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Helminth communities of *Leptodactylus latrans* (Anura: Leptodactylidae) from the Atlantic rainforest, south-eastern Brazil

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Abstract

The helminth fauna associated with *Leptodactylus latrans*, a large frog living in a disturbed environment of Atlantic rainforest in south-eastern Brazil, was evaluated. We found eight helminth taxa, including five nematode species, *Falcaustra mascula*, *Oswaldocruzia subauricularis*, *Physaloptera* sp., *Rhabdias* sp. and an unidentified cosmocercid, two trematodes, *Gorgoderina parvicava* and *Haematoloechus fuelleborni*, and one larval cestode. The overall prevalence of infection was 63.2% with a mean intensity of 11.3 ± 3.8. The cosmocercid nematode and *O. subauricularis* showed the highest prevalences, although the trematode *G. parvicava* was the most abundant and dominant parasite species. Host size positively influenced both the intensity of infection and parasite species richness. Our data suggest that the juvenile individuals of *L. latrans* are more susceptible to parasitic infection than the adults. The comparison of the similarity of this community component with that found in other studies in South America shows that, as well as the characteristics of the host, the sampling area also influences the parasitic fauna. Therefore, the results of this study agree that the helminth communities of frogs have relatively low species richness and dominance of generalist species.

Introduction

The number of studies focusing on knowledge of the helminth communities of amphibians in the Neotropical region has increased in recent years (Boquimpani-Freitas *et al.*, 2001; Luque *et al.*, 2005; Hamann *et al.*, 2006a, b; Schaefer *et al.*, 2006; Goldberg *et al.*, 2007; Holmes *et al.*, 2008; Campião *et al.*, 2009; Pinhão *et al.*, 2009; Santos & Amato, 2010; Klain *et al.*, 2011). Some of these studies have shown that different factors can influence the structure of the parasite community, including size and sex, diet composition, habitat preference and reproductive behaviour of the host (Bursey *et al.*, 2001; Hamann *et al.*, 2006a, b; Schaefer *et al.*, 2006; Santos & Amato, 2010).

This study evaluated the helminth component community of *Leptodactylus latrans* (Steffen, 1815), commonly known as Creole frog, and formerly referred to as *Leptodactylus ocellatus* (L., 1758). This species, revalidated by Lavilla *et al.* (2010) in a recent study, is a large-sized leptodactylid frog, widely distributed in South America, including Paraguay, Argentina, Uruguay and Brazil (Frost, 1985; Kwet & Di-Bernardo, 1999; Brusquetti & Lavilla, 2006; Achaval & Olmos, 2007). This species lives in litter or in the midst of grasses, but usually reproduces near temporary or permanent ponds, streams or marshes (Heyer *et al.*, 1990). *Leptodactylus latrans* is an opportunistic feeder as its diet is determined by the availability of

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prey in the environment (Duellman & Trueb, 1994). Little is known about the helminth parasites of *L. latrans*, for which some isolated studies in Atlantic rainforest have been published (Vicente & Santos, 1976; Rodrigues *et al.*, 1982, 1990; Stumpf, 1982). However, to our knowledge, no studies on the helminth community structure of this host species have been conducted.

The present aims are to describe helminth species richness and diversity at the component and infracommunity levels for comparison with other frogs within the genus *Leptodactylus*. In addition, the relationship between the parasitological descriptors and host body size is considered.

Materials and methods

Collection and examination of frogs

Frogs were sampled from a private property surrounded by Atlantic rainforest remnants located in the municipality of São Luiz do Paraitinga (23°13′S; 45°18′W), São Paulo State, south-eastern Brazil. A total of 38 specimens (29 juveniles and 9 adults) of *L. latrans* were sampled by active search at night, with five specimens recovered from February to November 2009 and 33 specimens in January and February 2010 under license from Instituto Brasileiro do Meio Ambiente – IBAMA (collection permit IBAMA/SISBIO 18240-1/2009) and deposited in the Museu de Zoologia da Universidade Estadual de Campinas 'Adão José Cardoso'. Frogs were transported live to the laboratory, and then euthanized with sodium thiopental solution. The snout–vent length (SVL) and body mass were recorded. Then they were necropsied and the gastrointestinal tract, lungs, kidneys, liver, urinary bladder, body cavity and musculature of the hind limbs were examined for the presence of helminths.

