

Community Structure of Helminth Parasites of *Leptodactylus bufonius* (Anura: Leptodactylidae) from Northeastern Argentina

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Monika Inés Hamann, Arturo Ignacio Kehr, and Cynthia Elizabeth González (2012) Community structure of helminth parasites of *Leptodactylus bufonius* (Anura: Leptodactylidae) from northeastern Argentina. *Zoological Studies* 51(8): 1454-1463. The main goals of this study were to determine the richness and diversity of helminth parasites of *Leptodactylus bufonius* at the component and infracommunity levels and evaluate whether the composition of the parasite community is determined by biotic and abiotic factors. In total, 76 specimens were collected near the city of Corrientes, Corrientes Province, Argentina. The helminth component community in *L. bufonius* in this area was comprised of 16 species. The predominant groups of helminth parasites (larval and adult) were trematodes (50%) followed by nematodes (38%); other groups of parasites were represented by only 1 species (Cestoda: *Cylindrotaenia* sp. and Acanthocephala: *Centrorhynchus* sp.). Helminth species showed unequal abundances with a typical aggregated pattern of distribution. The prevalence of infection was 93% in specimens of *L. bufonius* examined. The main helminth species in the community was *Aplectana hylambatis* (importance value: $I = 96.41$), followed by *Catadiscus inopinatus* ($I = 2.32$). At the infracommunity level, the mean individual species richness was no more than 3 helminth species per infected host; the diversity and equitability of helminths were 0.18 ± 0.14 and 0.44 ± 0.32 , respectively. The host body size was the main factor in determining the infrapopulation structure of various helminth species. Species richness was significantly and positively correlated with the host body size. Data revealed significant positive correlations between helminth species (*Cat. inopinatus*/*Centrorhynchus* sp. and *A. hylambatis*/*Centrorhynchus* sp.). A significant negative correlation was observed between *A. hylambatis* and *Cos. podicipinus*. Only 2 associations were found among the 7 species considered. The parasite community of *L. bufonius* showed wide variations in its helminth fauna and included helminths of aquatic and terrestrial habitats. The correspondence between host habits and parasite biology is reflected in the parasites harbored by these amphibian hosts. <http://zoolstud.sinica.edu.tw/Journals/51.8/1454.pdf>

Key words: Ecology, Parasites, Amphibian, Neotropical realm.

Studies on helminth communities of American amphibians have increased in recent years. These ecological studies suggested that the community structure depends on interactions of extrinsic factors such as the characteristic habitat of the host (Aho 1990, Muzzall 1991, McAlpine 1997, Kehr et al. 2000, Bolek and Coggins 2003, Luque et al. 2005, Hamann et al. 2006a b, Yoder and Coggins 2007, Campião et al. 2009) and anthropogenic perturbations (Hamann et al. 2006b,

McKenzie 2007, King et al. 2008, Marcogliese et al. 2009). At the same time, intrinsic factors such as the host body size play important roles in the helminth parasite composition (Goater et al. 1987, Aho 1990, Tucker and Joy 1996, McAlpine 1997, Joy and Pennington 1998, Gilliland and Muzzall 1999, Bolek and Coggins 2001, Hamann et al. 2006a b 2010, Santos and Amato 2010).

In Argentina, despite the existence of a highly diverse amphibian fauna (Frost 2011), studies

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related to the patterns and processes underlying the structure of helminth parasite communities are just beginning (Hamann 2011). In this sense, it is necessary to examine more amphibian hosts to ascertain if amphibian helminth communities generally correspond to the pattern described by Aho (1990). That author recognized that the parasite communities of amphibians are considered highly variable, depauperate, and non-interactive, and found that some of the most important factors affecting the structure of helminth communities include, as suggested by Kennedy et al. (1986), the various host habitats (e.g., terrestrial and aquatic), and different aspects of the host biology (e.g., diet, age, sex ratio, and vagility) and of the life cycle of the parasite. Additionally, local availability of parasitic species and colonization abilities of parasites can affect parasite diversity (Esch et al. 1988), which in turn could affect the degree of interspecific interaction and distribution of parasite species among infracommunities (see Holmes and Price 1986). Thus, the present study attempted to characterize helminth parasites of the Vizcacheras' white-lipped frog *Leptodactylus bufonius* Boulenger, 1894 at the component and infracommunity levels.

González and Hamann (2006) reported a preliminary study of the helminth parasite composition of *L. bufonius*; nevertheless, that study did not analyze the ecological implications of different biotic and abiotic factors. Based on certain assumptions, the approach employed in the current study predicted that (1) helminth parasite species are determined by the particular habitat and habits of the host; (2) the depauperate community is related to the host being ectothermic; and (3) large amphibians commonly have more individuals and species of helminths than do smaller amphibians. These premises were tested by analyzing specific compositions of larval and adult helminths present in *L. bufonius*. This anuran species occurs in southern Bolivia to northern Argentina, Paraguay, and central Brazil (Frost 2011); the adult frog lives and breeds in burrows (i.e., a fossorial habitat) and leaves them only to forage. The breeding season occurs in spring, summer, and autumn; eggs are placed in a mass of foam in the nest. Hatching and caring for embryos occur in the nest, and when the nest is flooded by rainfall, the larvae escape to the surrounding aquatic habitat (see Philibosian et al. 1974). *Leptodactylus bufonius* is considered a generalist feeder, with a diet dominated by isopteran and coleopteran; its foraging strategy

is intermediate between that of an ambush predator and an actively foraging predator (Duré and Kehr 2004).

The main goals of this study were to: 1) determine the richness and diversity of parasites at the component and infracommunity levels; 2) identify and examine species affinities in the helminth communities; and 3) analyze relationships between helminths and frog characteristics.

