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## Helminth communities from amphibians inhabiting agroecosystems in the Pampean Region (Argentina)

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### Abstract

The objectives of this study were to describe the composition and infection patterns of the helminth communities associated with four amphibians (*P. minuta*, *B. pulchella*, *L. latrans* and *R. fernandezae*) with different life-habits, and additionally to compare the helminth fauna associated with these amphibians in agroecosystems with different land use (crops and livestock). A total of 300 anuran individuals and twelve helminth taxa were collected in sites located northeast of the Province of Buenos Aires, Argentina. Helminth communities differed between amphibian species according to their habitat preference, digeneans were predominant in aquatic and arboreal amphibians (Hylidae) and nematodes with direct life cycle were found parasitizing semi-aquatic and terrestrial amphibians (Leptodactylidae and Bufonidae). In three of the frog species (*B. pulchella*, *L. latrans* and *R. fernandezae*), parasite prevalence did not vary significantly according to land use. The values of abundance and infracommunity species richness, meanwhile, varied according to land use, with the highest abundance values in crop sites and higher richness in livestock sites. Regardless of the use of land, *L. latrans* presented the highest values of prevalence, abundance, and richness of helminth species. This is the first study assessing the ecological aspects of parasite taxa in populations of *P. minuta*, *B. pulchella* and *L. latrans* that inhabit agroecosystems in Argentina.

### Keywords

Helminth communities, amphibians, Pampean Region, Argentina.

### INTRODUCTION

As established by Aho (1990), because amphibians have invaded a great variety of habitats and exhibit numerous patterns of life histories, reproductive modes, body size, and trophic relationships, they are excellent systems for exploring patterns and processes that influence the organization of helminth communities. By acting as generalist predators and as preys, amphibians play a role as intermediate and definitive hosts of a great variety of parasites in the aquatic and terrestrial food chains (Koprivnikar *et al.*, 2012).

Among the biological characteristics of the hosts, sex has been listed influencing the number and abundance

of parasitic species in vertebrates (Poulin, 1996). Differences in size, reproductive behaviour, microhabitat preference and hormonal levels related to sex, can affect the exposure of amphibians to the infective stages of parasites. This could be due to a larger colonization area (more space and resources for larger amphibians) or for a longer exposure time, for example, when males vocalize from water during the reproductive periods (Aho, 1990; McAlpine, 1997; Hamann *et al.*, 2006a, b, 2012, 2013, 2014b).

For just over a decade, and with the establishment of the environmental parasitology as a discipline, emphasis has been placed on the role of parasites as indicators of environmental changes, demonstrating significant effects

and interactions between different parasite indices and the presence and concentration of pollutants and/or environmental stressors (Poulin, 1992; Marcogliese & Cone, 1997; Marcogliese, 2005). Several authors suggest that changes in macroparasitic infections have potential as a tool in conservation research of amphibians (Sures, 2004; McKenzie, 2007; Marcogliese & Pietrock, 2011; Koprivnikar *et al.*, 2012).

The Pampean Region, one of the richest agricultural areas of the world, has undergone a drastic anthropogenic intervention during the nineties, with the intensification of land use, the replacement of lands used for livestock by soybean crops, and the concomitant increased in the use of pesticides (insecticides, herbicides) (Agostini & Burrowes, 2015). In addition, during the past two decades the peri-urban intensive production of vegetables has increased around big cities and, in search of greater productivity, it generates a greater use of chemical fertilizers to sustain these productive levels (Defensor del pueblo de la Provincia de Buenos Aires, 2015). These changes have increased the pesticide pollution of wetlands and aquifers, the risk of release to the environment of fertilizers residues and habitat loss, with a negative impact on habitat and wildlife (Herrera *et al.*, 2013).

The best represented and abundant anuran amphibians that inhabit this area belong to Hylidae, Leptodactylidae, and Bufonidae (Agostini, 2012). The hylids, *P. minuta* Günther and *B. pulchella* (Duméril & Bibron) are small to medium-sized frogs that use aquatic and arboreal habitats, respectively. The leptodactylid *Leptodactylus latrans* Steffen is a large semi-aquatic frog and the bufonid *Rhinella fernandezae* (Gallardo) is a medium sized species with terrestrial and fossorial habits (Cei, 1980).

Specifically, the objectives of this study were (a) to describe the infection pattern of the helminths (at both parasite population and community levels) associated with four native amphibian species, which exploit different life-habits, (b) to explore parasite-host relationships, and (c) to analyse the effects of land use (crops and livestock) on parasite prevalence, abundance and species richness.

## MATERIALS AND METHODS

### Study area

This study was carried out in nine sampling sites located northeast of the Province of Buenos Aires, Argentina. Five of these sites, which are called “crop” sites (C1-5), represent agroecosystems where intensive agriculture practices are carried out, mainly of soybeans and wheat, where pesticides like glyphosate, endosulfan, chlorpyrifos and cypermethrin are applied (Agostini, 2013). The remaining four, called “livestock” (L1-4), are natural grasslands used for low density dairy livestock pasture activities (Fig. 1).

### Collection and examination of amphibians

Sampling took place between 2011 and 2012, distributed in all seasons, and coincided with periods of rain and high amphibian activity. The authorization was provided by the Ministerio de Agroindustria, Buenos Aires Province (Exp. n° 225500-11319/10). Due to their twilight and/or nocturnal habits, hosts were captured manually at night, using the visual encounter surveys technique (Crump & Scott, 1994). Then were transported alive to the laboratory, euthanized with an overdose of 20% benzocaine by tegument impregnation and dissected following the usual parasitological techniques (see Goldberg & Bursey, 2007). Host samples were fixed in 10% formalin and preserved in 70% ethanol. Prior to the necropsy, sex and morphometric data (snout-vent length, weight) for each specimen were recorded.

### Parasitological analysis

The parasitological analysis included the examination of tegument, general cavity and respiratory, digestive, urinary and genital systems under a stereoscopic microscope. Helminths were quantified, fixed in cold 5% formaldehyde, and preserved in 70% alcohol. For its identification, nematodes and acanthocephalans were cleared in lactophenol or glycerin-alcohol and studied in temporary preparations. Trematodes and cestodes were compressed slightly between covers and submerged in 96% ethanol for approximately 24 hours. Then they were stained with hydrochloric Carmine diluted in 96% alcohol, dehydrated in an increasing series of alcohols, cleared in xylol, and mounted in Canada balsam in permanent slides. Parasites were examined under light microscopy and the taxonomic study was performed using specific keys (Yamaguti, 1961, 1963; Petrochenko 1971a, b; Anderson, 2000; Khalil *et al.*, 1994; Gibson *et al.*, 2002; Jones, 2005; Bray *et al.*, 2008). The voucher parasite specimens were deposited in the Helminthological Collection of the Museo de La Plata (MLP-He) under the catalog numbers MLP-He 7626-7637 and MLP-He 7680-7690.

