

# Biodiversity of trematodes associated with amphibians from a variety of habitats in Corrientes Province, Argentina

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## Abstract

The main goals of this study were to compare the richness of parasitic trematodes in amphibians with diverse habits (terrestrial, fossorial, semi-aquatic and arboreal), and to evaluate whether the composition of the trematode community is determined by ecological relationships. Specimens were collected between April 2001 and December 2006 from a common area (30 ha) in Corrientes Province, Argentina. Trematodes of amphibians in this area comprised a total of 19 species, and were dominated by common species. Larval trematodes presented highest species richness, with the metacercaria of *Bursotrema tetracotyloides* being dominant in the majority (7/9, 78%) of the parasite communities. Adults of the trematode *Catadiscus inopinatus* were dominant in the majority (6/9, 67%) of amphibians. The amphibians *Leptodactylus latinasus*, *Leptodactylus bufonius* and *Scinax nasicus* presented a high diversity of trematodes, whereas *Leptodactylus chaquensis* had the lowest diversity even though it presented with the highest species richness. The patterns of similarity among amphibian species showed groups linking with their habitats. Leptodactilid amphibians, with a generalist diet and an active foraging strategy showed highest infection rates with adult trematodes. The mean richness of trematode species related to host's habitat preferences was higher in semi-aquatic amphibians. Results suggest that semi-aquatic amphibians, present in both aquatic and terrestrial environments, present a greater diversity of parasites as they have a higher rate of exposure to a wider range of prey species and, hence, to diverse infective states. The trematode composition is related to the diets and mobility of the host, and habitat.

## Introduction

Parasites have been recognized as an important component of global biodiversity and can be excellent indicators of biodiversity in an ecosystem (Marcogliese & Cone, 1997). The life cycles of helminth parasites are generally complex and require one or more intermediate and definitive hosts, so that the presence of phylogenetically diverse species and appropriate densities in the community in a given area is a direct indicator of the existence of a variety of invertebrates and vertebrates, which, in turn, could act as an indicator of environmental

quality (Poulin & Morand, 2004; Marcogliese, 2005; Hudson *et al.*, 2006; King *et al.*, 2007). On the other hand, anthropogenic disturbances to watersheds or alterations to the landscape can affect the richness and abundance of parasitic infracommunities, as well as the distribution of helminth parasites (Hamann *et al.*, 2006a; McKenzie, 2007; King *et al.*, 2008; Marcogliese *et al.*, 2009).

Some research has related parasite occurrence to the habitat preference of their vertebrate hosts. In this sense, Aho (1990) described the richness pattern of intestinal helminth communities in amphibians and reptiles according to their respective habitat throughout their lifespan, and found that there was a trend for helminth species richness to peak in anurans with semi-aquatic life histories. Similarly, Bush *et al.* (1990) examined the

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Table 1. Sample size ( $n$ ), mean  $\pm$  1 SD snout-vent length (SVL; mm), and date of collection for nine amphibian species from Corrientes Province, Argentina.

Species	$n$	SVL (mean $\pm$ SD)	Date of collection	
			Years	Months
Leptodactylidae				
<i>Leptodactylus bufonius</i>	33	41.4 $\pm$ 9.1	2002–2006	Sep–Mar
<i>Leptodactylus chaquensis</i> <sup>a</sup>	40	44.0 $\pm$ 7.8	2002–2003	Sep–Nov
<i>Leptodactylus latinasus</i> <sup>b</sup>	36	24.5 $\pm$ 4.2	2002–2003	Sep–Nov
<i>Leptodactylus latrans</i>	11	57.1 $\pm$ 10.9	2001–2003	Aug–Jul
Cyclorhynchidae				
<i>Odontophrynus americanus</i>	19	28.1 $\pm$ 5.2	2002–2004	Jul–Sep
Bufonidae				
<i>Rhinella fernandezae</i>	20	35.1 $\pm$ 10.2	2002–2005	Apr–Feb
Leiuperidae				
<i>Physalaemus santafecinus</i>	46	21.7 $\pm$ 5.8	2001–2006	Aug–Mar
<i>Pseudopaludicola boliviana</i>	71	12.4 $\pm$ 1.3	2003–2005	May–Feb
Hylidae				
<i>Scinax nasicus</i> <sup>c</sup>	49	22.0 $\pm$ 7.1	2003–2004	Mar–Dec

Data from <sup>a</sup>Hamann *et al.* (2006a); <sup>b</sup>Hamann *et al.* (2006b); <sup>c</sup>Hamann *et al.* (2009).

richness pattern in communities of intestinal helminth parasites of fish, reptiles, birds and mammals, in relation to the number of species in a host population. They showed that terrestrial hosts have, on average, fewer parasitic species than aquatic hosts. Additionally, Kennedy *et al.* (1986) showed in the aquatic habitat that differences between freshwater fish and aquatic birds are

related to the complexity of their alimentary canals, diets and host vagility.

Numerous studies have also focused on the search for characteristics in amphibian hosts (e.g. diet, behaviour) that may determine the structure of helminth communities (Goater *et al.*, 1987; Aho, 1990; McAlpine, 1997; Muzzall *et al.*, 2001; Bolek & Coggins, 2003; Hamann *et al.*,

Table 2. Summary of ecology and biology of nine amphibians from Corrientes, Province of Argentina; the breeding season occurs in the spring, summer and autumn in all species except for *Scinax nasicus*, where breeding occurs in all seasons.

Species	Habitat preference <sup>a</sup>	Breeding site <sup>a</sup>	Feeding preferences/Foraging strategy <sup>b</sup>
<i>Leptodactylus bufonius</i>	Adult frogs live in a burrow, which they leave only to forage	Nest, deep building on dry land	Isopterans and coleopterans/ Between ambush predator and active predator
<i>Leptodactylus chaquensis</i>	Adult frogs live for a longer time near the shore of ponds, and also in flooded grass	Nest, foam building on top of water in shallow ponds or lagoon	Coleopterans, orthopterans and insect larvae/ Between ambush predator and active predator
<i>Leptodactylus latinasus</i>	Adult frogs live in a burrow, which they leave only to forage	Nest, deep building on dry land	Isopterans and coleopterans/ Between ambush predator and active predator
<i>Leptodactylus latrans</i>	Adult frogs live for a longer time near the shore of ponds, and also in flooded grass	Nest, foam building on top of water in shallow ponds or lagoon	Orthopterans and coleopterans/ Ambush predator and active predator
<i>Odontophrynus americanus</i>	Adult amphibians live on land with dry grass, leaving this habitat only to reproduce	On the bottom of shallow, temporary and semi-permanent ponds and flooded areas	Coleopterans and insect larvae/ Active predator
<i>Rhinella fernandezae</i>	Adult toads live in a burrow, which they leave to forage and to reproduce	On mounds and vegetation flooded or within the body of water in temporary and semi-permanent ponds	Ants and coleopterans/Active predator
<i>Physalaemus santafecinus</i>	Adult amphibians live for a longer time in the flooded grass and also near the shore of ponds	Nest, foam building on top of water in shallow ponds or lagoon	Ants/Active predator
<i>Pseudopaludicola boliviana</i>	Adult amphibians live near the shore of ponds, and also in flooded grass	On the bottom of shallow, temporary ponds and flooded areas	Dipterans and collembolans/ Active predator
<i>Scinax nasicus</i>	Adult frogs live on vegetation of height 1 m, leaving it to forage and to reproduce	On flooded vegetation near the shore or in ponds	Dipterans and coleopterans/ Sit-and-wait strategy

<sup>a</sup>Data from Schaefer *et al.* (2006), Schaefer (2007) or our observations. <sup>b</sup>Data from Duré (2004), Duré *et al.* (2004), Schaefer *et al.* (2006).

2006b; Yoder & Coggins, 2007). Furthermore, the presence and abundance of intermediate hosts have also been suggested as key determinants of which kinds of life cycles are possible, and ultimately parasite diversity, in a given host (Esch & Fernandez, 1994; Esch *et al.*, 2002).

On the other hand, Poulin (1998b) suggested that finding a parasite in various hosts does not mean that they all constitute equally suitable hosts for the species, but rather that host suitability should be gauged based on the reproductive rates of the parasite in each and the relative abundance of each in the hosts. He also defined

the specificity of a parasite as the degree of restriction on the number of host species used at a particular stage of its life cycle (Poulin, 1998b).