MATERIALS AND METHODS

Study area

The study area was located ~15 km east of the city of Corrientes (27°30'S, 58°45'W). Although limited to 30 ha, the predominant vegetation in the study area is forest with an herbaceous strata composed of grasslands, numerous cacti, and terrestrial bromeliads. The habitat is also characterized by temporary, semipermanent, and permanent ponds. The aquatic vegetation in a permanent pond (30 m long, 15 m wide, and 0.8 m deep) sampled in this study consisted of floating and submerged hydrophytes, surrounded by several species of grasses. The mean annual temperature for the area is 23°C, and the mean annual precipitation is 1500 mm, without a well-defined dry season, although periods of rain shortage occur every 4-6 yr (Carnevali 1994).

Collection and examination of amphibians

Seventy-six specimens of *Leptodactylus bufonius* collected between Sept. 2002 and Nov. 2010 were examined for the presence of helminths. Frogs were hand-captured, mainly at night, using the sampling technique defined as the "visual encounter survey" (Crump and Scott 1994). Specimens were transported live to the laboratory, killed in a chloroform (CHCl₃) solution, and their snout-vent length (SVL) and body weight (BW) were recorded. At necropsy, hosts were sexed and the esophagus, stomach, gut, lungs, liver, urinary bladder, kidneys, body cavity, musculature, integument, and brain were examined for parasites. Helminths were observed *in vivo*, counted, killed in hot distilled water, and preserved in 70% ethyl alcohol. Digeneans, cestodes, and acanthocephalans were stained with hydrochloric carmine, cleared in creosote, and mounted in Canada balsam. Nematodes were cleared in glycerin or lactophenol, and examined

as temporary mounts. Systematic determination of the helminths was carried out following Yamaguti (1961 1963 1971 1975), Anderson et al. (1974), Gibson et al. (2002), and Jones et al. (2005). Specimens of parasite species were deposited in the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Corrientes Province, Argentina (with accession numbers CECOAL, 04011507, *Glypthelmins repandum* (Rudolphi, 1819); 10021702, *Catadiscus inopinatus* Freitas, 1841; 10022306, *Haematoloechus longiplexus* Stafford, 1902; 06022411, *Bursotrema tetracotyloides* Szidat, 1960; 04011507, *Travtrema* aff. *stenocotyle* Cohn, 1902; 06022413, *Opisthogonimus* sp.; 09102809, *Strigea* sp.; 06030607, *Cylindrotaenia* sp.; 09102808, *Cosmocerca podicipinus* Baker and Vaucher, 1984; 10022310, *Cos. parva* Travassos, 1925; 10113010, *Aplectana hylambatis* (Baylis, 1927); 02123272, *Aplectana* sp.; 08022601, *Rhabdias elegans* Gutierrez, 1945; 02113228, *Ortleppascaris* sp.; and 10021703, *Centrorhynchus* sp.).

Statistical analysis

The infection prevalence, intensity, and abundance were calculated for helminths following Bush et al. (1997). Measurements of community richness and diversity employed included the total number of helminth species (= richness), Shannon index (H') (Shannon and Weaver 1949), and evenness (J') as H'/H' maximum (Pielou 1966, Zar 2010). The mean helminth species richness is the sum of helminth species per individual frog, including uninfected individuals, divided by the total sample size. The diversity index was used with decimal logarithms (\log_{10}). The Berger-Parker index of dominance (d) was used to determine the most abundant species (Magurran 2004). All values are expressed as the mean \pm standard deviation (SD). The helminth community structure was examined according to the methodology outlined by Thul et al. (1985), where helminth species are classified into 4 groups (dominant, codominant, subordinate, and unsuccessful) by taking into account the prevalence, intensity, and maturation factor, which is related to the degree of host specificity. The importance value (I) was calculated for each helminth species as follows:

$$I_j = (M_j) \frac{A_j B_j}{\sum_{i=1}^{16} A_i B_i} 100$$

where A_j is number of individual parasites in species j , B_j is number of hosts infected with parasite j , and M_j is a maturity factor equal to 1.0 if at least 1 mature specimen of species j was found and equal to 0 otherwise.

Helminth communities were classified at the infracommunity level (i.e., all helminth infrapopulations within a single Vizcacheras' white-lipped frog) and component community level (i.e., all of the different helminth infrapopulations within all Vizcacheras' white-lipped frogs in our area) (Esch et al. 2002). Spearman's rank test (r_s) was used to assess the relationship between the host body size and infracommunity descriptors and calculate possible correlations between the host body size and parasite abundances. Species co-variation was analyzed with the Spearman test correlation. Species associations were analyzed using a Chi-squared test (χ^2), with the Yates correction for continuity. The variance/mean ratio was used to determine the spatial distribution of parasites. The software used was XLstat 7.5 (Addinsoft 2004). For correlations and associations, only species that had at least 10% occurrences in each of the amphibian populations (7 species) were considered.

RESULTS

Community structure analysis

The infection prevalence was 93% for the 76 frogs examined. The helminth component community of this frog population consisted of 16 species of helminths (Table 1). The predominant groups of helminth parasites (larval and adult) were trematodes (50%) followed by nematodes (38%); other groups of parasites were represented by only 1 species (Cestoda: *Cylindrotaenia* sp. and Acanthocephala: *Centrorhynchus* sp.). In contrast, comparison of the richness of adult helminths showed an inverted pattern: 56% nematodes and 33% trematodes. Helminth diversity was 0.51 and evenness was 0.42. The nematode *A. hylambatis* was the dominant species ($d = 0.71$). Most helminth parasites (0.75%) showed aggregated patterns of distribution. Parasites were found in the small intestine, large intestine, lungs, mesenteries, and body cavity with a prevalence of $> 10\%$ (Table

1). Of all adult specimens of helminths examined, the primary site of infection was the digestive tract, except for *Haematoloechus longiplexus* and *Rhabdias elegans* that were found in the lungs. Three species were assumed to have indirect life cycles (*Glypthelmins repandum*, *Cat. inopinatus*, and *H. longiplexus*), and 6 were assumed to have

direct life cycles (*Cylindrotaenia* sp., *Cosmocerca podicipinus*, *Cos. parva*, *A. hylambatis*, *Aplectana* sp. and *R. elegans*) (Table 2).

