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Digenetic helminths of *Leptodactylus latrans* (Anura: Leptodactylidae) and *Rhinella dorbignyi* (Anura: Bufonidae) in southern Brazil

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RESUMO

Helminhos digenéticos de *Leptodactylus latrans* (Steffen, 1815) (Anura: Leptodactylidae) e *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Anura: Bufonidae) no sul do Brasil. Anuros são citados como hospedeiros para uma vasta diversidade de helmintos, entretanto as informações relativas a helmintofauna de anuros no Brasil, são pouco abordadas, sobretudo na região sul do Rio Grande do Sul. Este estudo tem como objetivo registrar os helmintos digenéticos parasitos de *Leptodactylus latrans* e *Rhinella dorbignyi* no extremo sul do Brasil. Para realização da pesquisa foram coletados entre agosto de 2017 e agosto de 2018, 60 espécimes de anuros, *L. latrans* (n= 30) e *R. dorbignyi* (n= 30), os quais foram necropsiados e os helmintos coletados, fixados e corados para identificação conforme bibliografia específica para cada grupo. Os índices de infecção estimados foram prevalência (P%), intensidade média de infecção (IMI) e abundância média (AM). Do total de anuros examinados, 28 indivíduos (46,66%) apresentaram helmintos digenéticos, totalizando 255 espécimes de parasitos. *Leptodactylus latrans* esteve parasitado por *Gorgoderina megacysta* (P = 40%), *Haematoloechus freitasi* (P = 23,33%), *Catadiscus* spp. (P = 30%), Plagiorchioideae gen. spp. (P = 63,33%), e *Halipegus* sp. (P = 3,33%), enquanto *R. dorbignyi* apresentou infecção por *Gorgoderina* sp. (P = 3,33%), *Haematoloechus* sp. (P = 3,33%), *Catadiscus* sp. (P = 3,33%), Plagiorchioideae gen. sp. (P = 3,33%), e Diplostomidae gen. spp. (metacercárias) (P = 6,66%).

Palavras-chave: Derogenidae, Diplodiscidae, Diplostomidae, Gorgoderidae, Haematoloechidae, Plagiorchioideae.

ABSTRACT

Even though anurans have been hosts to an array of helminths, data on the helminth fauna of anurans in Brazil are scarce. This study aims at reporting digenetic helminths on *Leptodactylus latrans* (Steffen, 1815) and *Rhinella dorbignyi* (Duméril & Bibron, 1841) in southern of Brazil. Sixty specimens of anurans *L. latrans* (n= 30) and *R. dorbignyi* (n= 30) were collected between August 2017 and August 2018. Then, necropsy was performed and helminths were collected, fixed and dyed to be identified in agreement with specific bibliography. Estimated infection indices were prevalence (P%), mean intensity of infection (MI) and mean abundance (MA). Twenty-eight anurans (46.66%) exhibited digenetic helminths, totalizing 255 parasite specimens. *Leptodactylus latrans* was infected with *Gorgoderina megacysta* Mañé-Garzón & González, 1978 (Gorgoderidae) (P = 40%), *Haematoloechus freitasi* Mané Garzón & Solares, 1959 (Haematoloechidae) (P = 23.33%), *Catadiscus* spp. (Diplodiscidae) (P = 30%), Plagiorchioideae fam. gen. spp. (P = 63.33%) and *Halipegus* sp. (Derogenidae) (P = 3.33%), whereas *R. dorbignyi* was infected with *Gorgoderina* sp. (P = 3.33%), *Haematoloechus* sp. (P = 3.33%), *Catadiscus* sp. (P = 3.33%), Plagiorchioideae fam. gen. sp. (P = 3.33%) and Diplostomidae gen. spp. (metacercariae) (P = 6.66%).

Keywords: Derogenidae, Diplodiscidae, Diplostomidae, Gorgoderidae, Haematoloechidae, Plagiorchioideae.

Anurans, which have been reported as hosts to a rich diversity of digenetic helminths, play important roles as definitive, intermediate and paratenic hosts. In many cases, they are fundamental to the development of the life cycle of these parasites (Aho, 1990; Campião et al., 2014; Fernandes & Kohn, 2014).

Specimens of *Leptodactylus latrans* (Steffen, 1815) (Leptodactylidae), whose size ranges from 120 mm to 140 mm, can be found in the south of Brazil. They can mainly be found in humid pasture, besides swamps and streams; however, they hide under rocks and logs in winter (Loebmann, 2005). Their diet consists of insects, crustacea, spiders, centipedes, earthworms, mollusks and even little amphibians (Pazinato et al., 2011). The species has been the focus of the largest number of studies and data on the diversity of helminths; several reports of Digenea species were found in Argentina, Brazil, Paraguay, Peru, Uruguay and Venezuela (Lent et al., 1946; Fernandes, 1958; Yamaguti, 1958; Fróes & Lima, 1974; Mañé-Garzón & González, 1978; Stumpf, 1982; Rodrigues et al., 1990; Goldberg et al., 2009; Lunaschi & Drago, 2010; Toledo et al., 2018).