Local studies made in recent years have contributed to the knowledge of the organization of helminth communities in different amphibian hosts (e.g. *Pseudis limellum*, *Leptodactylus chaquensis*, *Leptodactylus latinasus* and *Scinax nasicus*). These studies indicate that the presence or absence of helminth parasites in a host responds to various ecological factors, such as abiotic and biotic characteristics of the habitat (Kehr *et al.*, 2000; Hamann

Table 3. Survey of common and rare larval trematodes found in amphibians from Corrientes Province, Argentina. Prevalence (%), mean intensity (MI)/standard deviation (SD), and total number (*n*) of parasites.

Trematodes	Hosts	%	MI/SD	<i>n</i>
<b>Common larval species</b>				
<i>Travtrema</i> aff. <i>stenocotyle</i>	<i>Scinax nasicus</i> <sup>e</sup>	30	12.7 (1–101)*	190
	<i>Leptodactylus bufonius</i> <sup>b</sup>	15	12.0/12.7	60
	<i>Odontophrynus americanus</i> <sup>b</sup>	26	10.4/10.5	52
	<i>Rhinella fernandezae</i> <sup>b</sup>	10	3.0/1.0	6
	<i>Physalaemus santafecinus</i> <sup>b</sup>	9	4.6/2.2	19
	<i>Pseudopaludicola boliviana</i> <sup>b</sup>	8	2.5/1.6	42
	<i>Leptodactylus chaquensis</i> <sup>d</sup>	28	3.7/4.7	41
	<i>Leptodactylus latinasus</i> <sup>c</sup>	22	16.1/13.7	129
	<i>S. nasicus</i> <sup>e</sup>	41	11.3 (1–69)*	226
	<i>L. bufonius</i> <sup>b</sup>	6	16.5/5.5	33
<i>Opisthogonimus</i> sp.	<i>O. americanus</i> <sup>b</sup>	5	–	23
	<i>R. fernandezae</i> <sup>b</sup>	5	–	1
	<i>P. santafecinus</i> <sup>b</sup>	9	3.3/0.4	13
	<i>P. boliviana</i> <sup>b</sup>	8	2.3/1.6	14
	<i>L. chaquensis</i> <sup>d</sup>	25	3.1/2.3	19
	<i>L. latinasus</i> <sup>c</sup>	8	8.7/3.1	26
	<i>S. nasicus</i> <sup>e</sup>	16	2.9 (1–10)*	23
	<i>Leptodactylus latrans</i>	9	–	2
	<i>L. bufonius</i> <sup>b</sup>	3	–	1
	<i>O. americanus</i> <sup>b</sup>	11	2.0/0.0	4
<i>Styphlodora</i> sp.	<i>R. fernandezae</i> <sup>b</sup>	10	5.0/2.0	10
	<i>L. chaquensis</i> <sup>d</sup>	25	8.7/10.8	85
	<i>L. latinasus</i> <sup>c</sup>	8	1.3/0.5	4
	<i>S. nasicus</i> <sup>e</sup>	20	26.5 (1–110)*	265
	<i>L. bufonius</i> <sup>b</sup>	9	9.0/9.9	27
	<i>L. latrans</i>	9	–	260
	<i>O. americanus</i> <sup>b</sup>	26	12.0/11.0	60
	<i>R. fernandezae</i> <sup>b</sup>	40	4.9/2.6	39
	<i>P. santafecinus</i> <sup>b</sup>	4	1j1.3/6.9	34
	<i>P. boliviana</i> <sup>b</sup>	44	4.5/5.7	139
<i>Bursotrema tetracotyloides</i>	<i>L. chaquensis</i> <sup>d</sup>	75	110.7/354.7	3323
	<i>L. latinasus</i> <sup>c</sup>	19	22.6/18.5	158
	<i>S. nasicus</i> <sup>e</sup>	2	(1)*	1
	<i>L. chaquensis</i> <sup>d</sup>	13	3.4/3.4	17
	<i>L. latinasus</i> <sup>c</sup>	3	–	1
	<i>S. nasicus</i> <sup>e</sup>	2	(1)*	1
	<i>P. boliviana</i> <sup>b</sup>	1	–	1
	<i>S. nasicus</i> <sup>e</sup>	6	4.7 (1–9)*	14
	<i>S. nasicus</i> <sup>e</sup>	24	6.5 (1–22)*	78
	<i>Leptodactylus ocellatus</i> <sup>b</sup>	9	–	29
Unknown echinostomatid sp.	<i>P. santafecinus</i> <sup>b</sup>	9	26.3/5.6	105
	<i>P. boliviana</i> <sup>b</sup>	24	2.5/1.5	57
<b>Rare larval species</b>				
<i>Heterodiplostomum</i> sp.	<i>L. chaquensis</i> <sup>d</sup>	5	1.5/05	3
<i>Petasisger</i> sp.	<i>L. latinasus</i> <sup>c</sup>	6	3.5/1.5	7
Unknown strigeid species #2	<i>L. chaquensis</i> <sup>d</sup>	3	–	13

<sup>a</sup>Infects other hosts, see Hamann & Kehr (1998, 1999). <sup>b</sup>New host record. <sup>c</sup>Data from Hamann *et al.* (2006b). <sup>d</sup>Data from Hamann *et al.* (2006a). <sup>e</sup>Data from Hamann *et al.* (2009). \*Range of intensity.

Table 4. Survey of common and rare adult trematodes found in amphibians from Corrientes Province, Argentina. Prevalence (%), mean intensity (MI)/standard deviation (SD) and total number (n) of parasites.

Trematodes	Hosts	%	MI/SD	n
Common adult species				
<i>Glythelminis repandum</i>	<i>Leptodactylus latinasus</i> <sup>c</sup>	53	2.1/1.3	40
	<i>Leptodactylus chaquensis</i> <sup>d</sup>	33	3.5/3.0	44
	<i>Odonthophrynus americanus</i> <sup>b</sup>	11	1.1/0.0	2
	<i>Physalaemus santafecinus</i> <sup>b</sup>	2	–	2
	<i>Leptodactylus bufonius</i>	42	2.0/3.5	50
<i>Glythelminis palmipedis</i>	<i>Leptodactylus ocellatus</i>	27	1.7/0.9	5
	<i>Leptodactylus ocellatus</i>	27	2.0/1.4	6
	<i>Rhinella fernandezae</i> <sup>b</sup>	5	–	1
<i>Catadiscus inopinatus</i>	<i>Leptodactylus chaquensis</i> <sup>d</sup>	58	2.2/1.9	52
	<i>Scinax nasicus</i> <sup>e</sup>	10	1.4 (1–2)*	7
	<i>Leptodactylus bufonius</i>	18	4.0/3.1	24
	<i>Leptodactylus latrans</i>	73	6.4/7.1	51
	<i>Rhinella fernandezae</i> <sup>b</sup>	20	1.8/0.8	7
	<i>Physalaemus santafecinus</i> <sup>b</sup>	2	–	2
	<i>Pseudopaludicola boliviana</i> <sup>b</sup>	11	2.1/1.3	17
	<i>Leptodactylus chaquensis</i> <sup>d</sup>	55	6.0/8.8	133
	<i>Leptodactylus latinasus</i> <sup>c</sup>	19	4.0/1.9	28
	<i>Leptodactylus latrans</i> <sup>b</sup>	27	6.0/2.9	18
<i>Haematoloechus longiplexus</i>	<i>Rhinella fernandezae</i>	5	–	1
	<i>Pseudopaludicola boliviana</i> <sup>b</sup>	1	–	1
	<i>Leptodactylus chaquensis</i> <sup>d</sup>	35	7.2/6.3	101
	<i>Leptodactylus latinasus</i> <sup>c</sup>	8	4.0/4.2	12
	<i>Leptodactylus chaquensis</i> <sup>d</sup>	3	–	3
	<i>Gorgoderina rochalimai</i> <sup>a</sup>			
	<i>Gorgoderina parvicava</i>			
Rare adult species	<i>Leptodactylus latrans</i>	9	–	3
	<i>Leptodactylus chaquensis</i> <sup>d</sup>	20	3.3/3.5	26
	<i>Pseudopaludicola boliviana</i> <sup>b</sup>	8	1.2/0.4	7
<i>Gorgoderina cryptorchis</i>	<i>Scinax nasicus</i> <sup>e</sup>	2	(2)*	2

<sup>a</sup>Infects other hosts, see Hamann *et al.* (2010). <sup>b</sup>New host record. <sup>c</sup>Data from Hamann *et al.* (2006b).

<sup>d</sup>Data from Hamann *et al.* (2006a). <sup>e</sup>Data from Hamann *et al.* (2009). \*Range of intensity.