### Data analyses

To describe the infection patterns at the population level, the prevalence, mean abundance, and mean intensity of infection (followed by their respective standard errors) were calculated following Bush *et al.* (1997). Additionally, the Dispersion Index was calculated as the variance ( $S^2$ )/mean ratio (Krebs, 1999). In aggregated distributions the mean number of parasites is smaller than the variance, the ratio has a value higher than 1; in random distributions the value of this relationship is very close to 1 since the variance and the mean are almost equal in value; and in the regular or uniform distribution this ratio is smaller than 1. To describe the infection patterns at the community level, the species richness ( $S$ ), the diversity index of Shannon-Wiener ( $H'$ ) (Shannon-Weaver, 1949)

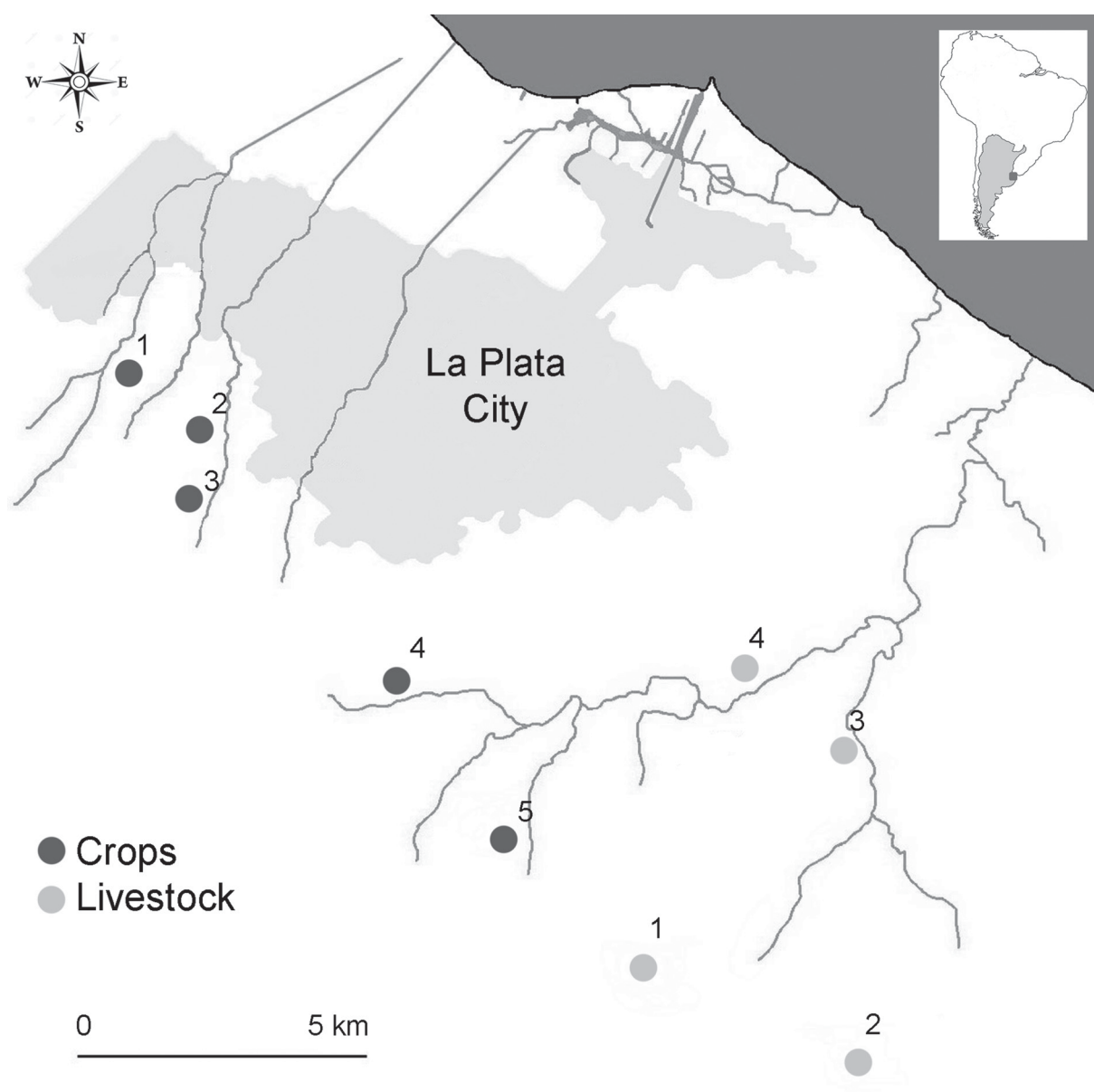


Fig. 1. Study area. C1-5: crop sites, L1- 4: livestock sites (C1: 34°55'13"S; 58°06'33"O; C2: 34°55'54"S; 58°04'29"O; C3: 34°57'36"S; 58°04'57"O; C4: 35°01'42"S; 57°59'44"O; C5: 35°03'06"S; 57°58'35"O; L1: 35°04'27"S; 57°57'23"O; L2: 35°07'46"S; 57°53'11"O; L3: 35°02'22,94"S; 57°48'58,8"O; L4: 35°02'23,2"S; 57°48'58,2"O).

and the Pielou evenness index ( $J'$ ) (Pielou, 1969) were calculated. Additionally, dominance ( $d$ ) was obtained using the non-parametric Berger-Parker Index (Berger & Parker, 1970). Dominant, codominant, subordinate, and unsuccessful pioneer species were distinguished according to the Importance index ( $I$ ) established by Thul *et al.* (1985). At the infracommunity level, cases of mono or polyspecific infections were recorded. Finally, Jaccard's index for qualitative similarity, based on the presence and absence of helminths, was used to evaluate the similarity of the parasitic infracommunities among the host species. The size and sex of the host were considered to explore parasite-host relationships. We used Mann Whitney

U test to test differences in size (snout-vent length, weight) between host males and females. Comparison of two proportions was calculated (Z test) to compare differences in parasite prevalence between host sexes. To prove the existence of significant differences between the abundance and intensity of infection by host sex the Mann Whitney U test was used, and the Spearman correlation when testing these parameters and its association with weight and the snout-ventral length (SVL). We conducted comparison of  $k$  proportions ( $\chi^2$ ) to test possible variations related to host sex and size in the abundance of the helminth species that presented prevalence greater than 10%.

To test the effects of land use on the prevalence, abundance, and richness of helminths associated with the amphibian species, generalized linear mixed models (GLMM) with different error distributions were applied (Zuur *et al.*, 2009). One model for each response variable was carried out, resulting in a total of three models. Prevalence was analysed employing Binomial distribution, using the term cbind (infected number, uninfected number), while the richness and abundance were analysed with the Poisson distribution and the log link function. The significance of the random effects (sites) was evaluated with a likelihood ratio (LR) test. For all cases, models were constructed with the same variables, considering as fixed effects: land use (crops, livestock) and host species (*B. pulchella*, *L. latrans* and *R. fernandezae*). At the same time, the effect of site variation was controlled considering it as a random effect (nested site in use). The Tukey a posteriori test was used to detect differences between host species. The statistical analyses were carried out with R software, Version 2.15.1 (R Development Core Team 2012) and the GLMM were built with the lme4 package (Bates *et al.*, 2012).