Helminths were fixed in alcohol–formaldehyde–acetic acid (AFA) and preserved in 70% ethyl alcohol. For species identification, cestodes and trematodes were stained with hydrochloric carmine and cleared with creosote. Nematodes were cleared in lactophenol and examined as temporary mounts. The systematic determination of the helminths was carried out following the approaches given by Yamaguti (1959, 1961), Vicente *et al.* (1991), Schmidt (1986), Anderson *et al.* (2009) and Gibbons (2010). The voucher species were deposited at the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB) at the Universidade Estadual Paulista (UNESP), São Paulo State, Brazil.

Data analysis

The overall prevalence, mean intensity of infection and mean abundance were calculated according to Bush *et al.* (1997). The hosts were classified as juveniles (SVL < 70.0 mm) and adults (SVL ≥ 70.0 mm) (Maneyro *et al.*, 2004). This classification was based on samples that were already reproductively mature. Fisher's exact test (P) and Z-test were used for comparing the infection between the juveniles and adults. The discrepancy index (D) was calculated following the method described by Poulin (1993). Berger–Parker's index of dominance (d) was used to determine the most abundant species (Magurran, 2004). The measures of community richness and diversity included the total number of helminth species (richness, S) and Shannon's index. Mean helminth species richness is the sum of helminth species per individual frog, including uninfected individuals, divided by the total sample size. Student's t-test was used to examine possible ontogenetic differences in richness of parasites (juveniles and adults) (Magurran, 1988). The Spearman coefficient of correlation (r_s) was used to evaluate the correlation between host body size and parasite richness, abundance and intensity of infection. To evaluate the similarity of helminth fauna between phylogenetic groups of *Leptodactylus* and their localities of origin, a cluster analysis was performed.

Statistical analyses were performed using BioEstat 5.0 (Ayres *et al.*, 2007), and MultiVariate Statistical Package (MVSP 3.1; Kovach, 1999). The Quantitative Parasitology 3.0 program (Rózsa *et al.*, 2000) was used to calculate discrepancy indices.

Results and discussion

Up to 24 of 38 frogs examined (63.2%) were infected with at least one parasite species and prevalence values were similar in juveniles (55.2%) and adults (88.9%) (Z-test $= 1.468$, $P = 0.142$). This was also confirmed by the Fisher's exact test (juveniles, $n = 16$ versus adults, $n = 8$; $P = 0.11$).

A total of 271 helminths were recovered, with a mean abundance of 7.1 ± 2.5 and a mean intensity of infection of 11.3 ± 3.8. The helminth component community in the population of *L. latrans* comprised eight taxa, six of which were adult parasites and two were in larval stages, a cestode and *Physaloptera* sp. (table 1). In view of the similarity between congeneric females and the absence of males, the identification of the cosmocercid nematode was not possible. The most abundant group was the Trematoda (48.0%), followed by the Nematoda (46.9%) and Cestoda (5.2%) (table 1). The most prevalent taxon was the unidentified Cosmocercidae, followed by *O. subauricularis* (table 1). *Gorgoderina parvicava* was the most abundant species ($d = 0.44$).

Although eight helminth taxa were identified, the mean richness was 1.2 ± 0.2 helminths/host, and greater richness ($S = 4$) was found in three hosts. The mean richness in adults ($S_{mean} = 2.2 \pm 0.5$) was higher than in juvenile hosts ($S_{mean} = 0.9 \pm 0.2$, $t = 3.1196$, df $= 36$, $P = 0.0036$). Adult frogs showed a lower diversity of parasites than the juvenile (H' juvenile $= 0.761$, H' adult $= 0.552$).

Host body size showed a very weak correlation with parasitic abundance (abundance versus length: $r_s = 0.4586$, $n = 37$, $P = 0.0043$; abundance versus weight: $r_s = 0.4764$, $n = 37$, $P = 0.0029$) and richness (richness versus length: $r_s = 0.4475$, $n = 37$, $P = 0.0054$; richness versus weight: $r_s = 0.4857$ $n = 37$, $P = 0.0023$), and the host body size was not significantly correlated with the intensity of infection (intensity of infection versus length: $r_s = 0.3161$, $n = 24$, $P = 0.1322$; intensity of infection versus weight: $r_s = 0.2895$, $n = 24$, $P = 0.1699$).