At the infracommunity level, the mean helminth richness was 2.49 ± 1.43 (maximum, 7) species per infected frog. Multiple infections were common, with 0, 1, 2, 3, 4, 5, and 7 species

Table 1. Helminths recorded in *L. bufonius*. The dispersion (S^2/\bar{x}), dominance (d), prevalence (%), mean abundance (MA) \pm 1 standard deviation (S.D.), mean intensity (MI) \pm 1 S.D., and site of infection are shown

Helminth	Stage	S^2/\bar{x}	d	Percent (%)	MA \pm 1 S.D.	MI \pm 1 S.D.	Site ^b
<i>Catadiscus inopinatus</i>	Adult	12.91	0.04	32.89	1.61 \pm 4.56	4.88 \pm 6.87	Li
<i>Glypthelmins repandum</i>	Adult	6.04	0.01	15.79	0.46 \pm 1.67	2.92 \pm 3.23	Si
<i>Haematoloechus longiplexus</i> ^a	Adult	1.00	0.00	1.32	0.01 \pm 0.11	1.00 \pm 0.00	Lu
<i>Bursotrema tetracotyloides</i> ^a	Larva	22.90	0.01	1.32	0.30 \pm 2.62	23.00 \pm 0.00	K
<i>Travtrema</i> aff. <i>stenocotyle</i> ^a	Larva	25.58	0.02	7.89	0.74 \pm 4.35	9.33 \pm 12.63	B M
<i>Opisthogonimus</i> sp. ^a	Larva	90.74	0.10	13.16	4.08 \pm 19.24	31.00 \pm 44.49	B M
<i>Strigea</i> sp. ^a	Larva	12.53	0.01	9.35	0.38 \pm 2.18	9.67 \pm 5.56	B
Strigeidae gen. sp. ^a	Larva	1.00	0.00	1.32	0.01 \pm 0.11	1.00 \pm 0.00	B
<i>Aplectana hylambatis</i>	Adult	42.45	0.71	73.68	29.80 \pm 35.57	40.45 \pm 35.87	Si Li
<i>Aplectana</i> sp.	Adult	6.43	0.01	3.95	0.21 \pm 1.16	5.33 \pm 2.63	Li
<i>Cosmocerca podicipinus</i>	Adult	6.89	0.02	19.74	0.72 \pm 2.23	3.67 \pm 3.79	Si Li
<i>Cosmocerca parva</i>	Adult	0.85	0.00	6.58	0.07 \pm 0.25	1.00 \pm 0.00	Li
<i>Rhabdias elegans</i>	Adult	3.11	0.01	15.79	0.38 \pm 1.09	2.42 \pm 1.61	Lu
<i>Ortleppascaris</i> sp.	Larva	6.94	0.00	3.95	0.16 \pm 1.05	4.00 \pm 3.56	L
<i>Cylindrotaenia</i> sp. ^a	Adult	1.00	0.00	1.32	0.01 \pm 0.11	1.00 \pm 0.00	Si
<i>Centrorhynchus</i> sp.	Larva	15.33	0.08	46.05	3.18 \pm 6.98	6.91 \pm 8.95	S M

^aNew host. ^bSite of infection: K, kidneys; B, body cavity; P, pharyngeal zone; Si, small intestine; Li, large intestine; S, serous of stomach; M, mesenteries; Lu, lungs; L, liver.

Table 2. Summary of helminth life cycles in *L. bufonius* from Corrientes Province, Argentina

Helminth	Host		Reference ^b
	Second intermediate	Definitive	
<i>Travtrema</i> aff. <i>stenocotyle</i>	Amphibian	Snake	Ostrowski de Núñez (1979a)
<i>Opisthogonimus</i> sp.	Amphibian	Snake	Grabda-Kazubaska (1963)
<i>Bursotrema tetracotyloides</i>	Amphibian	Mammal	Yamaguti (1975)
<i>Strigea</i> sp.	Amphibian	Bird	Yamaguti (1975)
Strigeidae gen. sp.	Amphibian	Bird, mammal	Yamaguti (1975)
<i>Glypthelmins repandum</i>	Amphibian	Amphibian	Smyth and Smyth (1980)
<i>Haematoloechus longiplexus</i>	Aquatic insect larva	Amphibian	Smyth and Smyth (1980)
<i>Catadiscus inopinatus</i>	Aquatic vegetation	Amphibian	Ostrowski de Núñez (1979b)
<i>Cosmocerca podicipinus</i>	-	Amphibian	Fotedar and Tikoo (1968)
<i>Cosmocerca parva</i>	-	Amphibian	Fotedar and Tikoo (1968)
<i>Rhabdias elegans</i>	-	Amphibian	Anderson (2000)
<i>Aplectana hylambatis</i>	-	Amphibian	Anderson (2000)
<i>Aplectana</i> sp.	-	Amphibian	Anderson (2000)
<i>Ortleppascaris</i> sp.	Amphibian ^a	Snake	Anderson (2000)
<i>Centrorhynchus</i> sp.	Amphibian ^a	Bird	Schmidt (1985)
<i>Cylindrotaenia</i> sp.	-	Amphibian	Prudhoe and Bray (1982)

^aParatenic host. ^bReferences: life cycles of congener species or species of the same family.

occurring in 5, 15, 21, 15, 15, 4, and 1 frogs, respectively. The observed distribution of helminth infracommunity species richness showed a good fit to the Poisson distribution ($\chi^2 = 5.29$, $d.f. = 6$, $p = 0.507$) (Fig. 1). The mean helminth diversity was 0.18 ± 0.14 , and evenness was 0.44 ± 0.32 .