## RESULTS

A total of 300 hosts were necropsied: 20 specimens of *P. minuta* with prevalence of infection of 50%, 150 *B. pulchella* with prevalence of 35%, 48 *L. latrans* with prevalence of 71%, and 82 *R. fernandezae* with prevalence of 27%. Quantitative descriptors of each helminth species are shown in Table 1. Most of the

helminth taxa found in this study are generalists, and were found in the intestinal tract, with the small intestine as the most parasitized organ.

The compound community associated with these four amphibian species comprised twelve helminth taxa: an acanthocephalan species, *Pseudoacanthocephalus* cf. *lutzi* (Hamann, 1891) Arredondo & Gil de Pertierra, 2009 (Echinorhynchidae); six nematode taxa, *Aplectana hylambatis* (Baylis, 1927) Travassos, 1931, *Aplectana* sp., *Cosmocerca parva* Travassos, 1925, *Cosmocercoides* sp. (Cosmocercidae), *Rhabdias elegans* Gutiérrez, 1945 (Rhabdiasidae), and *Schrankiana* sp. (Atractidae); a cestode taxon, *Cylindrotaenia* sp. (Nematotaeniidae) and four trematode taxa *Catadiscus uruguayensis* Freitas & Lent, 1939, *Catadiscus corderoi* Mañé-Garzón, 1958 (Diplodiscidae), *Rauschiella palmipedis* (Lutz, 1928) Sullivan, 1977, Macroderoididae gen. sp. (Macroderoididae). *Pseudoacanthocephalus* cf. *lutzi* and *C. uruguayensis* were the species with the largest host distribution, being found in three host species. For five of the taxa (42%), new parasite-host associations were registered.

Fig. 2 shows the contribution of each group of helminths to the proportion of the total of infected individuals per host species studied. The predominant group is that of the trematodes, with the other groups of helminths contributing to a lesser extent according to the host species and its life habit.

At the community level, similar values of diversity, evenness and dominance were observed in parasite communities, reflecting codominance and an equivalent

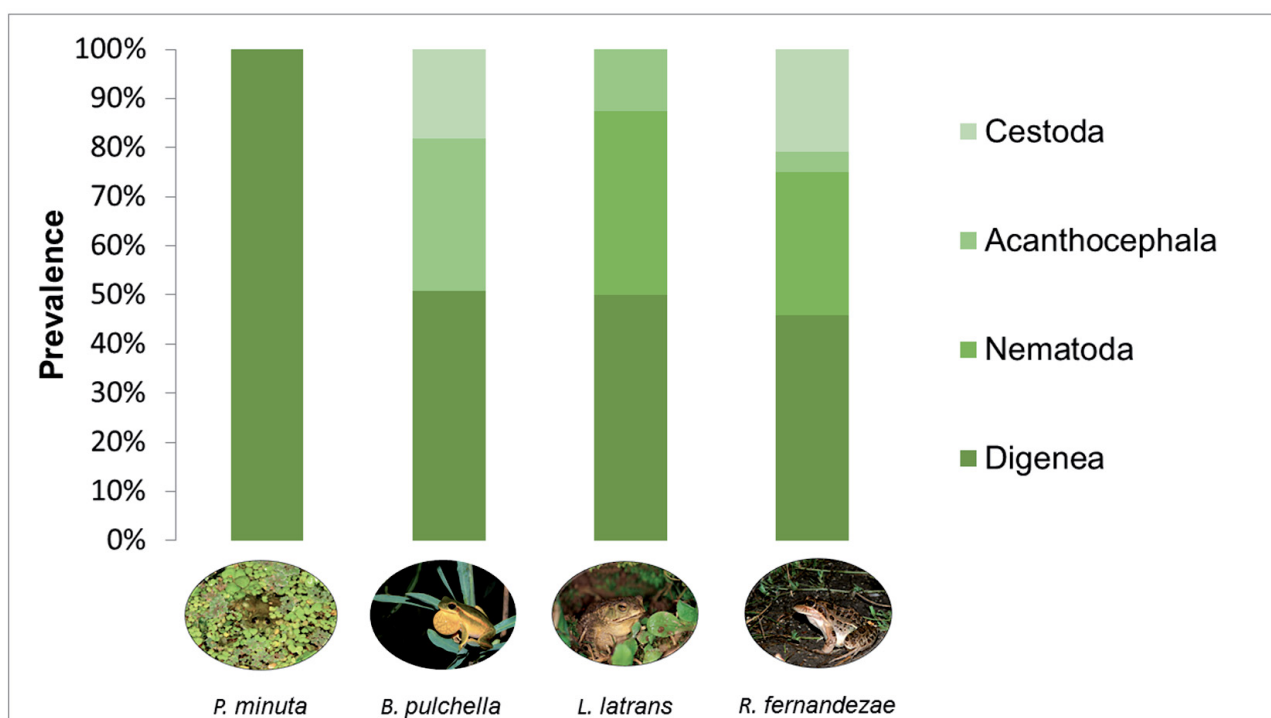


Fig. 2. Contribution of each group of helminths to the proportion of the total of infected individuals for each host species.



Table 1. Prevalence (%), mean intensity (MI)/ mean abundance (MA)  $\pm$  standard deviation (SD), total number (n) of parasites, infection site, dispersion index (Di), dominance index (d) and Importance index (I) of the helminth taxa found parasitizing four amphibian species from Buenos Aires, Argentina. Li= large intestine, Lu= lungs, Si= small intestine.