Rhinella dorbignyi (Duméril & Bibron, 1841) (Bufonidae) has been found in the area that stretches from eastern Rio Grande do Sul (RS) state to northern Argentina. It measures from 36 to 68 mm, finds shelter in excavated galleries in flooded areas with grass, where it preys on arthropods (Loebmann, 2005). However, little is known about helminths associated with *R. dorbignyi*, even though Yamaguti (1958) had reported *Gorgoderina cryptorchis* Travassos, 1924 (Digenea: Gorgoderidae) in Brazil and Paraguay.

This study aimed at reporting digenetic helminths on *L. latrans* and *R. dorbignyi* in southern Brazil. Thirty specimens of *L. latrans* (16 females; 14 males) and 30 of *R. dorbignyi* (18 males; 12 females) were analyzed. They were collected in channels and swamps in a peri-urban area in Pelotas (31°46'38.0"S 52°13'57.2"W), RS, Brazil, in August, September, October and December 2017, besides April and August 2018. Animal capture was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio 47397-5). Specimens were manually captured at dusk, the period of the day in which they are more active.

Animals were identified, kept in individual plastic containers and taken to the Laboratório de Parasitologia de Animais Silvestres (LAPASIL), which belongs to the Universidade Federal de Pelotas (UFPEL). Euthanasia was carried out in agreement with Resolution 1000/2012 issued by the Conselho Federal de Medicina Veterinária (CFMV, 2012) and approved by the Comissão de Ética em Experimentação Animal at the UFPEL (CEEA –1859/2015).

Before the necropsy, an external inspection, which included eyes, oral cavity and cloaca, was

carried out. Afterwards, trachea, esophagus, stomach, intestine, cloaca, gall bladder, lungs, liver, kidneys, bladder, spleen, heart and ovary/testicles were examined separately. Digenetic helminths were processed for identification in agreement with techniques recommended by Amato & Amato (2010). When they were collected alive, they were kept for 24 hours under cold action in a physiological solution to relax their muscles and later compressed between microscope slide and micro cover glasses and fixed in AFA (93% 70° ethanol, 5% commercial formalin and 2% glacial acetic) por 48h, kept in ethanol 70° GL and dyed with Langeron carmine, i. e., the so-called regressive process method was applied. After staining, helminths were mounted on permanent microscope slides in Canada balm for analysis under light microscopy. Helminths were identified in agreement with Travassos (1969); Yamaguti (1971); Mañé-Garzón & González (1978); Niewiadomska (2002); Gibson (2002); Jones (2005); Bray (2008); Campbell (2008) and Tkach (2008). Indices of prevalence (P%), mean intensity of infection (MII) and mean abundance (MA) were estimated as proposed by Bush et al. (1997). Vouchers were deposited in the helminth collection at the Laboratório de Parasitologia de Animais Silvestres (CHLAPASIL/UFPel), RS, Brazil (numbers: 792 – 799, 802 – 822).

Twenty-three (76.66%) out of all *L. latrans* specimens under investigation exhibited digenetic helminths which affected gastrointestinal, respiratory and urinary systems. The total number of digenetic helminths infecting this host species was 224, i. e., MII was 9.74 helminths/host. Regarding *R. dorbignyi*, only five individuals (16.66%) were infected with digenetic helminths. The total number of helminths was 31, i. e., MII was 6.2 helminths/host.

Digenetic helminths found in *L. latrans* were *Gorgoderina megacysta* Mañé-Garzón & González, 1978 (Gorgoderidae), *Haematoloechus freitasi* Mané Garzón & Solares, 1959 (Haematoloechidae), *Catadiscus* spp. (Diplodiscidae), *Halipegus* sp. (Derogenidae) and Plagiorchioideae fam. gen. spp. *Gorgoderina* sp. (Gorgoderidae), *Haematoloechus* sp. (Haematoloechidae), *Catadiscus* sp. (Diplodiscidae), Plagiorchioideae fam. gen. spp. and Diplostomidae gen. spp. were found in *R. dorbignyi*. Helminths and their respective infection sites and parasitological parameters are shown in Table 1.