*et al.*, 2006a, b, 2010). However, comparative analyses of trematode communities in amphibians with different habitat preferences from the same location are non-existent. The present research was undertaken to test the following hypotheses: (1) the parasite composition of a particular host is determined by its feeding characteristics and the habitat frequented; and (2) locally abundant parasite species can successfully infest a wide range of amphibian hosts. These premises are tested by analysing the specific composition of larval and adult trematodes present in the following host amphibians: *Pseudopaludicola boliviana*, *Physalaemus santafecinus*, *Rhinella fernandezae*, *Leptodactylus bufonius*, *Leptodactylus latrans* and *Odonthophrynus americanus*. This study also considers previously published information from the same geographical area about *L. chaquensis*, *L. latinasus* and *S. nasicus* (Hamann *et al.*, 2006a, b, 2009).

Amphibian hosts have different feeding characteristics (e.g. strategies and preferred food items). The diet of *S. nasicus* is dominated by insects, and it employs a sit-and-wait foraging strategy; *L. chaquensis*, *L. latrans*, *L. latinasus* and *L. bufonius* are generalist predators, using an intermediate strategy between active foraging and sit and wait predation; *R. fernandezae* has a diet intermediate between that of a generalist and a specialist, and is an actively foraging predator; *P. santafecinus* has a specialist diet, with active foraging; *O. americanus* and

*P. boliviana* are generalist predators (Duré, 2004; Duré *et al.*, 2004; Schaefer *et al.*, 2006).

The main objectives of this study were: (1) to determine mean species richness and diversity of trematode parasite communities; (2) to evaluate the distribution of parasites in nine amphibian species; and (3) to examine whether the feeding and habitat-use strategies of amphibians are important determinants of trematode species richness.

## 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 area was characterized by its variety of habitats, containing numerous temporary, semi-permanent and permanent ponds. A permanent pond sampled in this study (30 m long, 15 m wide and 0.8 m deep) presented vegetation consisting of: *Eichhornia azurea*, *Nymphaea* sp., *Hydrocleis nymphoides*, *Salvinia* sp. and *Ludwigia peploides*; the pond was surrounded by grasslands (*Andropogon lateralis*). Plant species in the forest sampled in this study consisted of quebracho (*Schinopsis balansae*), urunday (*Astronium balansae*) and ñandubay (*Prosopis affinis*), with a herbaceous stratum comprising patches of gramineous

Table 5. Summary of main results of the rarefaction method; (A) the number of individuals (*n*) was unified to obtain values of mean diversity; (B) probabilities of Bonferroni procedure were posteriorly analysed for nine amphibian species.

<i>n</i>	Lch	Oa	Pb	Lb	Rf	Ll	Lo	Sn	Ps
(A)									
5	0.34 ± 0.35	0.86 ± 0.26	0.92 ± 0.32	1.09 ± 0.26	0.79 ± 0.34	1.09 ± 0.25	0.63 ± 0.38	1.02 ± 0.27	0.78 ± 0.35
10	0.44 ± 0.31	1.01 ± 0.19	1.14 ± 0.26	1.34 ± 0.18	0.99 ± 0.29	1.33 ± 0.19	0.80 ± 0.33	1.25 ± 0.20	0.95 ± 0.26
15	0.48 ± 0.28	1.07 ± 0.16	1.22 ± 0.22	1.42 ± 0.14	1.09 ± 0.23	1.42 ± 0.14	0.86 ± 0.27	1.33 ± 0.17	1.02 ± 0.22
20	0.51 ± 0.26	1.10 ± 0.13	1.27 ± 0.20	1.46 ± 0.11	1.14 ± 0.20	1.46 ± 0.11	0.92 ± 0.24	1.37 ± 0.15	1.05 ± 0.19
25	0.53 ± 0.25	1.11 ± 0.12	1.28 ± 0.17	1.48 ± 0.09	1.15 ± 0.17	1.49 ± 0.09	0.93 ± 0.22	1.39 ± 0.14	1.07 ± 0.17
30	0.55 ± 0.23	1.13 ± 0.11	1.31 ± 0.16	1.51 ± 0.08	1.18 ± 0.15	1.50 ± 0.08	0.95 ± 0.20	1.42 ± 0.13	1.09 ± 0.16
35	0.56 ± 0.21	1.14 ± 0.10	1.33 ± 0.14	1.51 ± 0.07	1.20 ± 0.13	1.51 ± 0.07	0.97 ± 0.19	1.43 ± 0.12	1.10 ± 0.14
40	0.57 ± 0.21	1.15 ± 0.09	1.34 ± 0.14	1.53 ± 0.06	1.22 ± 0.11	1.52 ± 0.07	0.97 ± 0.18	1.44 ± 0.11	1.12 ± 0.13
45	0.59 ± 0.20	1.15 ± 0.08	1.34 ± 0.13	1.53 ± 0.06	1.22 ± 0.09	1.53 ± 0.06	0.99 ± 0.16	1.45 ± 0.11	1.11 ± 0.12
50	0.59 ± 0.18	1.16 ± 0.08	1.35 ± 0.12	1.54 ± 0.06	1.23 ± 0.07	1.53 ± 0.06	0.99 ± 0.15	1.47 ± 0.10	1.13 ± 0.11
55	0.60 ± 0.18	1.16 ± 0.07	1.36 ± 0.11	1.53 ± 0.05	1.24 ± 0.06	1.54 ± 0.05	1.01 ± 0.15	1.47 ± 0.10	1.12 ± 0.11
60	0.61 ± 0.17	1.17 ± 0.07	1.36 ± 0.11	1.54 ± 0.05	1.24 ± 0.04	1.54 ± 0.05	1.01 ± 0.14	1.48 ± 0.09	1.13 ± 0.10
65	0.61 ± 0.16	1.17 ± 0.07	1.37 ± 0.10	1.54 ± 0.05	1.25 ± 0.00	1.54 ± 0.05	1.00 ± 0.13	1.47 ± 0.09	1.14 ± 0.09

	Lch	Oa	Pb	Lb	Rf	Ll	Lo	Sn	Ps
(B)									
Lch	0								
Oa	-	0							
Pb	*	-	0						
Lb	*	*	-	0					
Rf	*	-	-	-	0				
Ll	*	*	-	-	-	0			
Lo	-	-	*	*	-	*	0		
Sn	*	-	-	-	-	-	*	0	
Ps	-	-	-	*	-	*	-	*	0

\**P* = 0.0014. Lch, *Leptodactylus chaquensis*; Lo, *Leptodactylus latrans*; Ps, *Physalaemus santafecinus*; Pb, *Pseudopaludicola boliviana*; Ll, *Leptodactylus latinasus*; Lb, *Leptodactylus bufonius*; Oa, *Odontophrynus americanus*; Rf, *Rhinella fernandezae*; Sn, *Scinax nasicus*.

plants (*Elyonurus muticus*) and terrestrial bromeliads (*Aechmea distichantha*, *Bromelia serra*). Mean annual temperature for the area is 23°C and the mean annual precipitation is 1500 mm, without a well-defined dry season, albeit with periods of rain shortage occurring every 4–6 years (Carnevali, 1994).

#### Collection and examination of amphibians

Adult amphibians (*n* = 325 specimens), comprising representatives of five families (Leptodactylidae: *L. latinasus*, *L. chaquensis*, *L. latrans* and *L. bufonius*; Leiuperidae: *P. santafecinus* and *P. boliviana*; Hylidae: *S. nasicus*; Bufonidae: *R. fernandezae* and Cycloramphidae: *O. americanus*), were collected between April 2001 and December 2006 (table 1). Specimens were hand-captured between 18.00 hours and 21.00 hours, using the sampling technique defined as 'visual encounters survey' (Crump & Scott, 1994). Frogs were transported live to the laboratory, killed in a chloroform (CHCl<sub>3</sub>) solution, and then dissected following standard protocols (Goater & Goater, 2001). The snout–vent length (SVL) and body weight of each specimen were recorded. During necropsy hosts were sexed and their oesophagus, stomach, gut, lungs, liver, urinary bladder, kidneys, body cavity,

