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Helminth parasites in the toad *Rhinella major* (Bufonidae) from Chaco region, Argentina

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Abstract. The present study describes the diversity of helminth parasites of *Rhinella major* (Anura: Bufonidae) in relation to their body size in 4 subhumid vs. semiarid sampling sites from the Argentine Chaco region. Helminths were found in 81% of the specimens examined (n = 85). Fifteen species (13 in subhumid and 7 in semiarid areas) of helminth parasites were found, and most of them were nematodes. Parasites were found in all the examined organs, with highest prevalence and intensity in the digestive tract. Parasite transmission to the toad host occurs by skin penetration or oral ingestion. Maximum helminth richness ranged between 2 and 4 species per infected toad. The most abundant species was *Aplectana hylambatis*. Body size of the host was the main factor in determining parasite richness. The helminth parasite fauna was rather different in hosts from subhumid vs. semiarid sites, but the dominant (*Aplectana hylambatis*) and codominant species (*Cylindrotaenia* sp. and *Rhabdias elegans*) were the same. Mean species richness and mean species diversity of helminths were significantly different between the zones. These results suggest that the amount of rainfall and associated humidity affects the distribution and development of the parasite fauna of this toad.

Keywords. Parasitic assembly, Helminth community, Habitat use, *Rhinella major*, Chaco region, Argentina.

INTRODUCTION

The small toad *Rhinella major* (Müller and Hellmich, 1936) is a member of the toad family Bufonidae, which can be found in South America. Its distribution in Argentina includes the provinces of Chaco, Formosa, Salta, Jujuy, Santiago del Estero, Santa Fe, and Tucumán (Frost, 2014), where it is able to withstand extremely dry conditions that predominate most of the year. *R. major* is a nocturnal species, living and feeding almost exclusively in terrestrial habitats. Its diet is neither generalised nor specialised, with predominance of formicids (i.e. insectivore) (Duré et al., 2009). Only the systematics of its helminth parasites have been reported (see Campião et al., 2014), whereas the respective host-parasite interactions are still poorly understood.

The diversity of helminth parasite species in amphibian hosts is often correlated with the habitat inhabited by the latter, e.g., terrestrial amphibian tend to host more nematode parasites with direct life cycles (Luque et al., 2005; Yoder and Coggins, 2007; Ibrahim, 2008; Santos and Amato, 2010, 2013; Hamann et al., 2006a, 2012, 2013a), while aquatic/semiaquatic amphibian are especially infected by trematode parasites, many of which have complex life cycles that require several hosts and may take place entirely within the aquatic habitat (Wetzel and Esch, 1997; McAlpine and Burt, 1998; Zelmer et al., 1999; Muzzall et al., 2001; Hamann et al., 2006b, 2013b; Campião et al., 2012). Such ecological factors may affect the likelihood of colonization by larval parasites in the intermediate hosts and among the vertebrate definitive hosts (Thieltges et al., 2008).

The present study of the toad *R. major* was performed in two areas from the Argentine Chaco region. One zone is subhumid with longer humid periods, and characterized by dense forests interspersed with open

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floodplain grassland, while the second zone is semiarid, with a shorter and relatively unstable humid period, and dominated by sparser, lower and less diverse forests combined with spiny shrubs.

In this context, our aim is to describe the helminth fauna infecting *R. major* in habitats contrasting for their climatic features (semiarid vs. subhumid habitats), and compare the level of parasitic infection of differently sized toads.

MATERIAL AND METHODS

Study sites

Toad populations were captured in four widely separated locations of the Chaco region of Argentina between October, 2011 and November, 2012. These locations span two different zones: subhumid zone (two localities: Concepción del Bermejo: 26°36'S, 60°57'W and Las Lomitas: 24°42'S, 60°35'W) and semiarid zone (two localities: Taco Pozo: 25°36'S, 63°15'W and Ingeniero Juárez: 23°54'S, 61°51'W) (Fig. 1).

Fig. 1. Map showing the location of the study area and sampling sites in the subhumid (Las Lomitas and Concepción del Bermejo) and semiarid (Ingeniero Juárez and Taco Pozo) zones.

Compared to the semiarid sampling sites, the subhumid zone is characterized by a more dense vegetation with hardwood trees (e.g., *Schinopsis balansae* and *Tabebuia avellanedae*), secondary woody vegetation (e.g., *Zizyphus mistol*, *Acacia caven*, *Bumelia obtusifolia* and *Acanthosyris falcata*), and herbaceous species comprising patches of grasses (e.g., *Elionurus muticus*) and terrestrial bromeliads (e.g., *Aechmea distichantha* and *Bromelia serra*). The climate of this zone is characterized by high temperatures and dry north winds in the months of August to November, whereas rainfall is often higher from December to March (Table 1). During the rainy season rivers overflow favours the development of numerous marshes, swamps and lagoons that occupy former riverbeds and meanders.

