showed that *L. podicipinus* presents with a high infection prevalence of *C. propinquus*. The genus *Catadiscus* includes several species commonly found in amphibians (Travassos *et al.*, 1969). However, *T. stenocotyle* and *I. infidum* are typically snake parasites (reviewed by Travassos *et al.*, 1969; Lunaschi & Drago, 2007). Considering that only one sample of each of these species was found in *L. podicipinus*, these might be cases of accidental parasitism.

The adults showed greater species richness of parasites than the younger ones, both in males and females. This difference between subadults and adults may be related to the fact that adults have greater surface area, which could facilitate the infection of direct life cycle parasites. Moreover, in adulthood, these frogs eat larger amounts of food, with a longer exposure to parasites, and are in the reproductive period, which can potentially increase the chances of infection. Yet, most of the adults were collected in the rainy season and this could have influenced the

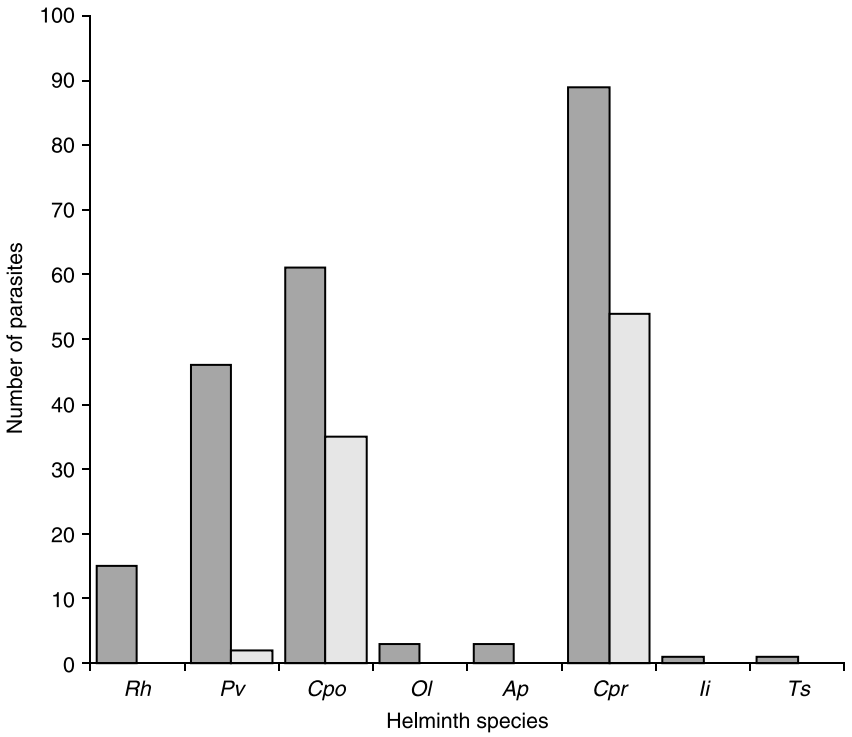

Fig. 1. Helminth species of *Leptodactylus podicipinus* from the riparian forest of the Miranda River, municipality of Corumbá, State of Mato Grosso do Sul, Brazil collected in the dry and rainy seasons. Dark bars, rainy season; light bars, dry season; *Rh*, *Rhabdias* sp.; *Pv*, *Physalopteroides venancioi*; *Cpo*, *Cosmocerca podicipinus*; *Ol*, *Oswaldocruzia lopesi*; *Ap*, *Aplectana* sp.; *Cpr*, *Catadiscus propinquus*; *Ii*, *Infidum infidum*; *Ts*, *Travtrema stenocotyle*.

chances of infection and consequently the prevalence and diversity of the helminth species. Even though the presented data suggest that *L. podicipinus* has higher richness of parasite species in the rainy season, statistical analysis to confirm this hypothesis could not be performed. Future studies will be conducted to investigate the possible influence of the season in structuring helminth parasite communities.

This study showed that in the region of the Miranda River, Pantanal, *L. podicipinus* show a high rate of helminth infection and that there is a significant difference in the pattern of infection among young and adults in this anuran. These results reinforce the importance of carrying out further studies in the region to obtain better understanding of the ecological relations of anurans and their helminth parasites.

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