Helminth by host	%	n	MI $\pm$ SD (range)	MA $\pm$ SD	Infection site			Di	d	I
					Lu	Si	Li			
<b><i>Pseudis minuta</i></b>										
<b>Trematoda</b>										
<i>Catadiscus uruguayensis</i>	50	64	6.4 $\pm$ 5.87 (1-15)	3.2 $\pm$ 0.29	---	x	x	8.5	0.98	1*
Macroderoididae gen. sp.	5	1	1	0.05	---	---	x	1	0.02	0**
<b><i>Boana pulchella</i></b>										
<b>Acanthocephala</b>										
<i>Pseudoacanthocephalus</i> cf. <i>lutzi</i> (new host)	11.3	61	3.59 $\pm$ 0.22 (1-12)	0.40 $\pm$ 0.02	---	x	x	6.93	0.40	0.41§
<b>Cestoda</b>										
<i>Cylindrotaenia</i> sp. (new host)	6.7	19	1.9 $\pm$ 0.087 (1-3)	0.12 $\pm$ 0.006	---	x	x	2.15	0.13	0.07§
<b>Trematoda</b>										
<i>C. uruguayensis</i>	15.3	57	2.43 $\pm$ 0.06 (1-5)	0.36 $\pm$ 0.008	---	x	x	2.79	0.37	0.46§
<i>Rauschiella palmipedis</i> (new host)	6.7	13	1.6 $\pm$ 0.07 (1-3)	0.10 $\pm$ 0.004	---	x	x	1.78	0.10	0.06§
<b><i>Leptodactylus latrans</i><sup>#</sup></b>										
<b>Acanthocephala</b>										
<i>P. cf. lutzi</i> (new host)	10.4	21	4.2 $\pm$ 0.93 (1-12)	0.44 $\pm$ 0.09	---	x	x	8.06	0.06	0.06§
<b>Trematoda</b>										
<i>C. uruguayensis</i>	18.8	43	4.78 $\pm$ 0.43 (1-13)	0.9 $\pm$ 0.08	---	x	x	6.8	0.12	0.22§
<i>R. palmipedis</i>	35.4	45	2.88 $\pm$ 0.15 (1-10)	1.02 $\pm$ 0.05	---	x	x	4.11	0.14	0.47§
<b>Nematoda</b>										
<i>Aplectana hylambatis</i>	4.2	23	7.5 $\pm$ 4.6 (1-14)	0.31 $\pm$ 0.19	---	x	x	13.1	0.04	0.02§
<i>Aplectana</i> sp.	2.01	1	1	1	---	x	---	1	0.003	0**
<i>Cosmocerca parva</i>	2.01	23	23	0.47	---	x	x	23	0.06	0.013§
<i>Cosmocercoides latrans</i> (new host)	4.2	159	79.5 $\pm$ 35.7 (29-130)	3.31 $\pm$ 1.49	---	---	x	110.6	0.46	0.18§
<i>Schrankiana</i> sp.	2.01	25	26	0.54	---	x	x	26	0.075	0.015§
<i>Rhabdias elegans</i> (new host)	10.4	7	1.4 $\pm$ 0.18 (1-3)	0.14 $\pm$ 0.018	x			1.75	0.02	0.02§
<b><i>Rhinella fernandezae</i></b>										
<b>Acanthocephala</b>										
<i>P. cf. lutzi</i>	1.2	1	1	0.01	---	x	---	1	0.02	0.003 <sup>æ</sup>
<b>Cestoda</b>										
<i>Cylindrotaenia</i> sp.	6.1	13	2.6 $\pm$ 0.36 (1-5)	0.16 $\pm$ 0.02	---	x	x	3.5	0.28	0.19§
<b>Trematoda</b>										
<i>Catadiscus corderoi</i> (new host)	13.4	21	1.91 $\pm$ 0.02 (1-7)	1.90 $\pm$ 0.16	---	x	x	3.26	0.46	0.69§
<b>Nematoda</b>										
<i>C. parva</i>	1.2	4	4	0.04	---	x	x	4	0.08	0.01§
<i>R. elegans</i> (new host)	7.3	7	1.2 $\pm$ 0.09 (1-2)	0.07 $\pm$ 0.005	x	---	---	1.28	0.13	0.09§

\*Dominant species, § codominant species, <sup>æ</sup> subordinate species, \*\* unsuccessful pioneer. # Taxonomic helminth data related to *L. latrans* was taken from Draghi *et al.* (2020).

representation of helminth taxa in most cases. The helminth community associated with *P. minuta* was the exception, with a specific richness equal to two. The values of the ecological indices confirmed that in this host the helminth species do not present a similar abundance, and therefore, *C. uruguayensis* is dominant in this community ( $H' = 0.09$  and  $J' = 0.09$ ). For *B. pulchella* the specific richness was equal to four. The diversity (1.69) and the value of evenness near 1 ( $J' = 0.84$ ) suggests that there is no dominant species in the community. The value of importance confirmed the codominance of parasitic species according to the methodology established by Thul *et al.* (1985) (see Table 1). Regarding the component community of *L. latrans*, the specific richness was equal to nine. The values of the ecological indices suggest that there is an intermediate evenness for the abundance of species ( $H' = 2.09$ ,  $J' = 0.65$ ). The species with the highest community dominance index value was the nematode species *Cosmocercoides latrans* ( $d = 0.46$ ). The values of the importance index indicate that 90% of the helminth species share the dominance of the community. Finally, for *R. fernandezae* the values of the ecological indices ( $H' = 1.83$ ,  $J' = 0.78$ ) suggest the existence of one dominant species, and the species with the highest value in terms of dominance index was *C. corderoi* ( $d = 0.46$ ). On the other hand, following Thul *et al.* (1985) *Cylindrotaenia* sp., *C. corderoi*, *C. parva* and *R. elegans* are codominant species in the community, and *P. cf. lutzi* becomes a subordinate and rare species.

At the infracommunity level monospecific infections always predominated. However, cases of multiple infections (two to three helminth species) were recorded in the four amphibians analysed. For *P. minuta* and *B. pulchella* 96% of monospecific infections were observed, and two cases of double parasitosis were recorded in the last one, one digenean-acanthocephalan (*C. uruguayensis* - *P. cf. lutzi*) and one between two digeneans species (*C. uruguayensis* - *R. palmipedis*), and also two cases of triple parasitosis digenean-acanthocephalan (*C. uruguayensis* - *R. palmipedis* - *P. cf. lutzi*). Monospecific infections predominated (79%) in *L. latrans* and in one host, the richness was equal to three, with two species of digeneans and one of nematode (*C. uruguayensis* - *R. palmipedis* - *R. elegans*) coexisting.

	1	2	3	4
<b>1- <i>P. minuta</i></b>	1	0.2	0.1	0
<b>2- <i>B. pulchella</i></b>	0.2	1	0.3	0.4
<b>3- <i>L. latrans</i></b>	0.3	0.3	1	0.27
<b>4- <i>R. fernandezae</i></b>	0	0.4	0.27	1

Finally, for *R. fernandezae* 97.5% of monospecific infections were observed.

In order to establish the values of similarity among the infracommunities of helminths related to the hosts under study, they were compared using the Jaccard coefficient. Fig. 3 shows the similarity matrix obtained and the corresponding dendrogram. According to this, *B. pulchella* and *R. fernandezae* shared a greater number of species ( $IJ = 0.4$ ).

When considering parasite-host relationships, results indicated that *Pseudis minuta* females were larger and heavier than males (Table 2) with significant differences found (USVL = 2,  $p_{0.05}, 2 = 0.029$  and UW = 31,  $p_{0.05}, 2 = 0.029$ , respectively). Prevalence was higher for females specimens of *P. minuta* ( $Z = 1.9$ ,  $p = 0.0024$ ), as well as the values of mean intensity (MI) and mean abundance (MA), but the differences in those cases were not statistically significant ( $U = 8$ ;  $p_{0.05}, 2 =$  and  $U = 7$ ;  $p_{0.05}, 2 = 1$ , respectively) (Table 3). Additionally, the abundance of *C. uruguayensis* (prevalence > 10%) correlated only with the size of females ( $r_s = 1.000$ ,  $p < 0.005$ ).

