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Metazoan parasites of *Odontophrynus americanus* (Anura: Odontophrynidae) from the Serra da Bodoquena mountain range, Mato Grosso do Sul, Brazil

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Amphibians can be definitive, intermediate and paratenic hosts of several metazoan parasites (Mcalpine, 1997; Muzzall et al., 2001; Brooks, et al. 2006; Imasuen et al., 2012). The route of infection varies and is influenced by many factors, from natural history traits (e.g. diet, biphasic life cycle, intermediate position in the food web, and reproductive mode) to abiotic conditions (e.g. micro-habitat, temperature and precipitation) (Aho, 1990; Hamann et al., 2006; Todd, 2007).

For many helminth parasite species, transmission may occur directly by active penetration of the parasite into the skin of the host, or through a complex life cycle, with one or more intermediate hosts required for transmission, which may be acquired by ingestion of larval forms and infected intermediate and paratenic hosts (Marcogliese, 2004; Hamann et al., 2013). Ectoparasites may attach to the skin of the host, but can also pass through the skin and encapsulate within the dermis spongy layer (Attademo et al., 2012). On the other hand, the transmission of intra and extracellular hemoparasites can be carried out by means of the contact of the hosts with infected hematophagous vectors (Desser, 2001; Leal et al., 2015).

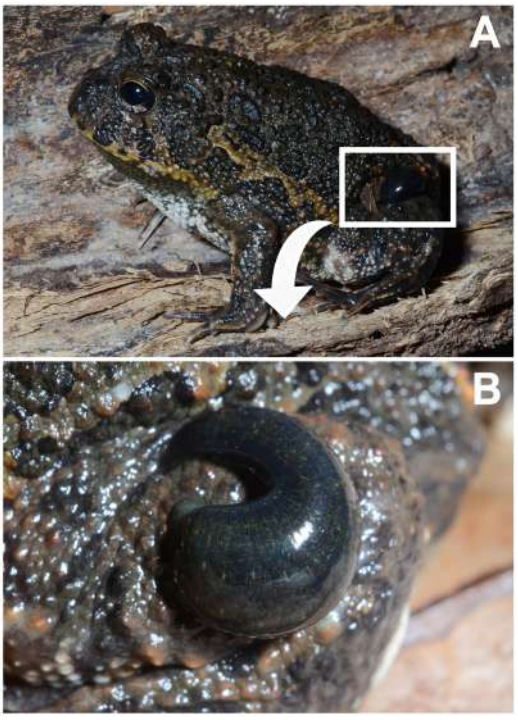

Figure 1. A. Leech Glossiphoniidae gen. sp. (arrow) feeding upon an adult male *Odontophrynus americanus*. B. The leech anchored on the thigh-skin during the blood feeding.

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Belonging to the family Odontophrynidae, *Odontophrynus americanus* (Duméril and Bibron, 1841) is a terrestrial and nocturnal toad widely distributed in Argentina, Paraguay, Uruguay, and Brazil (Frost, 2017). In this work, we present the first records of metazoan parasites of *O. americanus* from the Serra da Bodoquena mountain range, Mato Grosso do Sul state, Brazil.

We collected 10 adult males of *O. americanus* during nocturnal visual surveys (Heyer et al., 2014) on 14 May 2016, in a temporary pond in the pasture area (-21.114857°, -56.521811°, ~ 395m a.s.l., *datum*: WGS84) near to the campus of the Universidade Federal de Mato Grosso do Sul, located in the Bonito municipality, Mato Grosso do Sul state, central Brazil. The specimens were found during the breeding season, with several males vocalizing at the edge of the water body. Collected specimens were transported to the laboratory and euthanized with topic Xylocaine® 5% (CONCEA, 2013). Thereafter, we examined the body surface, eyes and oral cavity using a stereomicroscope. Subsequently, the specimens were necropsied and lungs, musculature, kidneys, liver, bladder, spleen, digestive tract and the coelomic cavity were also examined under stereomicroscope. The necropsied anuran specimens were fixed in 10% formalin, and preserved in 70% alcohol. Voucher specimens of all anurans and parasites are housed/stored at the Zoological Collection of the Universidade Federal de Mato Grosso de Sul (voucher specimens anurans ZUFMS-AMP 07615, 07616, 07617, 07618, 07619, 07620, 07621, 07622, 07623, 07624), and metazoan parasites (ANN 00002, NEM 00049-00050-00051, PLA 00013).

For species determination, the nematodes were fixed in 5% formalin, placed in 70% ethyl alcohol, clarified with glycerine and examined using temporary slides. Trematoda was compressed between lamina and coverslip, stained with Delafield's hematoxylin, diaphanised in beech creosote, and mounted on a permanent blade with Canada balsam. Leeches were also compressed between lamina and coverslip, later clarified on temporary slides of glycerine.

Four out of the 10 specimens of *O. americanus* had parasites of at least one morphospecies (Figure 1). We identified five morphospecies that belong to three nematodes (Ascarididae gen. sp., Cosmocercidae gen. sp., *Rhabdias* sp. (cf. *elegans*)), one trematoda (*Glypthelmins palmipedis*), and one leech (Glossiphoniidae gen. sp.). The leeches presented the highest prevalence (Figure 1), while the nematode *Rhabdias* sp. (cf. *elegans*) was the most abundant (Table 1).