Species infracommunity affinities

Two correlations between species were positive and significant: *Cat. inopinatus*/*Centrorhynchus* sp. and *A. hylambatis*/*Centrorhynchus* sp., and one was negative and significant: *Cos. podicipinus*/*A. hylambatis* (Table 3). Only 2 associations were found among the 7 species considered: *Cat. inopinatus*/*Centrorhynchus* sp. ($\chi^2 = 3.8$; $d.f. = 1$; $p < 0.05$) and *A. hylambatis*/*Centrorhynchus* sp. ($\chi^2 = 12.3$; $d.f. = 1$; $p < 0.05$).

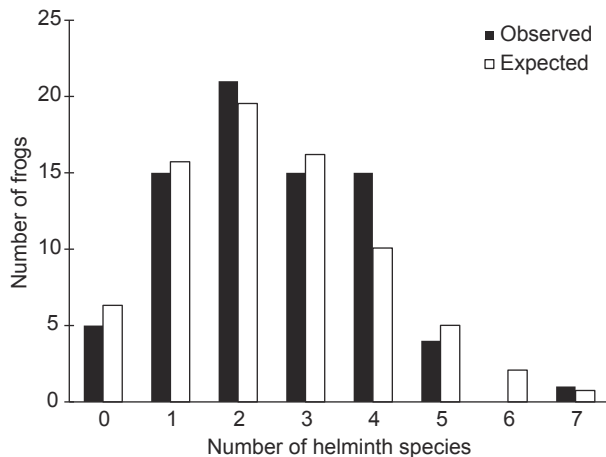


Fig. 1. Observed and expected frequency distributions of helminth infracommunity species richness in *L. bufonius*, according to a Poisson distribution pattern.

Infection in relation to host body size

The total lengths of frogs ranged 21.00-59.00 (43.65 ± 8.73) mm, and their weights ranged 0.93-38.10 (10.16 ± 5.38) g. The helminth infracommunity species richness was significantly correlated with the host body size (length: $r_s = 0.31$, $n = 71$, $p < 0.05$; and weight: $r_s = 0.32$, $n = 71$, $p < 0.05$); significant correlations were also found between the abundances of 5 helminth species and the host body size (Table 4).

Lengths of female frogs ranged 25.50-58.00 (42.41 ± 8.71) mm, and weights ranged 1.35-19.90 (9.25 ± 4.81) g. These variables were not significantly correlated with infracommunity descriptors; however, the host body size showed a significant correlation with the abundances of 3 helminth parasites (Table 4). Lengths of male frogs ranged 21.00-59.00 (44.26 ± 8.68) mm, and weights ranged 0.93-38.10 (10.61 ± 5.59) g. These variables were significantly correlated with the helminth infracommunity species richness, and also with abundances of 4 nematode species (Table 4).

Infection in relation to host specificity

Helminth species were classified according to community importance values (Table 5); 2 species were strongly characteristic of the community (dominant), and 5 species significantly contributed to the community, although to a lesser degree. Two species occurred infrequently and did not significantly contribute to the community, and 7 species that were able to enter the host but not to reach maturity contributed little to the community and are characteristic of another host.

Table 3. Co-variation based on Spearman coefficient correlations (r_s) among 7 common helminth species in *L. bufonius* from Corrientes Province, Argentina

Species	Table ID	(1)	(2)	(3)	(4)	(5)	(6)	(7)
<i>Rhabdias elegans</i>	(1)	1						
<i>Aplectana hylambatis</i>	(2)	0.042	1					
<i>Cosmocerca podicipinus</i>	(3)	-0.037	-0.274*	1				
<i>Catadiscus inopinatus</i>	(4)	-0.031	0.214	-0.120	1			
<i>Opisthogonimus</i> sp.	(5)	-0.095	0.222	-0.088	0.213	1		
<i>Glypthelmins repandum</i>	(6)	0.166	-0.231	-0.053	0.002	-0.181	1	
<i>Centrorhynchus</i> sp.	(7)	0.166	0.572*	-0.211	0.268*	0.098	-0.113	1

*Significant at $p < 0.05$.

DISCUSSION

In Argentina, previous reports found that the helminth communities of leptodactylids predominantly consisted of trematodes (Hamann et al. 2006a b). In the present study, *Leptodactylus bufonius* also presented large numbers of trematode species; nevertheless, when only adult helminths were compared, nematode species showed high richness, and this result was similar to previous studies in different species of the genus *Leptodactylus* from South America

(Table 6). Species causing helminth infections in *L. bufonius* showed unequal abundances and a typical aggregated pattern of distribution for many of the parasites (0.75%). The clumped (or overdispersed) distributions of most parasite species observed in *L. bufonius* were inferred by the heterogeneity between these amphibians in terms of their susceptibility to infection, i.e., possibly due to differences related to age, sex, and the immunological state (Anderson and Gordon 1982); and by its exposure to highly aggregated infective stages (Esch et al. 1988).