musculature, skin and brain examined for parasites. Trematode parasites were observed *in vivo*, counted and subsequently killed in hot distilled water and preserved in 70% ethyl alcohol. Digeneans were stained with hydrochloric carmine, cleared in creosote and mounted in Canada balsam, and identified taxonomically using Yamaguti (1971, 1975), Gibson *et al.* (2002), and Jones *et al.* (2005). Parasite specimens were deposited in the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL), Corrientes, Corrientes Province, Argentina [accession numbers CECOAL, 04012008, 03051608, 04062502, 06030604, 05022410, 03061301, 03032704, 03092404, *Travtrema* aff. *stenocotyle* Cohn, 1902; 04092904, 06022413, 02082201, 06030604, 05062918, 03061302, 03012904, 03022001, *Opisthogonimus* sp.; 04012003, 03070401, 06030607, 04090601, 04062503, 03051605, 05022430, *Styphlodora* sp.; 03120202, 06022411, 03070401, 04062502, 05022401, 06030615, 03061301, 03012904, 0309240, *Bursotrema tetracotylodes* Szidat, 1960; 04121324, 03052702, Unknown strigeid species #1; 04121318, *Nephrostomum* sp.; 04012014, Unknown echinostomatid sp.; 04121324, *Lophosicyadiplostomum* aff. *nephrocystis* (Lutz, 1928); 03091002, *Petasisger* sp.; 03042807, *Heterodiplostomum* sp.; 03092406, 03012904, 03061304, 02103022, 03051608, 01082294, *Glythelmins repandum* (Rudolphi, 1819); 01102346, 02042629, 03111804, *Glythelmins palmipedis* (Lutz, 1928); 04121330, 06022414, 03070401, 05022407, 02103022, 03070405, 02091101, 03092404, *Catadiscus inopinatus* Freitas, 1841; 03070401, 02042629, 03070415, 03111804, 03042402, *Haematoloechus longiplexus* Stafford, 1902; 03032704, *Gorgoderina rochalimai* Pereira & Cuocolo, 1940; 03070401, 03070402, *Gorgoderina parvicava* Travassos, 1922; 03070422, *Gorgoderina cryptorchis* Travassos, 1924; 04092904, *Glythelmins* sp.

#### Statistical analysis

Helminth communities were classified at two levels: infracommunity level (i.e. all trematode infrapopulations within a single host), and component community level (i.e. all trematode infracommunities within a population of a host species) (Esch & Fernandez, 1993). Prevalence, intensity and abundance of infection were calculated for



Table 6. Summary of main results of the rarefaction method; (A) the number of individuals ( $n$ ) was unified to obtain values of mean richness; (B) probabilities of Bonferroni procedure were posteriorly analysed for nine amphibian species.

$n$	Oa	Pb	Lb	Lch	Rf	Ll	Lo	Sn	Ps
(A)									
5	2.66 ± 0.63	2.92 ± 0.79	3.27 ± 0.76	1.70 ± 0.73	2.65 ± 0.85	3.08 ± 0.83	2.23 ± 0.79	3.07 ± 0.73	2.50 ± 0.79
10	3.23 ± 0.65	3.92 ± 0.89	4.35 ± 0.70	2.28 ± 0.99	3.61 ± 0.89	4.21 ± 0.98	3.12 ± 0.93	4.05 ± 0.80	3.35 ± 0.81
15	3.49 ± 0.66	4.49 ± 0.91	4.78 ± 0.54	2.78 ± 1.11	4.26 ± 0.89	4.95 ± 1.03	3.75 ± 0.92	4.56 ± 0.85	3.83 ± 0.77
20	3.72 ± 0.71	4.91 ± 0.88	4.97 ± 0.44	3.25 ± 1.19	4.71 ± 0.87	5.38 ± 1.02	4.19 ± 0.92	4.86 ± 0.88	4.14 ± 0.71
25	3.86 ± 0.68	5.17 ± 0.87	5.07 ± 0.41	3.77 ± 1.23	5.12 ± 0.85	5.77 ± 0.96	4.44 ± 0.95	5.09 ± 0.88	4.38 ± 0.71
30	3.99 ± 0.67	5.41 ± 0.81	5.15 ± 0.41	4.23 ± 1.26	5.32 ± 0.86	6.09 ± 0.95	4.83 ± 0.99	5.34 ± 0.93	4.52 ± 0.70
35	4.14 ± 0.67	5.61 ± 0.82	5.15 ± 0.37	4.54 ± 1.36	5.59 ± 0.84	6.33 ± 0.94	5.04 ± 0.96	5.54 ± 0.94	4.70 ± 0.72
40	4.23 ± 0.67	5.80 ± 0.76	5.16 ± 0.37	4.92 ± 1.30	5.86 ± 0.81	6.55 ± 0.91	5.29 ± 0.96	5.68 ± 0.91	4.77 ± 0.70
45	4.33 ± 0.64	5.91 ± 0.79	5.22 ± 0.42	5.25 ± 1.35	6.04 ± 0.80	6.74 ± 0.90	5.43 ± 0.99	5.82 ± 0.91	4.89 ± 0.70
50	4.41 ± 0.63	6.03 ± 0.77	5.26 ± 0.44	5.51 ± 1.41	6.36 ± 0.69	6.87 ± 0.90	5.69 ± 0.97	5.93 ± 0.91	4.97 ± 0.71
55	4.50 ± 0.60	6.11 ± 0.73	5.27 ± 0.45	5.75 ± 1.34	6.55 ± 0.62	7.06 ± 0.85	5.76 ± 1.00	6.10 ± 0.91	5.05 ± 0.70
60	4.56 ± 0.55	6.21 ± 0.70	5.30 ± 0.46	5.97 ± 1.37	6.77 ± 0.47	7.14 ± 0.84	5.89 ± 0.97	6.21 ± 0.90	5.15 ± 0.71
65	4.63 ± 0.52	6.26 ± 0.69	5.32 ± 0.47	6.32 ± 1.38	7.00 ± 0.00	7.22 ± 0.84	5.99 ± 0.96	6.37 ± 0.94	5.18 ± 0.70

$n$	Oa	Pb	Lb	Lch	Rf	Ll	Lo	Sn	Ps
(B)									
Oa	0								
Pb	*	0							
Lb	-	-	0						
Lch	-	-	-	0					
Rf	*	-	-	-	0				
Ll	*	-	-	-	-	0			
Lo	-	-	-	-	-	-	0		
Sn	*	-	-	-	-	-	-	0	
Ps	-	-	-	-	-	*	-	-	0

\* $P = 0.0014$ . Lch, *Leptodactylus chaquensis*; Lo, *Leptodactylus latrans*; Ps, *Physalaemus santafecinus*; Pb, *Pseudopaludicola boliviana*; Ll, *Leptodactylus latinasus*; Lb, *Leptodactylus bufonius*; Oa, *Odontophrynus americanus*; Rf, *Rhinella fernandezae*; Sn, *Scinax nasicus*.

trematode parasites following Bush *et al.* (1997). The measures of community richness and diversity employed included: the total number of helminth species (= species richness), Shannon index ( $H'$ ), and evenness ( $J'$ ) as  $H'/H'$  maximum (Zar, 2010). The Brillouin index ( $BH$ ) and evenness ( $E$ ) were used to compare trematode infra-communities among amphibian species. The diversity indices were used with decimal logarithms ( $\log_{10}$ ). Mean trematode species richness is the sum of trematode species per individual frog, divided by the total sample size. The Kruskal–Wallis test was used to compare diversity and richness of trematodes between hosts, and among hosts' habitat preferences. The probabilities were calculated according to the Bonferroni procedure, because it provides great control over Type I error. Since the sample sizes were different we used rarefaction methods for comparing the mean diversity and the mean richness. 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 1000 times to generate a mean and a variance of species diversity and richness. For this calculation, the

software used was EcoSim 7.7 (Gotelli & Entsminger, 2004). In this analysis the diversity indices used natural logarithms ( $\ln$ ).

The most dominant species were determined using the Berger–Parker index of dominance ( $d$ ; Magurran, 2004). The trematode species data were classified into common and rare categories. Those parasites that had been recorded as infecting more than one family of amphibians were considered as common; while those that had been recorded only from species within a single family of amphibians were considered as rare. To examine the distribution of trematode species through the host specificity, a regression analysis between prevalence and mean abundance (as a measure of parasite abundance) was applied across all hosts in which it occurred (as a measure of host specificity). This analysis was performed for parasite species for which at least three hosts were recorded. Regressions between these parasitological descriptors were performed using EcoSim 7 software. This process was repeated 1000 times to generate a standard linear regression for the data, and then randomization was used to test the null hypothesis that the slope, intercept or correlation coefficient equals 0.0 through a low sample number. To determine the qualitative similarity between component communities Jaccard's coefficient similarity index was used and a Cluster Analysis was then performed using the UPGMA (Unweighted Pair Group Method Average) method. Analyses were performed using the software packages Xlstat 7.5 (Addinsoft, 2004) and Bio Dap (Tomas & Clay, 2008).