For *B. pulchella* specimens, females were significantly larger than males (USVL = 2336,  $p_{0.05}, 2 < 0.0001$  and UW = 722,  $p_{0.05}, 2 < 0.0001$ ). Although from a descriptive approach the prevalence was slightly higher in females (Table 4), no significant difference was found between sexes ( $Z = 0.27$ ,  $p = 0.78$ ). Differences were statistically significant for MI and MA ( $U = 329$ ;  $p_{0.05}, 2 = 0$  and  $U = 344$ ;  $p_{0.05}, 2 \leq 0.0001$ , respectively). There was no relationship between the parasite abundance of species that presented a prevalence greater than 10% (*P. cf. lutzi* and *C. uruguayensis*), and the host's body parameters (considering the total of the hosts and separated by sex). On the other hand, males of *L. latrans* were more robust (UW = 100,  $p_{0.05}, 2 = 0.004$ ). There was no significant difference in prevalence between host sexes ( $Z = 0.25$ ,  $p = 0.802$ ). Similarly, MA and MI that were higher in males, did not present statistically significant differences ( $U = 105$ ,  $p_{0.05}, 2 = 0.41$  and  $U = 103$ ,  $p_{0.05}, 2 = 0.47$ , respectively) (Table 3). Of the species with prevalence greater than 10%, *C. uruguayensis* was the only one that presented a negative, high, and significant correlation with weight when considering the total number of hosts ( $r_s = -0.809$ ,  $p < 0.005$ ).

Finally, males of *R. fernandezae* were larger and heavier

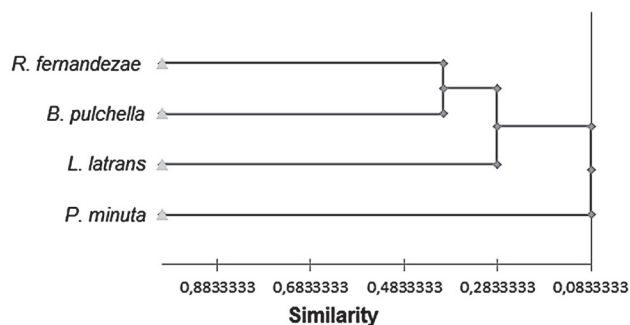


Fig. 3. Similarity matrix and dendrogram.

than females (USVL = 805;  $p_{0.05}$ ;  $2 < 0.0001$  and weight UW = 769;  $p_{0.05}$ ;  $2 < 0.0001$ ) (Table 3). From a descriptive perspective, both the prevalence of infection and the total number of helminths were slightly higher for males of *R. fernandezae*, but these differences were not statistically significant ( $Z = -0.09$ ,  $p = 0.92$ ). In contrast, values of MI and MA were higher in females ( $U = 6$ ,  $P_{0.05}$ ,  $2 = 0.011$ ) (Table 3). The abundance of the only species that presented a prevalence greater than 10% (*C. corderoi*) did not correlate with the host's body parameters. Table 4 shows the values of Prevalence (P) and abundance (MA) of the helminth taxa for three host

species (*B. pulchella*, *L. latrans* and *R. fernandezae*), distinguished by sampling site. The acanthocephalan *P. cf. lutzi* was found in the three hosts. Its maximum prevalence value was 56% parasitizing *B. pulchella* on the L2 site. It should be noted that as was not found in any of the crop sites, *P. minuta* was excluded from the GLMM analysis.

According to the GLMM, no significant effect of the sampling sites was detected, so models were corrected. In contrast, LR test showed significant random effects of sex for the models testing differences in helminths prevalence ( $\chi^2 = 4.06$ ,  $df = 1$ ,  $p = 0.04$ ) and abundance ( $\chi^2 =$

Table 2. Sample size (n), snout-ventral length (SVL; mm) and weight (W; g) for four amphibian species from Buenos Aires, Argentina. Mann Whitney U test, significant values ( $p < 0.05$ ) in bold.

	Sex	n	SVL, mean $\pm$ SD (min-max)	W, mean $\pm$ SD (min-max)
<i>P. minuta</i>	♀♀	3	<b>41.58 <math>\pm</math> 6.27 (35-47.5)</b>	<b>8.38 <math>\pm</math> 3.09 (5-11.1)</b>
	♂♂	11	34.28 $\pm$ 4.5 (29.5- 36.6)	4.5 $\pm$ 0.5 (3.8-6)
	§§	6	32.9 $\pm$ 3.34 (29.5- 38.5)	4.26 $\pm$ 0.95 (2.8-5.3)
<i>B. pulchella</i>	♀♀	26	<b>46.62 <math>\pm</math> 45.24 (33.61- 54.89)</b>	<b>5.91 <math>\pm</math> 2.18 (2.3-10.67)</b>
	♂♂	114	41.81 $\pm$ 3.5 (34-50)	4.11 $\pm$ 1.28 (1.96-11.5)
	§§	10	42.02 $\pm$ 2.52 (37.5-45.8)	3.81 $\pm$ 0.76 (2.11-4.95)
<i>L. latrans</i>	♀♀	15	78.37 $\pm$ 8.22 (61.64-93.03)	42.28 $\pm$ 15.17 (27.45-77.32)
	♂♂	23	83.31 $\pm$ 1.81 (58.62-104.06)	<b>69.20 <math>\pm</math> 32.08 (23.8-143.8)</b>
	§§	10	81 $\pm$ 11.77 (56.15-94)	59.58 $\pm$ 39.25 (17-97)
<i>R. fernandezae</i>	♀♀	18	50.53 $\pm$ 8.55 (13.3-61.8)	14.47 $\pm$ 9.25 (2.88-61.77)
	♂♂	56	<b>60.56 <math>\pm</math> 7.07 (41.34-69.7)</b>	<b>21.57 <math>\pm</math> 6.77 (7.6-32.15)</b>
	§§	8	56.5 $\pm$ 7.5 (46.42-64.5)	17.45 $\pm$ 6.38 (10.32-26.74)

§§ = not sexually differentiated.

Table 3. Indicators of parasite infection by sex of the host. Sample size (n), Prevalence (%), Mean Intensity (MI)/ Mean Abundance (MA)  $\pm$  standard deviation (SD), and total number (n) of parasites. Z test (%) and Mann Whitney U test (MI and MA) significant values ( $p < 0.05$ ) in bold.