Table 4. Spearman correlation (r_s) between helminth parasites and the body size of *L. bufonius* from Corrientes Province, Argentina

Helminth parasite	All frogs		Frog ♂		Frog ♀	
	Weight	Length	Weight	Length	Weight	Length
Infracommunity descriptors						
Diversity	0.206	0.193	0.216	0.215	0.137	0.074
Evenness	-0.030	-0.054	-0.001	-0.023	-0.089	-0.135
Richness	0.310*	0.324*	0.313*	0.342*	0.277	0.228
Parasitic abundance						
<i>Rhabdias elegans</i>	0.355*	0.217	0.434*	0.257	0.206	0.130
<i>Aplectana hylambatis</i>	0.512*	0.549*	0.428*	0.537*	0.584*	0.497*
<i>Cosmocerca podicipinus</i>	-0.485*	-0.400*	-0.460*	-0.430*	-0.420*	-0.327
<i>Catadiscus inopinatus</i>	0.209	0.239*	0.120	0.219	0.328	0.252
<i>Opisthogonimus</i> sp.	0.099	0.123	0.048	0.068	0.205	0.256
<i>Glypthelmins repandum</i>	0.145	0.033	-0.064	-0.075	0.481*	0.276
<i>Centrorhynchus</i> sp.	0.283*	0.418*	0.258	0.406*	0.296	0.373

*Significant at $p < 0.05$.

Table 5. Importance values (I) and classification of helminth parasite species in *L. bufonius* from Corrientes Province, Argentina

Helminth species	I	Classification
<i>Aplectana hylambatis</i>	96.410	Dominant
<i>Catadiscus inopinatus</i>	2.318	"
<i>Cosmocerca podicipinus</i>	0.627	Codominant
<i>Glypthelmins repandum</i>	0.319	"
<i>Rhabdias elegans</i>	0.265	"
<i>Aplectana</i> sp.	0.036	"
<i>Cosmocerca parva</i>	0.019	"
<i>Haematoloechus longiplexus</i>	0.001	Subordinate
<i>Cylindrotaenia</i> sp.	0.001	"
<i>Bursotrema tetracotyloides</i>	0.000	Unsuccessful pioneer
<i>Travtrema</i> aff. <i>stenocotyle</i>	0.000	"
<i>Opisthogonimus</i> sp.	0.000	"
<i>Strigea</i> sp.	0.000	"
Strigeidae gen. sp.	0.000	"
<i>Ortleppascaris</i> sp.	0.000	"
<i>Centrorhynchus</i> sp.	0.000	"

The helminth community in the present study was predominantly composed of generalist species; however, *A. hylambatis* which was one of the most important species ($I = 96.41$), uses *L. bufonius* as a specific definitive host; infection by this species is comparatively rare in other sympatric amphibians (Hamann et al. 2006a b). The high prevalence of *A. hylambatis* found in *L. bufonius* agrees with a previous report by Bursey et al. (2001) for other American terrestrial leptodactylids. These data suggest that adult *L. bufonius* that inhabits forests and lands with sandy soil makes such infections possible through the ingestion of or skin penetration by infective larval stages (Anderson 2000).

Adults of *L. bufonius* breed in burrows and enter the water for short periods as tadpoles, acting as intermediate and definitive hosts for various trematodes species (Table 2). Infection by larval trematodes showed low values as a consequence of the short period that *L. bufonius* is in the water, i.e., implying a lower probability of penetration by the Cercariae. It can be assumed that this amphibian plays an insignificant role in

trematode parasite life cycles compared to other anurans in the same area, e.g., *Leptodactylus chaquensis* (Leptodactylidae) and *Scinax nasicus* (Hylidae) which show high infection rates by Metacercariae that complete their development in mammals (e.g., *Bursostrema tetracotyloides*) and reptiles (e.g., *Opisthogonimus* sp.) (Hamann et al. 2006b 2010). Adult trematodes were represented by species with complex life cycles, where infection occurs by skin penetration of the Cercariae in tadpoles and adult frogs (e.g., *G. repandum*) or by Metacercariae ingested with aquatic vegetation (e.g., *Cat. inopinatus*) and aquatic insects (e.g., *H. longiplexus*) by a definitive host (Smyth and Smyth 1980). The uncommon occurrence of the latter species shows that the intermediate host (e.g., dragonfly larvae and adults) is not a main prey item in the diet of this host.

As predicted by Aho (1990), the adult infracommunities of *L. bufonius* showed low species richness. An individual frog generally harbored only 3 helminth species, with scarce evidence of species affinities. On the 1 hand, we noted associations for those larval and adult

Table 6. Helminth species richness at the component community level for *Leptodactylus* species from South America