To study the richness of trematode species associated with the hosts' habitat preferences, amphibian hosts were classified as fossorial, terrestrial, semi-aquatic and arboreal, according to their use of these habitats, following the classification proposed by Aho (1990). This characteristic was then linked to the variations in trematode communities present in each amphibian group, also taking into account that all amphibians return to water bodies for reproduction at some time during the year, with the time of permanence in the aquatic environment depending on the species' reproductive mode and pattern of reproductive activity. Accordingly,

Table 7. Ecological indices of trematode communities in nine amphibians from Corrientes Province, Argentina.

	Lch	Lo	Ps	Pb	Ll	Lb	Oa	Rf	Sn
<b>Larval and adult trematodes</b>									
Component community									
Richness	13	8	6	8	9	6	5	7	10
Diversity ( $H'$ )	0.30	0.46	0.50	0.61	0.68	0.68	0.52	0.54	0.67
Equitability ( $J'$ )	0.27	0.51	0.65	0.68	0.71	0.88	0.74	0.64	0.67
Diversity ( $HB$ )	0.30	0.44	0.48	0.59	0.66	0.66	0.49	0.48	0.66
Equitability ( $E$ )	0.27	0.50	0.64	0.68	0.71	0.88	0.74	0.63	0.67
Dominance ( $d$ )	0.84	0.70	0.69	0.50	0.39	0.31	0.68	0.60	0.33
Identification of dominant species	B	B	E	B	B	T	B	B	B
Infracommunity									
Mean richness	3.92	2.33	1.70	1.72	1.83	1.55	1.50	1.36	2.18
± SD	1.66	1.49	0.78	0.82	1.29	0.67	0.50	0.61	1.27
Mean diversity ( $HB$ )	0.26	0.14	0.13	0.11	0.10	0.09	0.06	0.06	0.16
± SD	0.16	0.11	0.14	0.11	0.14	0.10	0.07	0.11	0.16
Mean equitability ( $E$ )	0.54	0.43	0.47	0.43	0.27	0.34	0.29	0.26	0.43
± SD	0.32	0.33	0.48	0.44	0.33	0.39	0.35	0.42	0.42
<b>Larval trematodes</b>									
Component community									
Richness	7	3	4	5	6	4	4	4	8
Dominance ( $d$ )	0.95	0.89	0.61	0.55	0.49	0.50	0.43	0.70	0.33
Identification of dominant species	B	B	E	B	B	T	B	B	B
<b>Adult trematodes</b>									
Component community									
Richness	6	5	2	3	3	2	1	3	2
Dominance ( $d$ )	0.51	0.61	0.50	0.68	0.50	0.68	1.00	0.68	0.78
Identification of dominant species	C	C	C	C	G	G	G	C	C

Lch, *Leptodactylus chaquensis*; Lo, *Leptodactylus latrans*; Ps, *Physalaemus santafecinus*; Pb, *Pseudopaludicola boliviiana*; Ll, *Leptodactylus latinasus*; Lb, *Leptodactylus bufonius*; Oa, *Odontophrynus americanus*; Rf, *Rhinella fernandezae*; Sn, *Scinax nasicus*. E, unknown echinostomatid sp.; B, *Bursotrema tetracotylodes*; T, *Travtrema* aff. *stenocotyle*; C, *Catadiscus inopinatus*; G, *Glythelminis repandum*. Diversity data obtained with  $\log_{10}$ .

amphibians were grouped as: those frogs that live and breed in burrows, considered as typical fossorial species (*L. latinasus* and *L. bufonius*); those amphibians that live in burrows but come back to the water for reproduction, considered as terrestrial (*R. fernandezae* and *O. americanus*); those frogs that live strongly associated with the aquatic environment, considered semi-aquatic (*P. boliviiana*, *P. santafecinus*, *L. latrans* and *L. chaquensis*); and those frogs that live on small trees and shrubs, considered as arboreal species (*S. nasicus*) (table 2).

## Results

### Trematode fauna

Nineteen trematode species were found in representatives from five anuran families. The predominant groups of parasites were the metacercariae (*Opisthogonimus* sp., *Styphlodora* sp., *Travtrema* aff. *stenocotyle*, an unknown echinostomatid sp., *Nephrostomum* sp., *Petasisiger* sp., *Bursotrema tetracotylodes*, *Lophosicyadiplostomum* aff. *nephrocystis*, unknown strigeid sp. #1, unknown strigeid sp. #2 and *Heterodiplostomum* sp.) (table 3) compared to the adult digeneans (*Glythelminis repandum*, *Glythelminis palmipedis*, *Glythelminis* sp., *Catadiscus inopinatus*, *Haematoleechus longiplexus*, *Gorgoderina parvicava*, *Gorgoderina cryptorchis* and *Gorgoderina rochalimai*) (table 4). Of all individuals ( $n = 6300$ ) found in the amphibian hosts, 90% of them corresponded to metacercariae.

The trematode fauna were dominated by common species, with only five rare species (*Petasisiger* sp.,

unknown strigeid sp. #2, *Heterodiplostomum* sp., *G. parvicava*, *G. cryptorchis*) found in Leptodactylidae (*L. chaquensis*, *L. latinasus*, *L. latrans*) and Leiuperidae (*P. boliviiana*) frogs. Additionally, *G. rochalimai* and *L. aff. nephrocystis* may be considered as common species, as they have been found in other amphibians; namely *L. aff. nephrocystis* in *P. limellum*, from another area (table 3), and *G. rochalimai* in *S. nasicus* from the same study area, but during a different period (table 4).

### Comparison of infection between the nine hosts

Using a rarefaction method, the number of individuals was combined and the values of mean diversity (table 5A) and mean richness (table 6A) for each of the hosts were obtained. Mean species diversity of the trematodes between the nine hosts were different (Kruskal–Wallis K-test = 94.16;  $P = 0.0001$ ;  $df = 8$ ;  $n = 13$ ). Mean species richness of the trematodes between the nine hosts were also different (Kruskal–Wallis K-test = 32.72;  $P = 0.0001$ ;  $df = 8$ ;  $n = 13$ ). A comparison of diversity (table 5B) and richness (table 6B) between nine amphibians showed significant differences ( $P = 0.0014$ ) for some of them.

The diversity of the trematodes tended to be greatest in *L. latinasus* followed by *L. bufonius*, while *L. chaquensis* presented the lowest value (table 7). The host exhibiting the highest trematode richness ( $n = 13$ ) was *L. chaquensis*. The dominant species corresponded to *B. tetracotylodes* for most of the hosts (78%) followed by the unknown echinostomatid sp. and *Travtrema* aff. *stenocotyle*. On the