In the semiarid zone, the forest is adapted to dry conditions (xerophytic deciduous forest), with predominance of small-leaved deciduous and thorny species adapted to fluctuations in water availability, as well as to seasonal thermal variations. Woody vegetation (e.g., *Bulnesia sarmientoi*, *Prosopis ruscifoli*, *Stetsonia coryne* and *Trithinax biflabellata*) is sparse, and the herbaceous species are predominantly grasses (*Trichloris* sp., *Gouinia* sp. and *Setaria argentina*). The landscape is flat, gently sloping toward the east. The climate is characterized by low rainfall (mean anual about <700 mm), and high temperatures, at times exceeding 47 °C since this area comprises part of the South American Heat Pole (Naumann, 2006). Table 1 presents the values of precipitation and temperature at the study localities.

Collection and examination of toads and parasites

Samples of *R. major* were collected in subhumid (n = 61) and semiarid (n = 24) zones (Table 1). Toads were transported alive to the laboratory and then euthanized in chloroform ($CHCl_3$) solution. Their snout-vent length (SVL) was recorded. At necropsy, hosts were sexed and the esophagus, stomach, gut, lungs, liver, urinary bladder, kidneys, body cavity, musculature, skin, and brain examined for parasites. Helminths were observed in vivo, counted, and killed in hot distilled water and preserved in 70% ethanol. For identification, the digeneans, and cestodes were stained with hydrochloric carmine, cleared in creosote, and mounted in Canada balsam. Nematodes were cleared in glycerine or lactophenol, and examined as temporary mounts. Helminths were deposited in the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Corrientes city, Corrientes, Argentina [accession numbers CECOAL, 11101902, *Haematoloechus longiplexus*; 12110819, *Travtrema* sp.; 11100008, Strigeidae gen. sp. 1; 12110803, Strigeidae gen. sp. 6; 12110820, *Cylindrotaenia* sp.; 12110817, *Mesocestoides* sp.; 12110816, *Cosmocerca podicipinus*; 12110823, *Aplectana hylambatis*; 11101906, *Oswaldocruzia mazzai*; 12110825, *Schulzia travassosi*; 11101902, *Rhabdias elegans*; 12110813, *R. pseudosphaerocephala*; 12110814, *Porrocaecum* sp.; 11100003, *Physaloptera* sp.; 12110827, Ascarididae gen. sp.]. A sample of toad was deposited in the Herpetology Collection of CECOAL-CONICET, with accession number LHC, 5081.

Statistical analysis

Helminth fauna description. Prevalence, intensity, and abundance were calculated according to Bush et al. (1997). Helminth communities have been classified at the infracommunity level (all helminth populations within a single *R. major*), component community level (all helminth infracommunities within a population of the *R. major*) and parasite fauna (all the parasite species that exploit a given host across its geographic range)

Table 1. Variables recorded in *Rhinella major* and climatic data of two zones from the Chaco region, Argentina.

	Subhumid zone		Semiarid zone	
	Concepción del Bermejo Summer 2012	Las Lomitas Spring 2012	Taco Pozo Spring 2011	Ingeniero Juárez Spring 2011
Collected toads				
Number of toads	31	30	10	14
Males/Females	15/16	26/4	8/2	7/7
Mean length (mm)	44.68	57.23	51.60	39.48
Min-max length (mm)	37-61	48-68	42-60	32-47
Precipitations (mm) [1]				
Mean anual	937	919	620	689
Total annual	649	737	456	*805
Total season_	169	76	223	45
Temperature (°C) [2]				
Mean annual	20 to 22	22 to 24	20 to 22	22 to 24

[1]Data from Rohrmann H. (pers. comm.) and data from Gómez et al. (2013). [2]Data from Naumann (2006).*Exceptional situation.

(Bush et al., 1997; Poulin, 1997). The measures of community richness and diversity employed included: the total number of helminth species (richness), mean richness (the sum of helminth species, per individual toads, divided by the total sample size), Shannon index (H´) (Shannon and Weaver, 1949), evenness (J') as H´/H´ max (Pielou, 1966; Zar, 2010). The Berger-Parker index of dominance (d) was used to determine the most abundant species (Magurran, 2004).