Using a cluster analysis, hosts sampled from the same region of origin showed a closer similarity between

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Table 1. The prevalence (%), mean abundance (MA), mean intensity (MI), discrepancy index (*D*) and site of infection (SI) of the helminth parasites found in *Leptodactylus latrans* collected in São Luis do Paraitinga, SP, Brazil; range in parasite numbers given in brackets and SE = standard error.

Helminth taxon	%	MA ± SE	MI ± SE	*D*	SI*
Cestoda					
Cestoda (larvae)	8.3	0.4 ± 0.3	7 ± 6 (1–13)	0.945	SS, Mes
Trematoda					
Gorgoderina parvicava	20.8	3.2 ± 1.8	24 ± 10.1 (4–49)	0.903	U, K, LI
Haematoloechus fuelleborni	12.5	0.3 ± 0.2	3.3 ± 2.3 (1–8)	0.933	L
Nematoda					
Cosmocercidae unidentified	66.7	1.8 ± 0.6	4.1 ± 1.3 (1–23)	0.743	SI, LI
Falcaustra mascula	20.8	0.4 ± 0.2	3.2 ± 1.3 (1–8)	0.897	LI
Oswaldocruzia subauricularis	33.3	0.5 ± 0.2	2.4 ± 0.6 (1–5)	0.841	SI, LI
Physaloptera sp. (larvae)	4.2	0.4 ± 0.4	16 (16)	0.949	S
Rhabdias sp.	20.8	0.3 ± 0.1	2 ± 0.4 (1–3)	0.877	L

*Site of infection: S, mucosa of stomach; SS, serosa of stomach; Mes, mesentery; U, urinary bladder; K, kidneys; LI, large intestine; SI, small intestine; L, lungs.

L. petersii and *L. leptodactyloides*, belonging to the group melanonotus, although *L. pustulatus*, also in this group, showed a closer similarity to *L. fuscus* from the fuscus group (fig. 1). The helminth fauna of *L. latrans* was more similar and independent of region, except for populations from Parana and Tocantins.

Leptodactylus latrans has a wide range of helminth fauna characteristic of both aquatic and terrestrial hosts (Vicente & Santos, 1976; Stumpf, 1982; Goldberg *et al.*, 2009). In this study, the component helminth community the *L. latrans* included preferentially nematode and trematode taxa. The high richness of nematodes is likely to be related to their monoxenous life cycle (Anderson, 2000) and low host specificity, since these taxa are found in amphibian species of several phylogenetic groups.

Values of helminth species richness ($S = 8$) and mean richness per host ($S_{mean} = 1.2 \pm 0.2$) for *L. latrans* were lower than the average values reported by Aho (1990) ($S = 9$; $S_{mean} = 3.54 \pm 0.24$). Therefore, the present results suggest that helminth communities of amphibians have relatively low species richness and a dominance of generalist species (Aho, 1990). Helminth infracommunities of adult *L. latrans* showed low species diversity compared with the juveniles. Although amphibian and