Host Species	Sex	n	%	n	MI $\pm$ SD (range)	MA $\pm$ SD
<i>P. minuta</i>	♀♀	3	<b>100</b>	22	7.33 $\pm$ 2.34 (1-15)	7.33 $\pm$ 2.34
	♂♂	11	45.45	34	6.8 $\pm$ 1.33 (1-15)	3.09 $\pm$ 0.6
	§§	6	33.33	8	4 $\pm$ 2.12 (1-7)	1.33 $\pm$ 0.7
<i>B. pulchella</i>	♀♀	26	36.7	24	<b>2.4 <math>\pm</math> 0.18 (1-7)</b>	<b>0.9 <math>\pm</math> 0.07</b>
	♂♂	114	33.3	115	3.02 $\pm$ 0.08 (1-12)	1 $\pm$ 0.03
	§§	10	50	11	2.2 $\pm$ 0.22 (1-4)	1.1 $\pm$ 0.09
<i>L. latrans</i>	♀♀	15	73.3	55	5 $\pm$ 0.34 (1-12)	3.66 $\pm$ 0.26
	♂♂	23	69.56	252	15.75 $\pm$ 2 (1-130)	10.95 $\pm$ 1.4
	§§	10	50	40	8 $\pm$ 0.93 (3-15)	4 $\pm$ 0.47
<i>R. fernandezae</i>	♀♀	18	22.2	12	<b>3 <math>\pm</math> 1 (1-9)</b>	<b>0.66 <math>\pm</math> 0.22</b>
	♂♂	56	26.78	26	1.71 $\pm$ 0.084 (1-5)	0.46 $\pm$ 0.023
	§§	8	37.5	7	2.33 $\pm$ 0.5 (1-4)	0.87 $\pm$ 0.19

§§ = not sexually differentiated.



Table 4. Parasites per host and sampling site. Sample size (n), Prevalence (%), Mean Abundance (MA)  $\pm$  standard deviation (SD), C=crop sites, L= livestock sites.

Host	Helminth	Site	n	(%)	MA $\pm$ SD
<i>B. pulchella</i>	<i>Cylindrotaenia</i> sp.	C1	5	40	1 $\pm$ 0.14
		C4	4	8	0.16
		L1	1	3.84	0.038
		C3	5	13.63	0.22 $\pm$ 0.05
		C2	3	14.2	0.42
	<i>C. uruguayensis</i>	C4	3	8	0.12 $\pm$ 0.02
		L2	8	21.42	0.57 $\pm$ 0.1
		L3	21	34.78	0.91 $\pm$ 0.07
		L1	15	26.92	0.57 $\pm$ 0.04
		C3	8	13.63	0.36 $\pm$ 0.06
		L4	1	5.88	0.06
	<i>P. cf. lutzi</i>	C4	1	4	0.04
		L3	38	56	1.65 $\pm$ 0.13
		L4	13	11.76	0.76 $\pm$ 0.37
		C5	9	9.09	0.81
	<i>R. palmipedis</i>	C4	1	4	0.04
		L2	2	7.14	0.14
		L3	7	13.04	0.3 $\pm$ 0.045
		C3	1	4.54	0.045
		L4	1	5.88	0.058
C5		2	18.18	0.18	
<i>L. latrans</i>	<i>P. cf. lutzi</i>	L3	21	5	2.62 $\pm$ 1.06
	<i>C. uruguayensis</i>	L2	17	66.66	5.66 $\pm$ 2.12
		L3	1	12.5	0.125
		L4	1	20	0.2
		C2	1	14.28	0.14
		C3	23	20	1.15 $\pm$ 0.063
	<i>R. palmipedis</i>	C4	1	50	0.5
		L3	2	25	0.25
		C3	5	40	1 $\pm$ 0.14
		C2	11	28.6	1.57 $\pm$ 0.3
		L4	26	45	1.3 $\pm$ 0.15
		C5	4	50	2
	<i>A. hylambatis</i>	L4	15	10	0.75 $\pm$ 0.45
	<i>Aplectana</i> sp.	C1	1	100	1
	<i>C. parva</i>	C5	29	50	14.5
	<i>Cosmocercoides latrans</i>	L4	29	5	1.45
		C2	130	14.3	18.5
	<i>Schrankiana</i> sp.	L4	25	5	1.2
	<i>R. elegans</i>	C3	3	20	0.6
		L4	4	20	0.15

Table 4 (continued)

Host	Helminth	Site	n	(%)	MA±SD
<i>R. fernandezae</i>	<i>P. cf. lutzi</i>	L4	1	6.25	0.0625
		C4	5	3.84	0.19
	<i>Cylindrotaenia</i> sp.	L1	1	12.5	0.05
		C3	4	7.14	0.285
		L4	3	12.5	0.18±0.044
		C4	7	19.23	0.26±0.032
	<i>C. corderoi</i>	C3	11	28.57	0.78±0.035
		L4	3	12.5	0.1875±0.044
		C2	4	9.09	0.36
	<i>C. parva</i>	C2	4	9.09	0.36
<i>R. elegans</i>	C4	2	7.69	0.077	
	L4	2	12.5	0.125	
	C3	2	7.14	0.142	

4.49,  $df=1$ ,  $p=0.03$ ). The helminths prevalence did not vary significantly according to land use ( $Z = -0.71$ ,  $p = 0.09$ ). However, they were higher for the livestock sites in the three host species, standing out in L1 and L2 sites (Prevalence = 75% and 42%, respectively). Likewise, parasite prevalence was different across host species ( $Z = 5.42$ ,  $p < 0.05$ ), being higher for *L. latrans* (Tukey a posteriori test  $p < 0.05$ ) (Fig. 4A). When the effect of land use on helminth abundance was explored, the analyses showed that the highest abundances were found in crop sites ( $Z = 3.12$ ,  $p < 0.05$ ). In addition, abundances varied among host species ( $Z = -4.21$ ,  $p < 0.05$ ), with *L. latrans* presenting the highest values (Fig. 4B). The analyses on the infracommunities helminth richness showed a significant effect of land use ( $Z = 4.40$ ,  $p < 0.05$ ) with hosts from the livestock sites presenting the highest values. In turn, richness varied among host species ( $Z = -4.93$ ,  $p < 0.05$ ), again with *L. latrans* specimens presenting the highest values (Fig. 4C).

## DISCUSSION

In the present study, a marked relationship between the habit preference of the hosts and their helminth communities was observed. Digeneans predominated in aquatic and arboreal amphibians (Hylids). On the other hand, nematodes with a direct life cycle were present in semi-aquatic and terrestrial amphibians (Leptodactylids and Bufonids). However, trematodes always dominated in numbers among the parasite communities, showing the close relationship of this group of hosts with the aquatic environment.

Aho (1990) established a trend, positioning the species according to their habitat preference in a terrestrial-aquatic environmental transition. In this trend, species of semi-aquatic amphibians possess the highest species

richness of helminths (both at the population level and per individual host); terrestrial and aquatic species tend to have intermediate and similar values, while those with arboreal or fossorial habits have a lower helminth species richness. According to this and other authors, the type of diet is an additional factor that can lead to differences in the helminth communities of amphibian hosts and influence its development, (generalists vs. specialists) (Poulin, 2007; Poulin & Morand, 2004). In the present study, the helminth communities of *P. minuta* and *B. pulchella* were composed mainly of helminths with aquatic and indirect life cycles, with dominance of those whose transmission is achieved by a trophic route (e.g.: *C. uruguayensis*, *Cylindrotaenia* sp.).