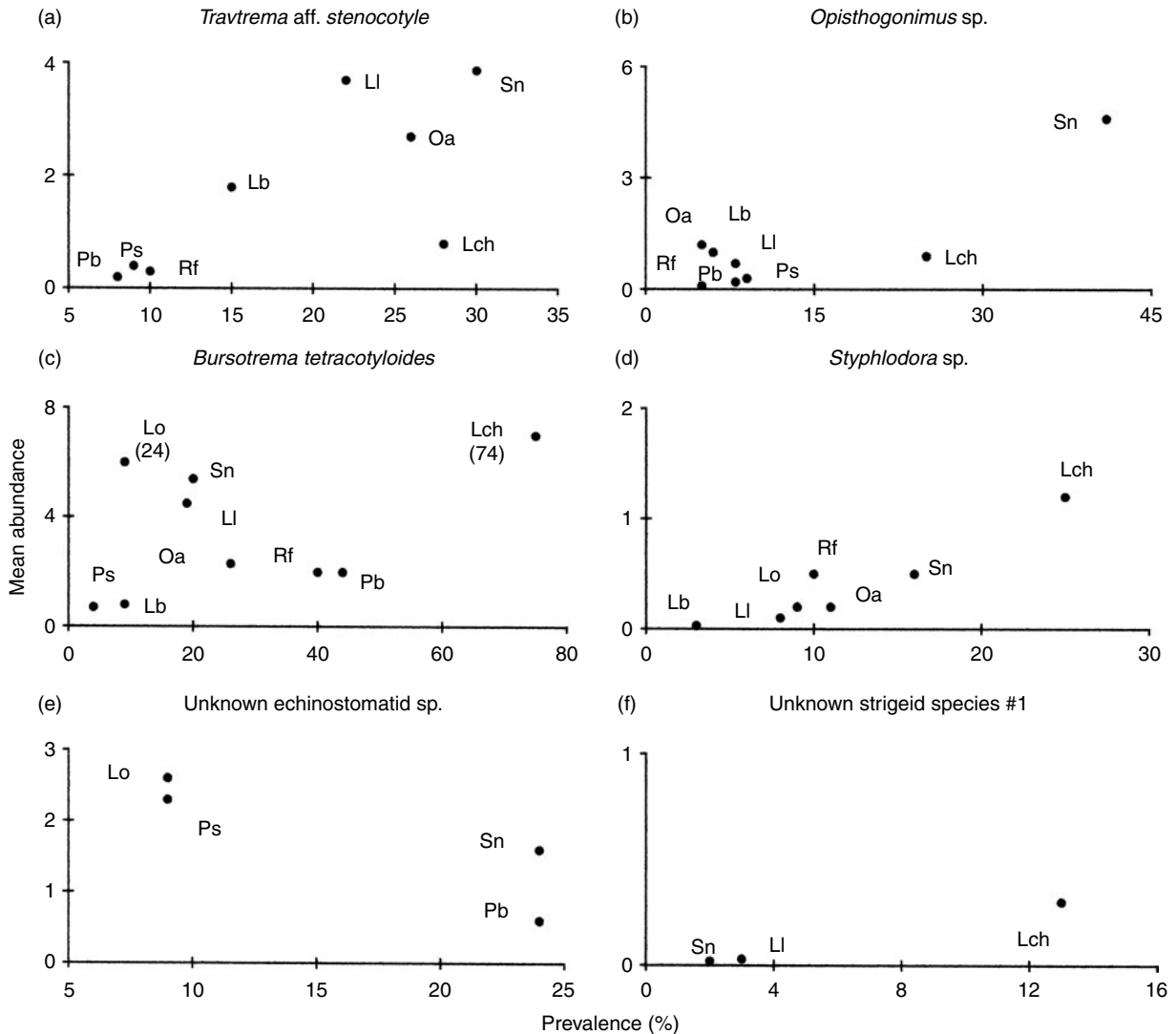


Fig. 1. Relationship between prevalence and mean abundance of common metacercariae, among host species. Pb, *Pseudopaludicola boliviana*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ll, *Leptodactylus latinasus*; Lch, *Leptodactylus chaquensis*; Rf, *Rhinella fernandezae*; Lb, *Leptodactylus bufonius*; Oa, *Odontophrynus americanus*; Lo, *Leptodactylus latrans*.

other hand, when only adult trematodes were analysed, the dominant species in most hosts (67%) was *C. inopinatus* followed by *G. repandum*.

Considering trematode infracommunities, the mean Brillouin's index and mean richness generally showed low values but tended to be comparatively greater in *L. chaquensis* (mean diversity =  $0.26 \pm 0.16$  and mean richness =  $3.92 \pm 1.66$ ).

#### Trematode distribution across specific hosts

Regressions between mean abundance and prevalence of metacercariae (as a measure of parasite abundance) among host species used by a parasite (as a measure of host specificity) revealed significant positive relationships for *Travtrema* aff. *stenocotyle* ( $r = 0.73$ ,  $P < 0.05$ , fig. 1a), *Opisthagonimus* sp. ( $r = 0.86$ ,  $P < 0.05$ , fig. 1b) and

*Styphlodora* sp. ( $r = 0.94$ ,  $P < 0.05$ , fig. 1d), but this relationships was non-significant for *B. tetracotyloides* ( $r = 0.70$ ,  $P > 0.05$ , fig. 1c) and unknown strigeid sp. #1 ( $r = 0.99$ ,  $P > 0.05$ , fig. 1f). The unknown echinostomatid sp. showed a non-significant negative correlation ( $r = -0.88$ ,  $P > 0.05$ , fig. 1e). Regressions between both descriptors of adult trematodes were all positively correlated but not always statistically significant (*G. palmipedis*:  $r = 0.04$ ,  $P > 0.05$ , fig. 2a; *H. longiplexus*:  $r = 0.99$ ,  $P < 0.05$ , fig. 2b; *G. repandum*:  $r = 0.89$ ,  $P < 0.05$ , fig. 2c; *C. inopinatus*:  $r = 0.56$ ,  $P > 0.05$ , fig. 2d).

#### Similarity analyses among host species

A cluster analysis based on the Jaccard's coefficient grouped the *R. fernandezae*, *O. americanus* and *L. bufonius* with highest similarity (1.00) followed by *P. boliviana* and



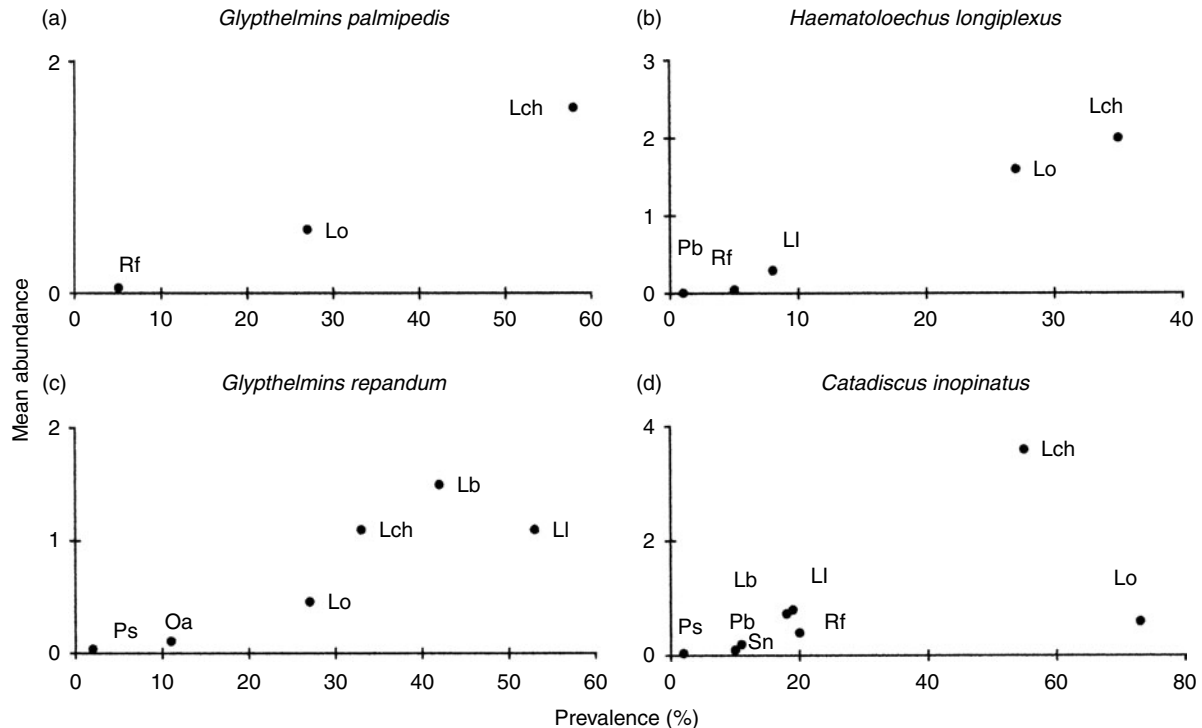


Fig. 2. Relationship between prevalence and mean abundance of adult common trematodes, among host species. Pb, *Pseudopaludicola boliviana*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; LI, *Leptodactylus latinasus*; Lch, *Leptodactylus chaquensis*; Rf, *Rhinella fernandae*; Lb, *Leptodactylus bufonius*; Oa, *Odontophrynus americanus*; Lo, *Leptodactylus latrans*.

*P. santafecinus* (0.80) in terms of metacercariae species composition (fig. 3a), while *L. bufonius* and *P. santafecinus* showed greater similarity (1.00) followed by *L. latrans* and *L. chaquensis* (0.83) in terms of species composition of adult digeneans (fig. 3b).