Host	n	Total richness	Adult helminth			Larval helminth				Locality	Reference
			N	T	C	n	t	c	a		
<i>L. bufonius</i>	76	16	5	3	1	1	5	-	1	Corrientes, Argentina	Present paper
<i>L. latinasus</i>	36	17	6	3	-	-	6	1	1	Corrientes, Argentina	Hamann et al. (2006a)
<i>L. chaquensis</i>	40	18	3	6	-	1	7	-	1	Corrientes, Argentina	Hamann et al. (2006b)
<i>L. chaquensis</i>	132	19	4	5	1	1	6	1	1	Corrientes, Argentina	Hamann et al. (2006b)
<i>L. ocellatus</i>	78	10	7	3	-	-	-	-	-	Rio de Janeiro, Brazil	Vicente and dos Santos (1976)
<i>L. podicipinus</i>	43	8	5	3	-	-	-	-	-	Mato Grosso, Brazil	Campião et al. (2009)
<i>L. mystaceus</i>	1	1	1	-	-	-	-	-	-	Pará, Brazil	Goldbeg et al. (2007)
<i>L. rhodomystax</i>	5	4	2	1	-	1	-	-	-	Pará, Brazil	Goldbeg et al. (2007)
<i>L. fuscus</i>	12	3	3	-	-	-	-	-	-	Pará, Brazil	Goldbeg et al. (2007)
<i>L. martinezi</i>	3	1	-	1	-	-	-	-	-	Pará, Brazil	Goldbeg et al. (2007)
<i>L. ocellatus</i>	31	7	3	1	-	2	-	-	1	Tocantins, Brazil	Goldbeg et al. (2009)
<i>L. fuscus</i>	15	3	3	-	-	-	-	-	-	Tocantins, Brazil	Goldbeg et al. (2009)
<i>L. petersii</i>	31	2	1	-	-	1	-	-	-	Tocantins, Brazil	Goldbeg et al. (2009)
<i>L. pustulatus</i>	19	4	3	-	-	1	-	-	-	Tocantins, Brazil	Goldbeg et al. (2009)
<i>L. mystaceus</i>	2	1	1	-	-	-	-	-	-	Tocantins, Brazil	Goldbeg et al. (2009)
<i>L. leptodactyloides</i>	9	3	1	-	-	1	-	-	1	Tocantins, Brazil	Goldbeg et al. (2009)
<i>L. lineatus</i>	2	1	1	-	-	-	-	-	-	Pastaza, Ecuador	McAllister et al. (2010)
<i>L. bolivianus</i>	14	6	4	-	-	2	-	-	-	Amazonia, Peru	Bursey et al. (2001)
<i>L. leptodactyloides</i>	14	4	3	-	-	1	-	-	-	Amazonia, Peru	Bursey et al. (2001)
<i>L. mistaceus</i>	12	5	4	-	-	1	-	-	-	Amazonia, Peru	Bursey et al. (2001)
<i>L. pentadactylus</i>	6	6	4	-	-	2	-	-	-	Amazonia, Peru	Bursey et al. (2001)
<i>L. petersii</i>	24	1	1	-	-	-	-	-	-	Amazonia, Peru	Bursey et al. (2001)
<i>L. rhodonotus</i>	22	3	2	-	-	1	-	-	-	Amazonia, Peru	Bursey et al. (2001)

Adult/ larval helminths: nematodes (N/n), trematodes (T/t), cestodes (C/c), and acanthocephalans (a).

species which were located in different organs of the frog (e.g., *Centrorhynchus* sp. + *A. hylambatis*); on the other hand, we found a negative correlation between 2 species at the same infection site, with the same guild (e.g., *Cos. podicipinus* vs. *A. hylambatis*). Similarly to other local amphibians (Hamann et al. 2006a b 2010), there was no discernible pattern or structure to *L. bufonius*'s infracommunities, although their proximity to the isolationist extreme of the continuum was demonstrated. This may also indicate that there are numerous vacant niches in the digestive tract of this frog species.

Regarding the host size, other researchers stated that frogs with larger bodies harbor more helminth species than do small bodied frogs (Muzzall 1991, McAlpine 1997, Hamann et al. 2006a b 2010, Yoder and Coggins 2007). In the present study, the size of amphibian individuals was a determining factor for the parasite species richness, which suggests that larger frogs ingest a more diverse diet and greater amounts of food, and also provide more surface area for parasite colonization, i.e., a greater probability of skin penetration by larval stages; a larger size is also related to a longer life and more time to become infected, which favor higher values of parasitism and species richness.

Additionally, the infrapopulations of some species increase in abundance in larger hosts; e.g., the adult *A. hylambatis* can occur through predation of a greater number of larval stages (L₃) accompanied by an increase in the intestinal area in hosts with a larger body size; nevertheless, the *Cos. podicipinus* abundance decreased in larger hosts. This suggests that skin penetration of larvae in adult frogs is presumably limited by its tegument (i.e., a thicker integument compared to juvenile frogs); and by the immune response of the host. Similarly, Hamann et al. (2006a) also found a negative correlation between this nematode species and the size of the frog *L. latinasus* (Leptodactylidae) from the same area of Corrientes Province. On the other hand, larger males were also more heavily parasitized (e.g., species richness), suggesting that reduced immune function and the differential behavior of males may also explain the increased levels of parasites (Folstad and Karter 1992, Poulin 1996).

In conclusion, *L. bufonius* showed a wide variation in its helminth fauna, including helminths characteristic of aquatic and terrestrial habitats. This community suggests that the feeding habits and particularly the diet of this frog

are determining factors of lower infections by helminths with complex life cycles. At the same time, the correspondence between low vagility and terrestrial habitat preferences of *L. bufonius* significantly contributed to the high occurrence of nematode infections, while its fauna showed low Metacercariae infections that result from acquisition of species by penetration of infective larvae into the host through natural orifices or the skin (Hamann and González 2009).

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REFERENCES

- Addinsoft. 2004. Xlstat for Excel. London: Addinsoft.
- Aho JM. 1990. Helminth communities of amphibians and reptiles: comparative approaches to understanding patterns and processes. In GW Esch, AO Bush, JM Aho, eds. Parasite communities: patterns and processes. London: Chapman and Hall, pp. 157-195.
- Anderson RC. 2000. Nematode parasites of vertebrates: their development and transmission. Wallingford, Oxford, UK: CABI International.
- Anderson R, D Gordon. 1982. Processes influencing the distribution of parasite numbers within host population with special emphasis on parasite-induced host-mortalities. *Parasitology* **85**: 373-398.
- Anderson RC, AG Chabaud, S Willmont. 1974. CHI. Keys to the nematodes parasites of vertebrates. Faruham Royal, Bucks, UK: Commonwealth Agricultural Bureaux.
- Bolek MG, JR Coggins. 2001. Seasonal occurrence and community structure of helminth parasites in green frogs, *Rana clamitans melanota*, from southeastern Wisconsin, U.S.A. *Comp. Parasitol.* **68**: 164-172.
- Bolek MG, JR Coggins. 2003. Helminth community structure of sympatric eastern American toad, *Bufo americanus americanus*, northern leopard frog, *Rana pipiens*, and blue-spotted salamander, *Ambystoma laterale*, from southeastern Wisconsin. *J. Parasitol.* **89**: 673-680.
- Burse CR, SR Goldberg, JR Parmelee. 2001. Gastrointestinal helminths of 51 species of anurans from Reserva Cuzco Amazónico, Peru. *Comp. Parasitol.* **68**: 21-35.
- Bush AO, KD Lafferty, JM Lotz, AW Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* **83**: 575-583.
- Campiã KM, RJ da Silva, VL Ferreira. 2009. Helminth parasites of *Leptodactylus podicipinus* (Anura: Leptodactylidae) from south-eastern Pantanal, State of Mato Grosso do Sul, Brazil. *J. Helminthol.* **83**: 345-349.
- Carnevali R. 1994. Fitogeografía de la provincia de Corrientes. Asunción, Paraguay: Litocolor.
- Crump ML, NJ Scott Jr. 1994. Visual encounters surveys. In WR Heyer, MA Donnelly, RW McDiarmid, LC Hayek, MS Foster, eds. Measuring and monitoring biological diversity - standard methods for amphibians. Washington DC: Smithsonian Institution Press, pp. 84-91.