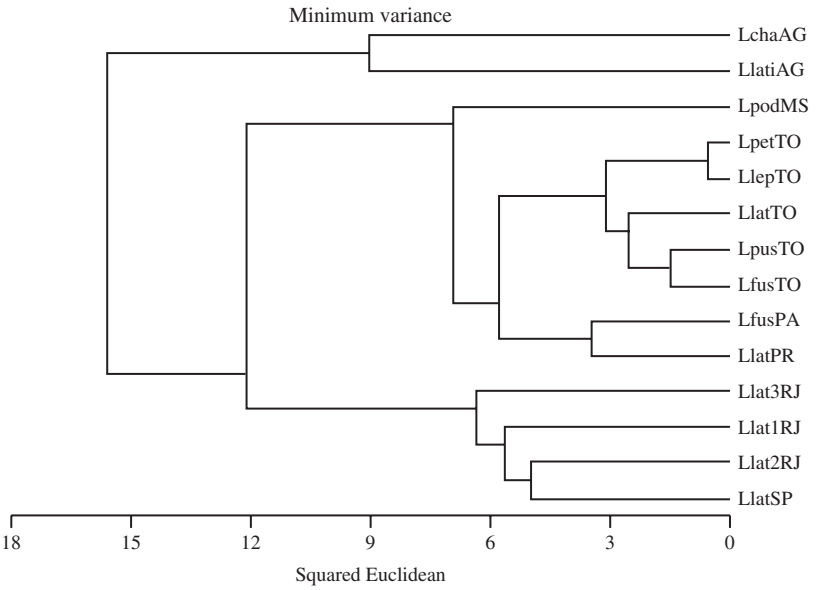

Fig. 1. Cluster analysis based on the composition of helminth parasites of *Leptodactylus* from nine locations in South America; LlatSP = *Leptodactylus latrans* (São Paulo; present paper); LlatPR = *L. latrans* (Paraná; Stumpf, 1982); Llat1RJ = *L. latrans* (Rio de Janeiro; Rodrigues *et al.*, 1990); Llat2RJ = *L. latrans* (Rio de Janeiro; Rodrigues *et al.*, 1982); Llat3RJ = *L. latrans* (Rio de Janeiro; Vicente & Santos, 1976); LfusPA = *L. fuscus* (Pará; Goldberg *et al.*, 2007); LfusTO = *L. fuscus*, LpusTO = *L. pustulatus*, LlatTO = *L. latrans*, LlepTO = *L. leptodactyloides*, LpetTO = *L. petersii* (Tocantins; Goldberg *et al.*, 2009); LpodMS = *L. podicipinus* (Mato Grosso do Sul; Campião *et al.*, 2009); LlatiAG = *L. latinasus* (Argentina; Hamann *et al.*, 2006a); LchaAG = *L. chaquensis* (Argentina; Schaefer *et al.*, 2006).

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reptile juveniles generally have less diversity than adults (Campião *et al.*, 2009), these data suggest that for this species, the juveniles are more susceptible to parasitic infection than the adults at the time and locality studied.

Frog body size has been recognized as an important correlate of parasite richness (Hamann & Kehr, 1998; Bolek & Coggins, 2003; Hamann *et al.*, 2006b). Our data indicated that richness and abundance of helminth infracommunities in *L. latrans* were influenced by host body size. Large frogs ingest substantial amounts of food (Duré, 1999) and, in addition, the larger surface area of the host may also increase opportunities for infection (Hamann *et al.*, 2006b). On the other hand, similar tests undertaken only on infected hosts showed no correlation, suggesting that all helminth taxa reported in the present study showed an aggregated distribution in the host population, i.e. when all parasites are found in a single host (Poulin, 1993).

A comparison of the helminth fauna of *L. latrans* with other species of *Leptodactylus* in Brazil showed that *L. latrans* presented high species richness in the component community level, similar to that found by Rodrigues *et al.* (1990) and Goldberg *et al.* (2009) for *L. latrans*, and Campião *et al.* (2009) for *L. podicipinus*. It was also observed that infection by parasites is more closely related to the location rather than host species of the group in which they are included. Some groups of species were phylogenetically similar but only from the same locality or sampling area. Hosts from the same location, even those of different species and genera, might share some helminth taxa once they are exposed to similar ecological conditions (Aho, 1990).

According to Janovy *et al.* (1992), the community structure of amphibian and reptile parasites is influenced mainly by the probability of individual hosts acquiring the various species of parasites, which in turn is influenced by the ecological requirements of each host species. This can lead to differing assemblages of parasites between hosts, and the present results seem to agree with this affirmation. The present study, apart from contributing to our knowledge of the helminth fauna of Atlantic rainforest amphibians, has shown that *L. latrans* has a high rate of helminth infection and that there is a significant difference in the pattern of infection among juveniles and adults in this frog species.

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Conflict of interest

None.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and has been approved by the institutional committee Comissão de Ética no Uso de Animais (CEUA) do Instituto de Biociências de Botucatu.

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