In comparison with other species of *Pseudis* previously studied (Kehr & Hamann, 2003; Campião *et al.*, 2010), the specific richness for *P. minuta* in the present study was extremely low, with only one species of digenean (*C. uruguayensis*) in the adult stage. *Pseudis minuta* was assumed abundant in the study area, but was found only in two livestock sites, and was completely absent from the crop sites. According to Agostini (2013) this can be related to the effects of pesticides as well as the presence of different stress factors in these ecosystems. Characteristics of this host as its low population density, small body size and other hosts with which it interacts could be affecting its parasitic communities (Price, 1990). *Boana pulchella* normally lives in the axillary pods of the long leaves of marsh plants belonging to the genus *Eryngium* sp. (Apiaceae), hunting and feeding mainly on diptera (Langone, 1994). The arboreal and climbing habit of this species could reduce the chances of infection by directly transmitted parasites (from the soil or the aquatic environment), causing the occurrence of trophically transmitted helminths through the ingestion of infected hosts, free infective stages (cercariae) or cystic (metacercariae). As was found by Hamann *et*

*al.* (2009) and Campião *et al.* (2015) for climbing amphibians, the predominant group of helminths was that of trematodes, with two species: *C. uruguayensis* and *R. palmipedis*. As for the species with the highest

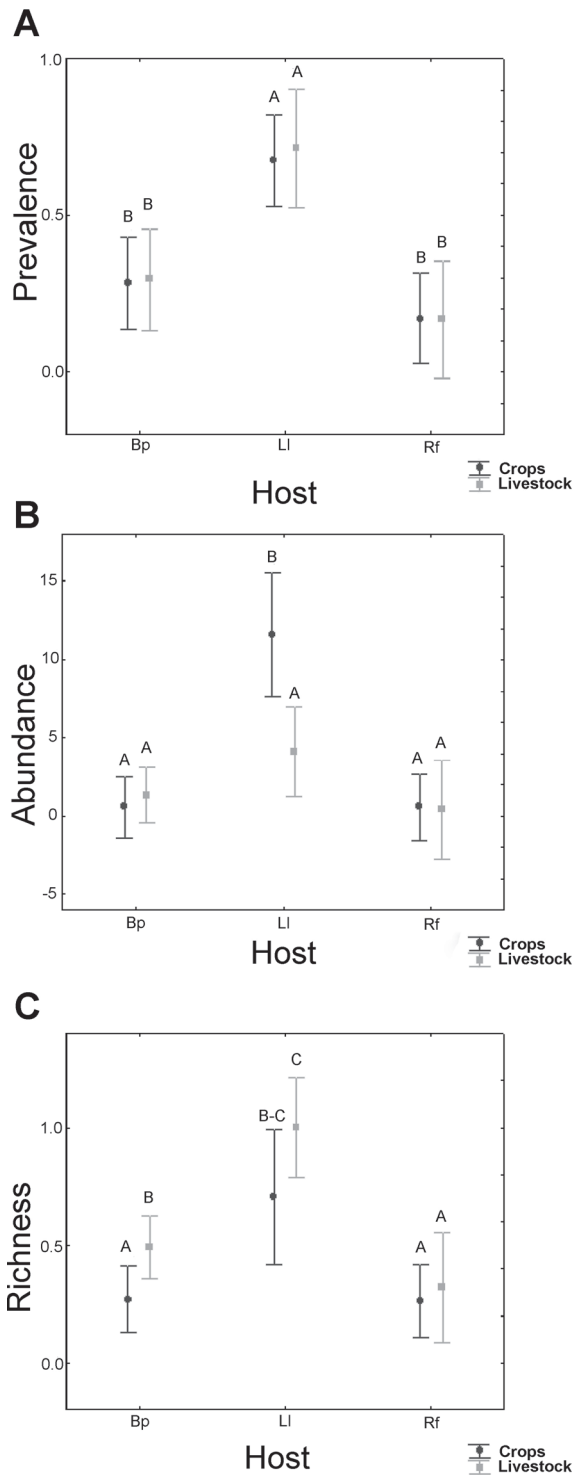


Fig. 4. Helminth prevalence (A), abundance (B) and richness (C) at infracommunity level, in relation to land use and host species. *B. pulchella* (Bp), *L. latrans* (LI), *R. fernandaeze* (Rf).

prevalence of infection, *Pseudoacanthocephalus cf. lutzii* and *C. uruguayensis*, both have an indirect life cycle that need an aquatic intermediate host to be complete and are transmitted by trophic route. At least one species of *Pseudoacanthocephalus* has as intermediate host terrestrial isopods (Nakao, 2016), arthropods in close relationship with humid environments. There were often isopods in the stomach and intestinal contents of *B. pulchella* in our study and these arthropods have been cited as dietary components in other populations of *B. pulchella* in Argentina (Antoniazzi *et al.*, 2013) and Uruguay (Maneyro *et al.*, 2004). In addition, it is presumed that for the *Cylindrotaenia* species (nematoda cestodes with direct life cycle) infection also occurs by trophic route, but in this case by the ingestion of proglottids with embryonated eggs (Stumpf, 1981/1982; Brooks *et al.*, 2006). With respect to *R. palmipedis*, a digenean with an indirect and aquatic life cycle, it presented low prevalence (6.7%) and this could also be related with the habit preference of *B. pulchella*. The terrestrial habitat and the mobility of *R. fernandaeze* associated with the active search for prey (mainly ants), can favour the active penetration of infective nematode larvae (*C. parva*, *R. elegans*), resulting in a more diverse community of parasites, where the four groups of helminths were represented. Previous reports in South America indicate that amphibian helminth communities belonging to *Rhinella* are mostly composed of nematode species (Hamann *et al.*, 2013, 2014a). For *R. fernandaeze* populations, this dominance of nematodes with direct life cycle is due to the terrestrial habits of the host since the adult frogs lives in burrows and only leaves to feed (Hamman *et al.*, 2012). In the present study, the helminth community presented a richness score of five. Trematodes were the best represented group numerically, with *C. corderoi* (aquatic life cycle) as the dominant species ( $d = 0.46$ ). *Cylindrotaenia* sp. also has an aquatic life cycle, but in this case, it is assumed to be direct; and the nematode species *C. parva* and *R. elegans* are characterized by presenting monoxenous life cycles and inhabiting both wet and dry soils with infection occurring through penetration by the larval stage through the skin of the host (Anderson, 2000). The low prevalence of nematodes found in *R. fernandaeze* may be because adults of this toad species possess a thick tegument, which could be a limiting factor for the penetration of infective larvae.