#### Richness of trematode community and host's habitats

Using a rarefaction method, the number of individuals was combined and the values of mean richness of trematodes for each of the localities were obtained (table 8A, C, E). Mean species richness of the trematodes between the four environments were different (adult: Kruskal–Wallis  $K$ -test = 9.83,  $P$  = 0.02,  $df$  = 3; larval:  $K$  = 21.28,  $P$  = 0.001,  $df$  = 3; larval + adult:  $K$  = 8.34,  $P$  = 0.03,  $df$  = 3). Adult trematode communities showed a greater value in semi-aquatic amphibians than in terrestrial, arboreal and fossorial amphibians (fig. 4a), nevertheless, a significant difference ( $P$  = 0.0083) was only observed between semi-aquatic and arboreal amphibians (table 8B). The larval trematode trend was different, with a higher species richness in arboreal amphibians (fig. 4a), where there was a significant difference ( $P$  = 0.0083) between this group and terrestrial and fossorial hosts (table 8D). Larval and adult trematodes showed no significant differences between host and habitat preferences (table 8F).

Trematode infracommunities between the four habitats were different (adult: Kruskal–Wallis  $K$ -test = 35.12,  $df$  3,  $P$  < 0.001; larval:  $K$  = 8.49,  $df$  3,  $P$  < 0.05; larval + adult:

$K$  = 20.55,  $df$  3,  $P$  < 0.001). The results revealed similar trends to those reported at component community levels (fig. 4b).

#### Discussion

Trematode parasites of amphibians from the area studied comprised an assemblage of 19 species. Considering the component community, species richness varied between 5 and 13 species of trematodes, whereas the infracommunity richness was no more than 4 species of trematodes per infected host. Larval trematodes had the highest species richness; among them, the metacercaria of *B. tetracotyloides* was dominant in the parasite component community of the amphibians studied. The high occurrence of these larvae would suggest that these amphibians are the principal intermediate hosts in the trematode's life cycle. The life cycles of the other metacercariae found in this study are also complex and require generally more than one intermediate host (table 9), so the presence of these larval stages is a direct indicator of the existence of several different definitive hosts (Hamann *et al.*, 2010).

On the other hand, *C. inopinatus* was the dominant adult trematode species in the component communities. This parasite employs a different strategy of infection, in which cercariae encyst on the skin of frogs and tadpoles, or on aquatic plants (Smyth & Smyth, 1980). Plants are always available as vectors of infection and the cercariae

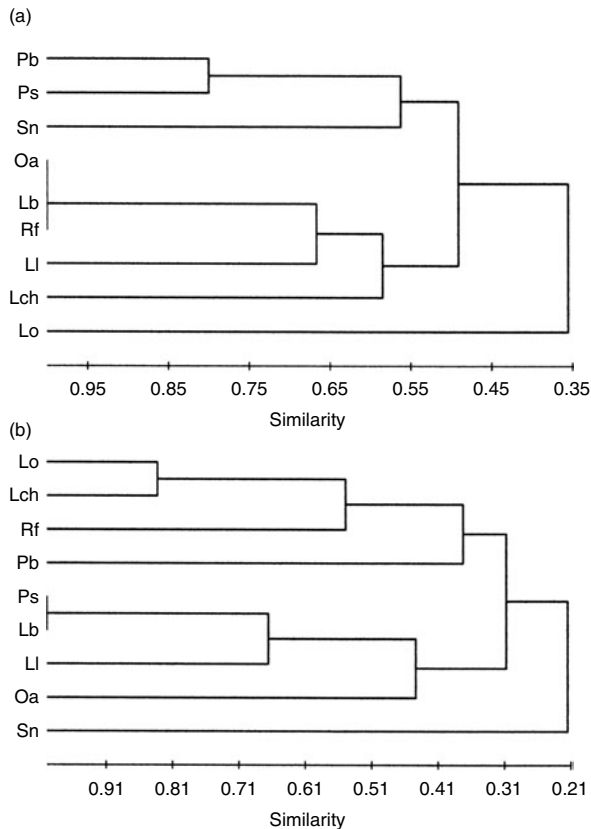


Fig. 3. Similarity among nine species of amphibians with respect to their component communities of larval (a) and adult (b) trematodes. Pb, *Pseudopaludicola boliviana*; Ps, *Physalaemus santafecinus*; Sn, *Scinax nasicus*; Ll, *Leptodactylus latinasus*; Lch, *Leptodactylus chaquensis*; Rf, *Rhinella fernandezae*; Lb, *Leptodactylus bufonius*; Oa, *Odontophrynus americanus*; Lo, *Leptodactylus latrans*.

emerge at any hour of the day or night (i.e. arrhythmic emergences; Bouix-Busson *et al.*, 1985), possibly explaining its dominance.

At the infracommunities level, amphibian species had low values for diversity and species richness of trematodes. The depauperate nature of these communities was also shown by other studies in temperate (Goater *et al.*, 1987; Aho, 1990; Muzzall, 1991; Bolek & Coggins, 2003; Yoder & Coggins, 2007) and tropical (Luque *et al.*, 2005; Hamann *et al.*, 2006a, b, 2010; Ibrahim, 2008) regions. All of these studies suggest that trematode communities are responsive to host and parasite life histories at particular sites.

Factors related to host susceptibility may explain the distribution of parasites (Bush & Holmes, 1986; Holmes & Price, 1986; Stock & Holmes, 1987; Poulin, 1998a, 1999). By focusing on this relationship and applying it to our dataset, it becomes evident that those species with relatively high prevalences and mean abundances, e.g. *G. repandum*, are characterized by some degree of host specificity. Other species, such as *B. tetracotylodes* and *C. inopinatus*, which do not reach high abundances in their various hosts, are common species. Among the species

with low prevalence and mean abundance, there were some that infected few hosts (e.g. unknown echinostomatid sp.) and others that were characterized by a wide range of hosts (e.g. *Styphlodora* sp.). These results provide evidence of their proximity to the pattern observed in bird parasites, in which parasites are supported by increased resource amplitude, where species with high prevalence and abundance have a wide range of local hosts and establish a positive relationship with them (Poulin, 1998b, 1999). In this sense, generalist parasites exploiting hosts belonging to different amphibian families or orders would require adaptations to counteract a wider range of immune responses, and this would hinder their high abundance in any or all of their hosts. In contrast, specialist parasites that exploit congeneric species in which immune responses are likely to be similar, might have a competitive advantage and reach high abundances in their various hosts (Poulin, 1998b).

Regarding the species composition of larval trematodes, the patterns of similarity among the studied amphibian species showed two groups: the first group consisted of species that occupy terrestrial and fossorial habitats, and the second was a group represented by amphibians that share a semi-aquatic habitat. These results indicate that the composition of larval parasites in both groups is a consequence of the length of time that these hosts remain exposed to the larval infections in an aquatic habitat. Additionally, these infections can occur during the breeding season or in the tadpole stage (Hamann & González, 2009; Hamann *et al.*, 2010). Adult trematodes also showed two similar groups; the first set was represented by amphibians that use fossorial and semi-aquatic habitats, e.g. *P. santafecinus* which is associated for a longer time with flooded grass (table 2); all of these host species were infected by a small number of adult species of trematodes, which are characterized by having encysted larval stages in different substrates (*C. inopinatus*) or infective cercariae to penetrate the host (*Glythelminis* spp.). However, the second group was represented by amphibians that exploit semi-aquatic habitats, and these host species presented the highest values of trematode species richness. Of these, the genera *Haematolechus* and *Gorgoderina* use the larvae of insects (e.g. dragonfly) as second intermediate hosts in their life cycle (Smyth & Smyth, 1980); consequently, infection by these genera was directly related to the hosts' feeding behaviour and diet.

In relation to the importance of the habitat-use made by hosts (Aho, 1990), the richest and most diverse adult trematode community was found in semi-aquatic amphibians, which may indicate that these hosts are exposed to a greater diversity of parasites because they are exposed to their prey, and, hence, to trematode infective stages in both the aquatic and terrestrial environments. These results are consistent with previous findings by other studies of helminth communities in temperate amphibians; where hosts that exhibit a gradient in preference for aquatic habitats show parasitism dominated by adult digeneans (Muzzall, 1991; McAlpine, 1997). Similar conclusions were reported by Bolek & Coggins (2003) and Yoder & Coggins (2007) who demonstrated that infection by trematodes in semi-aquatic frogs is more abundant than in terrestrial frogs.

Table 8. Summary of main results of the rarefaction method; the number of individuals ( $n$ ) was unified and values of mean richness  $\pm 1$  SD were obtained for adult (A), larval (C) and larval + adult (E) trematodes; probabilities according to Bonferroni procedure were posteriorly analysed for adult (B), larval (D) and larval + adult (F).