This study characterizes the first reports of helminths on *L. latrans* in Rio Grande do Sul State, since these digenetic helminths had already been reported on this host in other Brazilian regions (Lent et al., 1946; Yamaguti, 1958; Travassos et al., 1969; Yamaguti, 1971; Rodrigues et al., 1990; Goldberg et al., 2009; Campião et al., 2014; Toledo et al., 2015, 2018) and in other countries in South America (Lent et al., 1946; Yamaguti, 1958; Mañé-Garzón & González, 1978; Lunaschi & Drago, 2010). Most studies have a taxonomic nature and there is little information about infection rates. Toledo et al. (2015), in São Paulo, registered only *Gorgoderina parvicava* Travassos, 1922 and *Haematoloechus fuelleborni* (Travassos & Darriba, 1930), whose prevalence of helminths was lower than that found in the present study.

Table 1. Digenetic helminths found in *Leptodactylus latrans* (Steffen, 1815) (Leptodactylidae) (n = 30) and *Rhinella dorbignyi* (Duméril & Bibron, 1841) (Bufonidae) (n = 30) in Rio Grande do Sul, Brazil, and their respective sites of infection (SI), prevalence (P%), mean intensity of infection (MII), mean abundance (MA) and infection intensity range (R).

Hosts	Helminths	SI	P(%)	MII	MA	R
<i>Leptodactylus latrans</i>	<i>Gorgoderina megacysta</i>	Urinary bladder	40	3.25	1.30	1-7
	<i>Haematoloechus freitasi</i>	Trachea and lung	23	1.80	0.43	1-6
	<i>Catadiscus</i> spp.	Intestine	30	4.22	1.26	1-9
	<i>Halipegus</i> sp.	Oral cavity	3	1.00	0.03	1
	Plagiorchioideae fam. gen. spp.	Esophagus, stomach, intestine	63	7.05	4.46	1-8
<i>Rhinella dorbignyi</i>	<i>Gorgoderina</i> sp.	Urinary bladder	3	2.00	0.06	2
	<i>Haematoloechus</i> sp.	Lung	3	1.00	0.03	1
	<i>Catadiscus</i> sp.	Intestine	3	5.00	0.16	5
	Plagiorchioideae fam. gen. spp.	Intestine	3	1.00	0.03	1
	Diplostomidae gen. spp. (metacercariae)	Kidney	7	11.00	0.73	1-21

Concerning *R. dorbignyi*, only one study that reports *Gorgoderina cryptorchis* in Brazil and Paraguay was found (Yamaguti, 1958). The study described by this paper introduces four new reports of digenetic helminths infecting this anuran: Plagiorchioideae fam. gen. sp., *Haematoloechus* sp., *Catadiscus* sp. and metacercariae of Diplostomidae.

Helminthological studies of anurans in RS have been restricted to the northern region, where Santos & Amato (2010) examined 90 specimens of *Rhinella fernandezae* (Gallardo, 1957), which were infected by *Catadiscus* sp., *Gorgoderina festoni* (Mata-López et al., 2005), *Gorgoderina* sp. and metacercariae of Diplostomidae, which had low infection indices, as observed by this study in the case of helminths belonging to the same taxonomic groups.

Studies of the biology of some species of *Gorgoderina*, *Haematoloechus* and *Halipegus* have shown that these helminths use Odonata larvae as intermediate hosts, which must be ingested by anurans to keep the life cycle (Krull, 1935; Olsen, 1974; Jourdane et al. 1975; Zelmer & Esch, 1998; Bolek et al., 2010). Therefore, studies of helminth fauna and diet complement each other and help understand the biology of organisms under investigation.

Jourdane et al. (1975) described the cycle of *Gorgoderina rochalimai* Pereira and Cuocolo, 1940, and stated that Odonata larvae are its second intermediate host. The authors observed that *Bufo marinus* (Linnaeus, 1758) acts as the definite host of the parasite and infects itself when it ingests Odonata larvae infected with metacercariae. Jourdane et al. (1975) concluded that the life cycle of *G. rochalimai* is similar to the one of other Gorgoderidae species. *Gorgoderina megacysta* was described on *L. latrans* in Uruguay, where Mañé-Garzón & González (1978) reported *Gorgoderina* spp. with prevalence of 53.84% when both species, *G. parvicava* Travassos, 1922 and *G. megacysta*, were

considered. Therefore, this study is the second report of *G. megacysta* infecting *L. latrans*.

Prevalence of *Haematoloechus freitasi* was 23% in *L. latrans*, whereas, in *R. dorbignyi*, its low rates and the quality of the material did not enable the digeneans of this group to be identified. About 50 species of *Haematoloechus* have been described worldwide. These parasites are found in frog lungs, as well as in other amphibians (León-Règagnon, 2017). Mollusks and Odonata nymphs act as first and second intermediate hosts, respectively (Olsen, 1974).