- Duré MI, Al Kehr. 2004. Influence of microhabitat on the trophic ecology of two leptodactylids from northeastern Argentina. *Herpetologica* **60**: 295-603.
- Esch GW, A Barger, KJ Fellis. 2002. The transmission of digenetic trematodes: style, elegance, complexity. *Integr. Compar. Biol.* **42**: 304-312.
- Esch GW, CR Kennedy, AO Bush, JM Aho. 1988. Patterns in helminth communities in freshwater fish in Great Britain: alternative strategies for colonization. *Parasitology* **96**: 519-532.
- Folstad I, AJ Karter. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Mid. Nat.* **139**: 603-622.
- Fotedar DN, R Tikoo. 1968. Studies on the life cycle of *Cosmocerca kashmirensis* Fotedar, 1959, a common oxyurid nematode parasite of *Bufo viridis* in Kashmir. *Indian Sci. Congr. Assoc. Proc.* **55**: 460.
- Frost DR. 2011. Amphibian species of the world: an online reference. Vers. 5.5. New York: American Museum of Natural History electronic database. Available at <http://research.amnh.org/vz/herpetology/amphibia> Accessed 31 Aug. 2012.
- Gibson DI, A Jones, RA Bray. 2002. Keys to the Trematoda. London: CABI Publishing and Natural History Museum.
- Gilliland MG, PM Muzzall. 1999. Helminths infecting froglets of the northern leopard frog (*Rana pipiens*) from Foggy Bottom Marsh Michigan. *J. Helminthol. Soc. Wash.* **66**: 72-77.
- Goater TM, GW Esch, AO Bush. 1987. Helminth parasite of sympatric salamanders: ecological concepts at infra-community, component and compound community levels. *Am. Midl. Nat.* **118**: 289-300.
- Goldberg SR, CR Bursey, JP Caldwell, DB Shepard. 2009. Gastrointestinal helminths of six sympatric species of *Leptodactylus* from Tocantins State, Brazil. *Comp. Parasitol.* **76**: 258-266.
- Goldberg SR, CR Bursey, JP Caldwell, LJ Vitt, GC Costa. 2007. Gastrointestinal helminth from six species of frog and three species of lizard simpatics in Pará State, Brazil. *Comp. Parasitol.* **74**: 327-342.
- González CE, MI Hamann. 2006. Helminths parásitos de *Leptodactylus bufonius* Boulenger, 1894 (Anura: Leptodactylidae) de Corrientes, Argentina. *Rev. Esp. Herpetol.* **20**: 39-46.
- Grabda-Kazubaska B. 1963. The life cycle of *Metalephthophallus gracillimus* (Lühe, 1909) and some observations on the biology and morphology of developmental stages of *Letophalus nigrovenosus* (Bellingham, 1844). *Acta Parasitol. Pol.* **11**: 349-370.
- Hamann MI. 2011. Current state of knowledge of helminth parasites of amphibians in Argentina: a taxonomic and ecological approach. *In Anais do XXII Congresso Brasileiro de Parasitologia, São Paulo. Rev. Patol. Trop.* **40 (Supplement 2)**: 1-3.
- Hamann MI, CE González. 2009. Larval digenetic trematodes of tadpoles of six amphibian species from northeastern Argentina. *J. Parasitol.* **95**: 623-628.
- Hamann MI, CE González, Al Kehr. 2006a. Helminth community of *Leptodactylus latinasus* (Anura: Leptodactylidae) from Corrientes, Argentina. *Acta Parasitol.* **51**: 294-299.
- Hamann MI, Al Kehr, CE González. 2006b. Species affinity and infracommunity ordination of helminths of *Leptodactylus chaquensis* (Anura: Leptodactylidae) in two contrasting environments from northeastern Argentina. *J. Parasitol.* **92**: 1171-1179.
- Hamann MI, Al Kehr, CE González. 2010. Helminth community structure of *Scinax nasicus* (Anura: Hylidae) from a South American subtropical area. *Dis. Aquat. Organ.* **93**: 71-82.
- Holmes JC, PW Price. 1986. Communities of parasites. *In* DJ Anderson, J Kikkawa, eds. *Community ecology: pattern and process.* Oxford, UK: Blackwell Scientific Publications, pp. 187-213.
- Jones A, RA Bray, DI Gibson. 2005. Keys to the Trematoda. London: CABI Publishing and Natural History Museum.
- Joy JE, JL Pennington. 1998. Ecology of *Megalodiscus temperatus* (Digenea: Paramphistomatidae) in red-spotted newts, *Notophthalmus v. viridescens*, from West Virginia. *J. Helminthol. Soc. Wash.* **65**: 205-211.
- Kehr Al, BFJ Manly, MI Hamann. 2000. Coexistence of helminth species in *Lysapsus limellus* (Anura: Pseudidae) from an Argentinean subtropical area: influence of biotic and abiotic factors. *Oecologia* **125**: 549-558.
- Kennedy CR, AO Bush, JM Aho. 1986. Patterns in helminth communities: Why are birds and fish different? *Parasitology* **93**: 205-215.
- King KC, AD Gendron, JD McLaughlin, I Giroux, P Brousseau, D Cyr et al. 2008. Short-term seasonal changes in parasite community structure in northern leopard froglets (*Rana pipiens*) inhabiting agricultural wetlands. *J. Parasitol.* **94**: 13-22.
- Luque JZ, AA Martins, LER Tavares. 2005. Community structure of metazoan parasites of the yellow Cururu toad *Bufo ictericus* (Anura, Bufonidae) from Rio de Janeiro, Brasil. *Acta Parasitol.* **50**: 215-220.
- Magurran AE. 2004. Measuring biological diversity. Oxford, UK: Blackwell Publishing.
- Marcogliese DJ, KC King, HM Salo, M Fournier, P Brousseau, P Spear et al. 2009. Combined effects of agricultural activity and parasites on biomarkers in the bullfrog, *Rana catesbeiana*. *Aquat. Toxicol.* **9**: 126-134.
- McAllister CT, CR Bursey, PS Freed. 2010. Helminth parasites of selected amphibians and reptiles from the Republic of Ecuador. *Comp. Parasitol.* **77**: 52-66.
- McAlpine DF. 1997. Helminth communities in bullfrogs (*Rana catesbeiana*), green frogs (*Rana clamitans*), and leopard frogs (*Rana pipiens*) from New Brunswick, Canada. *Can. J. Zool.* **75**: 1883-1890.
- McKenzie VJ. 2007. Human land use and patterns of parasitism in tropical amphibian hosts. *Biol. Conserv.* **137**: 102-116.
- Muzzall PM. 1991. Helminth infracommunities of the frogs *Rana catesbeiana* and *Rana clamitans* from Turkey Marsh, Michigan. *J. Parasitol.* **77**: 366-371.
- Ostrowski de Núñez M. 1979a. Ungewöhnliche Xiphidiocercarie aus *Ampullaria canaliculata* nebst Bemerkungen ubre *Travtrema stenocotyle*. *Angew. Parasitol.* **20**: 46-52.
- Ostrowski de Núñez M. 1979b. Fauna de agua dulce de la república Argentina. IX. Sobre representantes de la fauna Paramphistomatidae (Trematoda). *Physis* **38**: 55-62.
- Philibosian R, R Ruibal, VH Shoemaker, LL McClanahan. 1974. Nesting behavior and early larval life of the frog *Leptodactylus bufonius*. *Herpetologica* **30**: 381-386.
- Pielou EC. 1966. The measurement of diversity in different types of biological collections. *J. Theor. Biol.* **13**: 131-144.
- Poulin R. 1996. Sexual inequalities in helminth infections: a