Helminth fauna in semiarid vs. subhumid habitats. Sorensen similarity qualitative index was used for comparing the degree of similarity in helminth communities between habitats. The statistic Z was used to compare 2 proportions (prevalence) of helminth infection. A t-test was used to test for significant difference in the $\log_{10}$ helminth diversity (Hutcheson, 1970) between zones. The importance of each parasite in the community was examined according to the methodology outlined by Thul et al. (1985), in which helminth species are classified into four groups (dominant, codominant, subordinate and unsuccessful pioneer species) by taking into account their prevalence, intensity, and maturity factor (equal to 1.0 if at least 1 mature specimen of species j is found and equal to 0 otherwise), which is related to the degree of host specificity. Since the samples sizes were different in semiarid vs. subhumid habitats, we used rarefaction methods (implemented in the software EcoSim 7.7; Gotelli and Entsminger, 2004) for comparing the mean diversity and the mean richness of the helminth species. Rarefaction uses probability theory to derive expressions for the expectation and variance of species diversity and richness for a sample of a given size. This method "rarefies" its samples down to a common abundance level and then compares species diversity and richness. The process was repeated 1,000 times to generate a mean and a variance of species diversity and richness. Wilcoxon's V-test was used to compare differences in species richness between semiarid and subhumid habitats.

Helminth fauna and *R. major* size. Spearman's Rank test (r_s) and Pearson's coefficient correlation (r) were used to indicate the relationship between host body size and helminth species richness.

All statistical analyses were performed using Xlstat 7.5 (Addinsof, 2004) and MVSP (Kovach, 1999).

RESULTS

General patterns

Fifteen helminth species were found in the 85 *R. major* individuals collected in the Chaco region. The predominant groups of parasites were nematodes (9 species), followed by trematodes (4 species) and cestodes (2 species). Parasites were found in all major organs, with highest prevalence and intensity in the small intestine (anterior and middle portion of intestine), large intestine and stomach (Table 2). However, no parasites were found in the muscles. Eight species were found at the adult stage, and for these *R. major* is the definitive host; 7 species were at a larval stage and infect *R. major* as intermediate or paratenic hosts. Parasite transmission to the toad host occurs by skin penetration (60% of the species) or oral ingestion (40% of the species) (Table 2).

Comparison of the parasite fauna in semiarid vs. subhumid habitats

The relative importance of species within the parasite community was as follows: *A. hylambatis* was strongly characteristic of the community in both habitat types (Table 3). Seven codominant species contributed to a

Table 2. Helminths recorded in *Rhinella major* from the Chaco region, Argentina. Site of infection: SI-small intestine, LI-large intestine, PZ-pharyngeal zone, GB-gall bladder, M-mesentery, GM-gastric mucosa, LU-lung; stage (S): adult (A) and larval (L); mode of transmission of parasites (T): oral ingestion (I), skin penetration (P); prevalence: %; mean intensity: MI ± SD; total number: #; prevalence in Chaco region: CR.

Helminths and site of infection	S	T	Subhumid zone						Semiarid zone						CR
			Las Lomitas			Concepción Bermejo			Ingeniero Juárez			Taco Pozo			
			%	MI ± SD	#	%	MI ± SD	#	%	MI ± SD	#	%	MI ± SD	#	%
Trematoda															
Haematoloechus longiplexus LU	A	I										10	1.0	1	1
Travtrema sp. M	L	P	3	1.0 ± 0.0	1										1
Strigeidae gen. sp.1 M	L	P				3	3.0 ± 0.0	3							1
Strigeidae gen. sp. 6 M	L	P	3	9.0 ± 0.0	9				7	1.0 ± 0.0	1				2
Cestoda															
Cylindrotaenia sp. SI	A	I	17	5.2 ± 1.5	26							70	2.1 ± 1.4	15	14
Mesocestoides sp. M	L	I	10	1.3 ± 0.5	4										4
Nematoda															
Cosmocerca podicipinus LI/ SI	A	P	23	6.0 ± 5.5	42										8
Aplectana hylambatis LI/SI	A	I/P	97	142.7 ± 77.0	4139	55	54.9 ± 55.5	934	86	17.0 ± 21.2	204	90	45.6 ± 38.2	410	79
Oswaldocruzia mazzai SI	A	P										10	2.0 ± 0.0	2	1
Schulzia travassosi SI/ GB	A	P	10	6.0 ± 4.1	18	3	23.0 ± 0.0	23							5
Rhabdias elegans LU	A	P				7	3.5 ± 0.5	7				30	3.0 ± 2.2	9	6
R. pseudosphaerocephala LU	A	P	17	1.6 ± 0.5	8										6
Porrocaecum sp. PZ	L	I	3	1.0 ± 0.0	1										1
Physaloptera sp. GM	L	I	30	4.3 ± 3.3	39	3	1.0 ± 0.0	1	21	1.30 ± 0.5	4				15
Ascarididae gen. sp. SI	L	I	3	1.0 ± 0.0	1										1