Finally, the high species richness of helminths present in *L. latrans* would correspond not only to their habitat preference but also to its larger size, which would provide a greater colonization surface, and a greater energy requirement, translated into a greater food intake (Duré & Kehr, 1999; Hamman *et al.*, 2006a). *Leptodactylus latrans* is a generalist predator, which uses an intermediate strategy between active foraging and what is known as “sit and wait”. Their diet consists of earthworms, snails, spiders, insects, and other amphibians

(Langone, 1994). According to the present study, it can be confirmed that it feeds both on land (*P. cf. lutzii*) and in the aquatic environment (*C. uruguayensis*). Habitat and mobility (water-ground) of the host, associated with the search for prey, could favour the penetration of the infective stages of the helminths, resulting in a richer and more diverse parasitic community (Hamann *et al.*, 2013b). These data agree with those obtained by different authors for populations of *L. latrans* (Vicente & Santos, 1976; Stumpf, 1981/1982; Goldberg *et al.*, 2009; Toledo *et al.*, 2015) and are similar to those reported for other leptodactylids in Argentina (Hamann *et al.*, 2006a, b, 2012). *Leptodactylus latrans* has a wide range of associated helminths, characteristic of both aquatic and terrestrial environments.

When comparing the parasite infracommunities associated with the four amphibian hosts here studied, *B. pulchella* and *R. fernandezae* shared a greater number of species and presented, therefore, similar parasite communities. In contrast, *P. minuta* and *R. fernandezae*, amphibians located at the ends of the water-land continuum, did not present species in common.

Sexual dimorphism in size may influence the intensity of infection or the richness of parasite species (McAlpine, 1997). A larger body would provide more area for the colonization of parasites and would allow a higher probability of cercariae penetration (Hamann *et al.*, 2010). In this sense, there was a correlation between the parasitic abundance of *C. uruguayensis* and the body length values of *P. minuta* females. In addition, prevalence was higher for females. Cercariae of *Catadiscus* species emerge from the gastropod that acts as an intermediate host, encyst in different substrates (aquatic vegetation and other substrates) and are ingested by the definitive host in which they mature (Ostrowski de Núñez, 1978/1979). Zank *et al.* (2010) observed a very marked location for males of *P. minuta*, they are found in more remote and deeper places away from the shore of the water body. Meanwhile females remain in places close to shore. These differences in the use of microhabitats were also observed for the population of *P. minuta* from the sample area studied. The spatial distribution of freshwater gastropods that could act as intermediate hosts for digeneans, like planorbids, is mostly aggregated. Also, these gastropods are littoral mollusks associated with vegetation (Gutiérrez Gregoric *et al.*, 2006). Cercariae that emerge are short-lived (Esch *et al.*, 2002), encysting in the nearby vegetation or on some substrate. Because they are closer to shore, females of *P. minuta* would have a higher probability of encountering the infective stages of the parasites, and in addition, they have a larger body size that represents a greater colonization surface.

A similar situation was observed for *R. fernandezae*, no significant differences were found in relation to the prevalence between sexes. Nevertheless, in parasitized females the intensity and abundance of infection was higher. This could be that the encounter probability is

higher for females due to variability in behaviour or their habitat selection; or could also be inferred that the probability of encountering with infective stages was similar for males and females, but in the latter, perhaps due to issues related to their feeding and / or time in water or land, the infection was more intense. However, when relating size and weight to infection indicators only for infected females, no correlation was observed: females of larger size and weight were not more parasitized. This confirmed that helminths presented an aggregate distribution in the host population.

For *B. pulchella*, the situation was the opposite. Although females are larger, values corresponding to the number of parasites, the mean intensity and the average abundance of infection were slightly higher for males. These differences could be attributed to a differential behaviour between sexes, either in relation to the permanence in the water body or to their prey items.

Lastly, for *L. latrans* the parasite prevalence was similar between males and females, so the probability of encountering with infective stages of helminths would seem to be equal between sexes. In some amphibian hosts, the body size has been related to the richness of helminths (Hamann & Kehr, 1998; Bolek & Coggins, 2003; Hamann *et al.*, 2006b), but this was not this case, since the host's body parameters did not relate to parasitic indicators of infection. The only helminth species whose abundance correlated with the weight of the hosts was *C. uruguayensis*, and according to its life cycle, it could be assumed that a larger host ingests larger amounts of food (Duré & Kehr, 1999).

Beyond the intrinsic characteristics of each species, hosts that share the same location, even those belonging to different genera or species, can share helminth taxa because they are exposed to the same ecological conditions (Aho, 1990). The development of agricultural activities is identified as one of the main causes that contribute to the loss of biodiversity, not only due to the reduction and fragmentation of the habitat, but also because of the agrochemicals used (Agostini, 2013). In our study the prevalence of infection in the host species that were present in both types of land uses (crops and livestock) was not related to land use. Should the prevalences be determined by the probability of parasite-host encounter, we can assume that the occurrence and abundance of larval stages and intermediate hosts in both types of sites is similar.

In general, parasite abundance seems to be negatively affected by environmental disturbances (Hamann *et al.*, 2006a; Koprivnikar *et al.*, 2006; McKenzie, 2007; Hartson *et al.*, 2011). In the present study, it varied significantly according to land use, with abundance being higher for crop agroecosystems as compared to livestock sites. This could be related to the high abundances observed for the cosmocercids nematodes with direct life cycle (*A. hylambatis*, *Aplectana* sp., *C. parva*, *C. latrans*) which also presented higher prevalences at the crop sites.



Within their hosts, nematodes present an aggregated distribution, most infected hosts have few parasites and a few hosts have many parasites (Anderson & May, 1978). For these parasites, with direct and monoxenous life cycle, the interactions with the different stressors are less predictable. If the stressor has a strong and negative effect on the hosts defences, it will intensify the impact of the parasites (Lafferty & Kuris, 1999; Johnson & Chase, 2004; Johnson & Sutherland, 2003; Johnson *et al.*, 2007), and this could be happening in crop sites.

The species richness of helminths at infracommunity level, on the other hand, was significantly higher in amphibian specimens collected at livestock sites. The presence of a greater variety of parasitic species may correspond to a healthier ecosystem (Marcogliese, 2005) since a community rich in parasites should reflect the number of host species available (Hudson *et al.*, 2006). The amphibian communities in the livestock sites are richer and the populations of the three host species more abundant (Agostini, 2013) and this could be related to the greater richness of helminths in these sites.

Finally, the three variables studied were significantly related to the host species. *Leptodactylus latrans* presented the highest prevalence, abundance, and species richness at infracommunity level. As discussed above, characteristics of this host such as its size, habit (semi-aquatic) and diet (generalist) were related to its associated parasite community, and these results agree with previous ecological studies carried out on the helminth fauna of this host species in the Neotropical region (Toledo *et al.*, 2015).

According to Campião *et al.* (2015) an average of four studies is needed to describe 50% of the parasite richness in anuran amphibians and only 22% of South American anurans presently match this criterion. The present study is a contribution to the description of helminth diversity in amphibians and is the first involving ecological aspects of the parasite groups for populations of *P. minuta* and *B. pulchella* throughout their distribution area, and the first one for *L. latrans* in Argentina. It also represents the first approach to communities of helminths associated with anurans that inhabit agroecosystems of the Pampean Region.

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