Studies of the life cycle of *Halipegus eccentricus* Thomas, 1937 show that it needs four hosts to complete its cycle: anurans (definite host), mollusks (first intermediate host), ostracods (second intermediate host) and dragon-fly larvae (paratenic host) (Bolek et al., 2010). *Halipegus occidualis* can be found on frogs (definite hosts); mollusks and microcrustacea (copepods and ostracods) act as first intermediate hosts while Odonata larvae act as either second intermediate hosts or paratenic ones. Adult amphibians are infected when they ingest dragon-fly larvae with metacercariae of the parasite (Krull, 1935; Zelmer & Esch, 1998).

Catadiscus is represented by species that act as parasites in the intestine of amphibians and reptiles; it has also been reported on anurans in South America (Campião et al., 2014). The study reported by this paper found three morphotypes of *Catadiscus*; two of them in *L. latrans* and one in *R. dorbignyi*. Differentiation among morphotypes is mainly related to the position of the vitellarium, testicles and size of the ventral sucker. Helminths that belong to this genus usually infect *L. latrans* in South America, since there have been reports of *C. corderoi* Mañé-Garzón, 1958; *C. freitaslenti*, Ruiz, 1943; *C. inopinatus*, Freitas, 1941; *C. marinholutzi* Freitas & Lent, 1939 and *C. uruguayensi* Freitas & Lent, 1939 (Lent et al., 1946; Yamaguti, 1958; Goldberg et al., 2009; Lunaschi & Drago, 2010; Campião et al., 2014). However, since no reports of *R. dorbignyi* were found, this is the pioneer one in which *R. dorbignyi* is the host of helminths that belong to *Catadiscus*. Little has been known about the life cycle of *Catadiscus* species, however representatives of this genus were found to use mollusks as intermediate hosts; young ones are released to the environment, where tadpoles are infected by ingesting metacercariae encysted in aquatic plants (Kehr & Hamann, 2003; Hamann, 2004).

Species that belong Plagiorchioideae use an array of vertebrates as definite hosts (Olsen, 1974). Some of them are *Choledocystus* Pereira & Cuocolo, 1941, *Plagiorchis* Lühe, 1899 and *Glypthelmins* Stafford, 1905. When they are adults, they infect organs of the digestive system of amphibians (Campião et al., 2014; Gomes, et al., 2017). Plagiorchioideae have a quite polemic classification, since researchers have not reached a consensus about morphological differences that differentiate their families, genera and species. To define Plagiorchioideae families, there are two classifications: the one based on morphological characteristics and aspects of their life cycles and the most recent one that based family

classification on knowledge of phylogeny that results from molecular studies (Bray, 2008). In this study, specimens found on anurans have characteristics that are similar to the ones of *Choledocystus*, *Plagiorchis* (Plagiorchiidae) and *Glypthelmins* (Glypthelminthidae). However, in order to identify these genera, helminths must be observed by electronic microscopy and molecular studies must be used (Razo-Mendivil & Ponce de León, 2008; Gomes et al., 2017). Therefore, these studies introduce and broadly discusses digenetic helminths that belong to this group. Visible morphological differences were observed in the collected specimens, a fact that suggests the occurrence of more than one species of Plagiorchioideae on *L. latrans*. Martin (1969) studied the life cycle of *Glypthelmins hyloreus* (Martin, 1969) and reported that adults live in the intestine of *Pseudacris regilla* (Baird & Girard, 1852) (Hylidae) which are infected when cercariae abandon mollusks (intermediate host), enter *H. regilla* tadpoles through their nostrils and reach the celom, where they develop as non-hardened mobile metacercariae that penetrate and ripen in the intestine of anurans.

There have been reports of Diplostomidae on anurans in South America (Campião et al., 2014). According to Hamann & Gonzáles (2009), anurans may get the infection when they are tadpoles as the result of direct penetration of cercariae. Therefore, finding metacercariae of Diplostomidae, even at low infection indices, implies that *R. dorbignyi* acts as the second intermediate host of helminths of this group in the region. Transmission to definite hosts should occur through the trophic chain.

All helminths found on *R. dorbignyi* have been reported for the first time in this host, while the digenetic helminth *G. megacysta* has been reported, for the first time, in *L. latrans*, in Brazil. Data shown by this study highlight the need to keep carrying out parasitological studies of anurans in Rio Grande do Sul and to expand knowledge about the diversity of helminths associated with this group of vertebrates so as to generate information that may help understand parasite-host relations.

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