lesser degree in the helminth community, and two of them (*Cylindrotaenia* sp. and *R. elegans*) were common to the semiarid and subhumid zones. Two species were uncommon or rare in the community (e.g., *H. longiplexus*). Seven that were able to enter the host but not to reach maturity contributed little to the community and are characteristic of different hosts (e.g., *Physaloptera* sp.).

In both zones, helminths exhibited a low diversity with a less equitable distribution (Tabla 4). The most abundant species (d) was *A. hylambatis*. There were 5256 individuals of diverse helminth species in the subhumid zone, while 646 individuals were found in the semiarid zone. The value of Sorensen qualitative similarity index was low (40%). Infection prevalence showed no significant differences between zones ($Z = -0.94$; df = 1; $P > 0.05$).

Using a rarefaction method, the number of individuals was combined and the values of mean richness and mean diversity for each of the zones were obtained (Table 5). Mean species richness of helminth parasites differed between the two habitats, i.e., it was greater in the subhumid zone (Wilcoxon V-test = 85; $P = 0.003$; $n_1 = 13$; $n_2 = 13$). Mean species diversity of helminth parasites also differed between the two habitats, being higher in the semiarid zone (Wilcoxon V-test = 0; $P = 0.0002$; $n_1 = 13$; $n_2 = 13$).

In both habitats, the helminth parasites were similar in a set of 4 species (*A. hylambatis*, *Physaloptera* sp., *R. elegans* and Strigeidae gen. sp. 6). Diversity of these helminth parasites showed significant difference between zones (t-test = -1.983; V = 732.19; $P < 0.05$); the subhumid zone (H' = 0.030) showed greater inequality of species abundance compared to the semiarid zone (H' = 0.054).

Helminth species richness related to host body size

Of the 85 toads examined from the two zones, infection prevalence was 81%, and significant positive correlation was found between toad body length and helminth species richness ($r = 0.43$; $n = 69$; $P < 0.0001$).

Toads from the subhumid zone showed high infection prevalence (79%), and parasite species richness was significantly correlated with body length of the host ($r_s = 0.46$; $n = 48$; $P = 0.001$). Toads from the semiarid zone presented even higher infection prevalence (88%), and also significant correlation between parasite species richness and body length of the host ($r_s = 0.59$; $n = 21$; $P < 0.01$).

Table 3. Classification of helminth species in *Rhinella major* from the Chaco Region, Argentina. Dominant species (I ≥ 1.0), codominant species (0.01 ≤ I < 1.0), subordinate species (0 < I < 0.01), unsuccessful pioneer species (I = 0). Combined data (C).

Classification of helminth*	Subhumid zone			Semiarid zone		
	Las Lomitas	Conc. del Bermejo	C	Ingeniero Juárez	Taco Pozo	C
Dominant species						
Aplectana hylambatis	99.262	99.742	99.544	99.472	96.471	98.865
Cylindrotaenia sp.	-	-	-	-	2.745	-
Codominant species						
Cylindrotaenia sp.	0.108	-	0.055	-	-	0.805
H.longiplexus	-	-	-	-	0.026	-
Cosmocerca podicipinus	0.243	-	0.125	-	-	-
Schulzia travassosi	0.045	0.144	0.070	-	-	-
Oswaldocruzia mazzai	-	-	-	-	0.052	0.015
Rhabdias elegans	-	0.088	-	-	0.706	0.207
R. pseudosphaerocephala	0.033	-	0.017	-	-	-
Subordinate species						
Rhabdias elegans	-	-	0.006	-	-	-
H. longiplexus	-	-	-	-	-	0.008
Unssucesfull pioneer species						
Porrocaecum sp.	0.000	-	0.000	-	-	-
Physaloptera sp.	0.000	0.000	0.000	0.000	-	0.000
Ascarididae gen. sp.	0.000	-	0.000	-	-	-
Travtrema sp.	0.000	-	0.000	-	-	-
Strigeidae gen. sp. 1	-	0.000	0.000	-	-	-
Strigeidae gen. sp. 6	0.000	-	0.000	0.000	-	0.000
Mesocestoides sp.	0.000	-	0.000	-	-	-

*According to the methodology from Thul et al. (1985)

Table 4. Summary of ecological indices of helminth communities in *Rhinella major* from Chaco region, Argentina. Combined data (C).