- cost of being a male? *Am. Nat.* **147**: 287-295.
- Prudhoe S, RA Bray. 1982. Platyhelminth parasites of the amphibian. London: British Museum (Natural History), Oxford Univ.
- Santos VGT, SB Amato. 2010. Helminth fauna of *Rhinella fernandezae* (Anura: Bufonidae) from the Rio Grande do Sul coastland, Brazil: analysis of the parasite community. *J. Parasitol.* **96**: 823-826.
- Schmidt GD. 1985. Development and life cycles. In DWT Crompton, BB Nickol, eds. *Biology of the Acanthocephala*. Cambridge, UK: Cambridge Univ., pp. 273-305.
- Shannon CE, W Weaver. 1949. *The mathematical theory of communication*. Urbana, IL: Univ. of Illinois, Press.
- Smyth JD, MM Smyth. 1980. Frogs as host-parasite systems. I. An introduction to parasitology through the parasites of *Rana temporaria*, *R. esculenta* and *R. pipiens*. London: Macmillan.
- Tucker RB, JE Joy. 1996. Relationships between *Glypthelmins pennsylvaniensis* (Trematoda: Digenea) infections and host size. *J. Helminthol. Soc. Wash.* **63**: 42-46.
- Thul JE, DJ Forrester, CL Abercrombie. 1985. Ecology of parasitic helminths of wood ducks, *Aix sponsa*, in the Atlantic Flyway. *Proc. Helminthol. Soc. Wash.* **52**: 297-310.
- Vicente JJ, E dos Santos. 1976. Fauna helmintológica de *Leptodactylus ocellatus* (L., 1758) de Volta Redonda, Estado do Rio de Janeiro. *Atas Soc. Biol. Rio de Janeiro* **18**: 27-42.
- Yamaguti S. 1961. *Systema helminthum. The nematodes of vertebrates*. New York: Interscience.
- Yamaguti S. 1963. *Systema helminthum. The Acanthocephala of vertebrates*. New York: Interscience.
- Yamaguti S. 1971. *Synopsis of the digenetic trematodes of vertebrates*. Tokyo: Keigaku Publishing.
- Yamaguti S. 1975. *A synoptical review of life histories of digenetic trematodes of vertebrates*. Tokyo: Keigaku Publishing.
- Yoder HR, JR Coggins. 2007. Helminth communities in five species of sympatric amphibians from three adjacent ephemeral ponds in southeastern Wisconsin. *J. Parasitol.* **93**: 755-760.
- Zar JH. 2010. *Biostatistical analysis*. Englewood Cliffs, NJ: Prentice-Hall.