Currently, at least 11 morphospecies of helminths parasites are known to infect populations of *O. americanus*, six nematodes (*Aplectana membranosa*, *A. meridionalis*, *Cosmocerca parva*, *C. podicipinus*, *C. uruguayensis* and *Rhabdias elegans*), four trematodes (Echinostomatidae gen. sp., *Styphlodora* sp., *Travtrema* aff. *stenocotyle*, *Opisthogonimus* sp.), and one Acanthocephala (*Oligacanthorhynchus* sp.). The myxosporid *Myxidium immersum* also is known to infest *O. americanus*, with all these records made in Argentina, Paraguay and Uruguay (Cordero, 1928; Smales, 2007; González and Hamann, 2009).

Among the parasites already found in *O. americanus*, *Rhabdias elegans* is the only one that has a previous report for this host, from the municipality of Corrientes, Argentina (González and Hamann, 2009). In our study, the specific determination of the *Rhabdias* parasite was not possible, since the parasite found here is a female and the most reliable diagnostic characters can only be found on male specimens (Kuzmin et al., 2003).

Table 1. Prevalence (P%) with a 95% confidence interval, mean abundance (MA), and mean intensity (MI) of the metazoan parasites of *Odontophrynus americanus* collected in the Serra da Bodoquena mountain range, Mato Grosso do Sul state, central Brazil. SD – standard deviation.

Metazoan parasites	Site of infection	P(%)	MA ± SD	MI ± SD
Nematoda				
Ascarididae gen. sp.	Small intestine	10 (0.002-0.445)	0.01 ± 0.32	1.00 ± 0.40
Cosmocercidae gen. sp.	Small intestine	10 (0.002-0.445)	0.01 ± 0.32	1.00 ± 0.40
Rhabdias sp. (cf. *elegans*)	Lung	10 (0.002-0.445)	0.04 ± 1.26	4.00 ± 1.62
Trematoda				
Glypthelmins palmipedis	Small intestine	10 (0.002-0.445)	0.01 ± 0.32	1.00 ± 0.40
Annelida				
Glossiphoniidae gen. sp.	Body surface	30 (0.066-0.652)	0.03±0.48	1.00 ± 0.50

Following a more conservative assumption, we named this recorded parasite as *Rhabdias* sp. (cf. *elegans*). However, we highlight that the individual found here presents characteristics very similar to *R. elegans* nominal species.

Nematodes of the *Rhabdias* genus are pulmonary parasites, commonly found in the lungs of amphibians and reptiles, via direct transmission and the infection occurs by active penetration into the skin of the hosts (Langford and Janovy, 2009; Kuzmin et al., 2015). In our study we discovered this parasite on the amphibians' lungs.

Only female specimens of the nematodes Ascarididae and Cosmocercidae were found, precluding us from reaching a more specific identification, as the main diagnostic morphological characters can only be found in male specimens (Mcallister et al., 2010; Campião et al., 2016). Ascarididae and Cosmocercidae are intestinal parasites acquired through the diet or by larvae penetration through the skin (Vicente et al., 1990; Anderson, 2000).

The nematodes registered parasitizing *O. americanus* may be present to the fossorial habit of this amphibian species, a natural history trait that can facilitate eventual transmission by infective forms that live in the soil (Barton, 1999; Bolek and Coggins, 2003; González and Hamann, 2009).

Aquatic parasites (i.e. trematoda and leeches) can parasitize the specimens of *O. americanus* during the *O. americanus'* reproductive behaviour, when both males and females move to water bodies to spawn (Bertoluci, 1998; Eterovick and Sazima, 2000). Trematodas of the *Glypthelmins* genus infect amphibians by active penetration of the cercarias through the skin, or by the ingestion of infectious encysted metacercarias on aquatic vegetation (Hamann et al., 2009).

The leeches of the Glossiphoniidae family found here are also principally acquired in aquatic environments, many of which are known as potential vectors of some pathogens, such as trypanosomes and fungi (Chivers et al., 2001; Hopkins et al., 2014). Members of the Glossiphoniidae family have already been registered as ectoparasites in another eleven species of anurans (*Bombina variegata*, *Bokermannohyla hylax*, *Bufo spinosus*, *Lithobates catesbeianus*, *L. pipiens*, *L. sphenocephalus*, *L. sylvaticus*, *Pseudacris crucifer*, *Rana cascadae*, *R. pretiosa* and *R. temporaria*), both in adults and tadpoles, with occurrences in Bosnia and Herzegovina, Brazil (state of São Paulo), the United States, Italy and Tunisia (Riggs and Ulmer,1983; Berven and Boltz, 2001; Briggler et al., 2001; Chivers et al., 2001; Bertoluci et al., 2003; Siddall and Bowerman, 2006; Tiberti and Gentilli, 2010; Stead and Pope, 2010; Hassine and Escoriza, 2014; Zimić, 2015; Watermolen, 1996).

The parasitic helminths found in *O. americanus* are common parasites, previously recorded in other amphibian hosts (Campião et al., 2014). All the records of these parasites in their adult stage show *O. americanus* acting as their definitive host.

In general, our results indicate that the presence of terrestrial and aquatic metazoan parasites is probably a reflection of the habits of *O. americanus*, due to the environment which the host inhabits being a factor that favours the transmission of certain species of parasites. Anurans of fossorial habit remain buried during periods of drought and usually host parasitic communities composed of nematodes which are transmitted by active penetration through the host's skin or by ingestion of the infecting larval forms (Anderson, 2000). However, *O. americanus* also inhabits water bodies during reproductive periods, which facilitates contact with aquatic-transmission parasites.

Our data suggests an intricate relationship between *O. americanus* and its environment. The new occurrences of parasites for *O. americanus* found in this study highlight the need to maintain the integrity of the landscape in this unique and highly diversified region.

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