Variables	Subhumid zone			Semiarid zone		
	Las Lomitas	Conc. del Bermejo	C	Ingeniero Juárez	Taco Pozo	C
Component community						
Richness	11	5	13	3	5	7
Diversity (H')	0.09	0.08	0.10	0.05	0.13	0.12
Evenness (J)	0.09	0.11	0.09	0.11	0.18	0.14
Dominance (d)	0.96	0.96	0.95	0.99	0.94	0.97
Identification of (d)	*Aplectana*	*hylambatis*		*Aplectana*	*hylambatis*	
Infracommunity						
Range richness	1 - 4	0 - 2	0 - 4	0 - 2	0 - 4	0 - 4
Mean richness	2.17 ± 0.93	1.22 ± 0.42	1.81 ± 0.91	1.33 ± 0.47	2.33 ± 0.94	1.76 ± 0.89
Mean diversity (HB)	0.06 ± 0.08	0.02 ± 0.07	0.05 ± 0.08	0.06 ± 0.10	0.13 ± 0.11	0.08 ± 0.10
Mean evenness (E)	0.17 ± 0.24	0.08 ± 0.22	0.13 ± 0.24	0.20 ± 0.33	0.39 ± 0.37	0.28 ± 0.37

DISCUSSION

In general, the results of the present study indicate that the helminth fauna (both adults and larvae) of *R. major* is relatively rich, with a composition similar to those previously described for other bufonid toads (*Rhinella fernandezae* and *Melanophryniscus klappenbachi*) from the Chaco region of Argentina (Hamann et

Table 5. Summary of main results of the rarefaction method. The number of individuals (n) was unified to obtain values of mean diversity (MD) and mean richness (MR) 1 ± SD for subhumid and semiarid zones.

n	Subhumid zone		Semiarid zone	
	MD ± SD	MR ± SD	MD ± SD	MR ± SD
50	0.16 ± 0.12	2.47 ± 1.03	0.22 ± 0.12	2.78 ± 0.95
100	0.18 ± 0.09	3.70 ± 1.23	0.24 ± 0.08	3.76 ± 0.95
150	0.19 ± 0.08	4.51 ± 1.26	0.24 ± 0.07	4.39 ± 0.96
200	0.20 ± 0.06	5.27 ± 1.28	0.25 ± 0.05	4.81 ± 0.92
250	0.20 ± 0.05	5.68 ± 1.27	0.26 ± 0.04	5.28 ± 0.92
300	0.20 ± 0.05	6.21 ± 1.25	0.26 ± 0.04	5.56 ± 0.87
350	0.20 ± 0.05	6.55 ± 1.27	0.26 ± 0.03	5.81 ± 0.84
400	0.20 ± 0.04	6.90 ± 1.25	0.26 ± 0.03	6.05 ± 0.76
450	0.20 ± 0.04	7.11 ± 1.21	0.26 ± 0.03	6.28 ± 0.71
500	0.21 ± 0.04	7.39 ± 1.18	0.26 ± 0.02	6.50 ± 0.61
550	0.21 ± 0.04	7.59 ± 1.22	0.26 ± 0.02	6.68 ± 0.52
600	0.21 ± 0.03	7.83 ± 1.22	0.26 ± 0.01	6.84 ± 0.39
650	0.21 ± 0.03	8.00 ± 1.22	0.26 ± 0.00	7.00 ± 0.00

al., 2013a, 2014). The helminthological fauna of *R. major* is dominated by nematode parasites. This pattern is also observed in other bufonid toads from temperate region (Bolek and Coggins, 2003; Yoder and Coggins, 2007) and tropical region (Bursey et al., 2001; Goldberg and Bursey, 2003; Luque et al., 2005; Ibrahim, 2008; Santos and Amato, 2010, 2013; Hamann et al., 2013a), in which the helminth community consisted mainly of nematodes. An exception is the study by Hamann et al. (2014) who found that nematode species show low infection levels in the toad *M. klappenbachi*; these results were related essentially to the thicker integument of this particular host, as well as by the presence of alkaloids in the skin glands that could avoid the parasitic attack.

The species *A. hylambatis* was the dominant parasite in *R. major*. This had also been observed by Hamann et al. (2012) for *Leptodactylus bufonius* (Leptodactylidae) from Corrientes Province in Argentina, a frog with a more terrestrial habit, whose microhabitat preference significantly contributed to the high infection by *A. hylambatis*. Likewise, in other reports for *Rhinella fernandezae* (Bufonidae) from Brazil (Santos and Amato, 2010), and for the toad *Amietophrynus regularis* (Bufonidae) from Egypt (Ibrahim, 2008) the parasitic community was dominated by the genus *Aplectana* (Cosmocercidae). In particular, the fact that *A. hylambatis* -a nematode with direct life cycle- predominates in *R. major* could indicate a relationship with the mainly terrestrial habits of this amphibian. Infection by aquatic metacercariae (*Travtrema* sp., Strigeidae gen. sp. 1 and Strigeidae gen. sp. 6) showed low richness, reflecting the fact that *R. major* spends only little time in the water. Thus, the present study confirms that the parasite communities of *R. major* exhibit the general characteristics of a terrestrial amphibian, coinciding that terrestrial amphibians contain greater proportion of nematode parasites (Bursey et al., 2001; Goldberg and Bursey, 2003; Bolek and Coggins, 2003; Luque et al., 2005; Yoder and Coggins, 2007I; Ibrahim, 2008; Santos and Amato, 2010, 2013; Hamann et al., 2013a).

The infections recorded in *R. major* show that this anuran species represents the definitive host for more than 50% of the species found parasitizing them; apart from this, this toad had a minor role as paratenic and/or intermediate host, reflected by the presence of larvae at different infection sites, e.g. infection by larvae of *Physaloptera* sp., which persist in the gastric mucosa for varying periods of time without reaching the adult stage (see Anderson, 2000), or by larvae of *Mesocestoides* sp. which remain encapsulated in the mesenteries (see Prudhoe and Bray, 1982). These infections occur when toads eat invertebrates (e.g., insects and oribatid mites), and subsequently the ingested larval forms of these parasites culminate their biological life cycles in some birds and mammals (e.g., by predation of *R. major* by fox; see Schalk and Morales, 2012). Many of the helminth species recorded in this toad were generalists, given that its presence has been reported in amphibian species belonging to other families in our region (see Campião et al., 2014; Hamann et al., 2014).

Proportionately, each zone had a similar number of dominant and codominant helminth species in the community, and the three species (*A. hylambatis*, *Cylindrotaenia* sp. and *R. elegans*) shared by toads from both zones were categorized the same way (Table 3); i.e., the relative importance of these species was similar in both habitats. Nevertheless, more larval species were present in toads from the subhumid zone. This difference may be influenced in part by low water availability in the semi-arid zone given by shorter humid periods. Thus, because of this factor some invertebrates that act as intermediate hosts are not active, and therefore the development and survival of infective stages does not progress to allow completion of the parasite life cycle in this zone. In the same way, soil with high contents of sand combined with scarce humidity can also affect the survivorship and transmission of infective nematode larvae (see Pizzatto et al., 2013).

On the other hand, the dominant species (*A. hylambatis*) confers a relatively high level of predictability regarding the parasite communities of this toad, such that any sample of *R. major* from a single locality is likely to include this common helminth. This cosmocercid species that reaches high local prevalence also tends to have a wide geographical range (see Poulin, 2007). Finally, the helminthological composition showed little similarity between the sampling sites, and this result clearly highlights the important role of local biotic and abiotic factors in determining the proportion of infected toads in a population and the number of helminth species infecting them.

Available information suggests that parasite species richness increases with host size. For instance, several authors in North America (Bolek and Coggins, 2003; Yoder and Coggings, 2007) have found a positive relationship between species richness and size of *Anaxyrus americanus* (Bufonidae), and Ibrahim (2008) in Egypt has shown that host age of *A. regularis* played a significant role in determining community species richness. The results of the present study also indicate that host size plays an important role in parasitic infections, in agreement with the results of previous analyses of Argentine amphibians (Hamann et al., 2010, 2013a, 2014); these results suggest that larger (i.e., older) toads have been exposed to parasites for a longer time and also expose a larger surface area for parasitic attack. In the present study, this is supported by the fact that in the subhumid zone the amphibians present larger body sizes with higher infection level.

In conclusion, this study revealed that the size and habitat of *R. major* influence its helminth infections. In particular, our results suggest that climate (e.g. amount of rainfall and soil moisture) is an important factor affecting the distribution and development of the parasite fauna of *R. major*.

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