(A)				
$n$	Fossorial	Arboreal	Terrestrial	Semi-aquatic
1	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
2	1.55 $\pm$ 0.50	1.40 $\pm$ 0.49	1.59 $\pm$ 0.49	1.72 $\pm$ 0.45
3	1.86 $\pm$ 0.54	1.56 $\pm$ 0.50	2.01 $\pm$ 0.66	2.26 $\pm$ 0.63
4	2.06 $\pm$ 0.58	1.74 $\pm$ 0.44	2.34 $\pm$ 0.74	2.67 $\pm$ 0.72
5	2.21 $\pm$ 0.55	1.84 $\pm$ 0.37	2.63 $\pm$ 0.73	2.98 $\pm$ 0.75
6	2.29 $\pm$ 0.52	1.90 $\pm$ 0.30	2.90 $\pm$ 0.71	3.29 $\pm$ 0.79
7	2.40 $\pm$ 0.53	1.97 $\pm$ 0.17	3.18 $\pm$ 0.67	3.55 $\pm$ 0.81
8	2.49 $\pm$ 0.52	2.00 $\pm$ 0.00	3.39 $\pm$ 0.61	3.76 $\pm$ 0.87
9	2.52 $\pm$ 0.51	2.00 $\pm$ 0.00	3.66 $\pm$ 0.51	3.86 $\pm$ 0.83

(C)				
$n$	Fossorial	Arboreal	Terrestrial	Semi-aquatic
20	3.43 $\pm$ 0.66	4.70 $\pm$ 0.77	3.73 $\pm$ 0.45	2.68 $\pm$ 0.98
40	3.95 $\pm$ 0.75	5.33 $\pm$ 0.74	3.96 $\pm$ 0.20	3.71 $\pm$ 1.02
60	4.27 $\pm$ 0.77	5.67 $\pm$ 0.68	4.00 $\pm$ 0.07	4.38 $\pm$ 1.02
80	4.58 $\pm$ 0.74	5.90 $\pm$ 0.65	4.00 $\pm$ 0.00	4.81 $\pm$ 0.96
100	4.75 $\pm$ 0.69	6.07 $\pm$ 0.61	4.00 $\pm$ 0.00	5.17 $\pm$ 0.96
120	4.96 $\pm$ 0.68	6.18 $\pm$ 0.60	4.00 $\pm$ 0.00	5.45 $\pm$ 0.92
140	5.08 $\pm$ 0.62	6.27 $\pm$ 0.58	4.00 $\pm$ 0.00	5.65 $\pm$ 0.92
160	5.26 $\pm$ 0.63	6.36 $\pm$ 0.61	4.00 $\pm$ 0.00	5.87 $\pm$ 0.89
180	5.32 $\pm$ 0.58	6.39 $\pm$ 0.62	4.00 $\pm$ 0.00	5.94 $\pm$ 0.88
195	5.39 $\pm$ 0.55	6.51 $\pm$ 0.62	4.00 $\pm$ 0.00	6.09 $\pm$ 0.85

(E)				
$n$	Fossorial	Arboreal	Terrestrial	Semi-aquatic
20	5.43 $\pm$ 0.89	4.83 $\pm$ 0.84	4.61 $\pm$ 0.83	4.11 $\pm$ 1.36
40	6.26 $\pm$ 0.88	5.68 $\pm$ 0.88	5.43 $\pm$ 0.84	6.13 $\pm$ 1.39
60	6.79 $\pm$ 0.87	6.23 $\pm$ 0.91	5.94 $\pm$ 0.86	7.31 $\pm$ 1.43
80	7.06 $\pm$ 0.89	6.59 $\pm$ 0.91	6.38 $\pm$ 0.85	8.20 $\pm$ 1.44
100	7.37 $\pm$ 0.80	6.88 $\pm$ 0.87	6.74 $\pm$ 0.82	9.04 $\pm$ 1.30
120	7.58 $\pm$ 0.79	7.16 $\pm$ 0.89	7.01 $\pm$ 0.80	9.46 $\pm$ 1.32
140	7.80 $\pm$ 0.72	7.38 $\pm$ 0.88	7.27 $\pm$ 0.73	9.96 $\pm$ 1.25
160	7.88 $\pm$ 0.71	7.51 $\pm$ 0.86	7.54 $\pm$ 0.62	10.28 $\pm$ 1.21
180	8.04 $\pm$ 0.62	7.68 $\pm$ 0.87	7.74 $\pm$ 0.48	10.61 $\pm$ 1.25
206	8.13 $\pm$ 0.62	7.75 $\pm$ 0.88	8.00 $\pm$ 0.00	10.92 $\pm$ 1.20

(B)				
	F	A	T	S
F	0			
A	-	0		
T	-	-	0	
S	-	*	-	0

(D)				
	F	A	T	S
F	0			
A	*	0		
T	-	*	0	
S	-	-	-	0

(F)				
	F	A	T	S
F	0			
A	-	0		
T	-	-	0	
S	-	-	-	0

\* $P = 0.0083$ .

All of these studies associated these results with a set of variables that are linked to aquatic and terrestrial productivity, e.g. a wider variety of aerial, terrestrial and aquatic invertebrates contributing to a more diverse trematode fauna. Also, other factors determine the biological interactions that occur, such as frog vagility and transmission dynamics of trematodes.

Similarly, studies of larval trematode communities show the same general pattern, with terrestrial hosts being infected with fewer species. This may be related to a short period in water for reproductive activities, i.e. implies a lower probability of penetration by larval trematodes, and so this factor can favour lower values of parasitism. Nevertheless, arboreal amphibians show high larval infections, resulting from acquisition of parasites when the frogs visit the water at different seasons of the year, coinciding with prolonged breeding (see Hamann *et al.*, 2009). Additionally, infections by metacercariae in

all the amphibian species studied can occur throughout ontogenetic development (tadpole, juvenile and adult), and suggests that these stages are suitable secondary intermediate hosts for most of these metacercariae. In fact, this may represent an alternative strategy of the parasites, to ensure transmission, since amphibians occupy an intermediate position in the food web, constituting easy prey for potential definitive hosts, such as snakes, birds and mammals (e.g. the case of *Styphlodora* sp., *Opisthogonimus* sp. and *Travtrema* aff. *stenocotyle*, which infect reptiles as the final host). It may be expected that the metacercariae that infect short-lived hosts (e.g. tadpoles) are obliged to maintain an ability to infect a broad spectrum of hosts to ensure their transmission. These results would suggest that the composition of larval parasites in these host groups is a consequence of local physical and biological characteristics of the habitat (Esch *et al.*, 1990; Marcogliese, 2001).

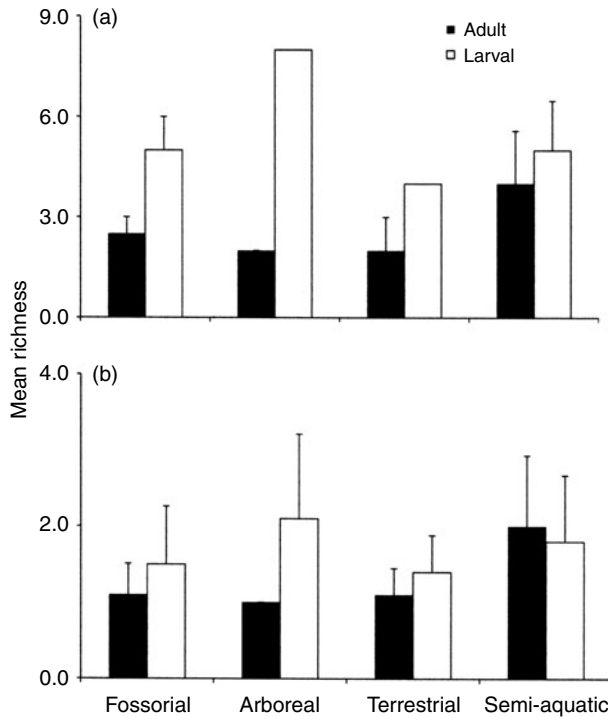


Fig. 4. Comparison of host habitat preferences with the mean richness ( $\pm 1$  SD) of adult and larval trematodes at component community (a) and infracommunity (b) levels.

Aho (1990) states that amphibian hosts with an ambush feeding strategy tend to have fewer parasites, whereas hosts that forage actively show richest and most diverse helminth communities. Therefore, ecological differences in feeding strategies could easily contribute to differences in the complexity of the helminth community within each and between amphibian families. The patterns arising when comparing different amphibian feeding strategies showed that the probability of encounter between parasite and host was higher for those amphibians that actively seek prey and have higher vagility (e.g. *L. chaquensis*) than for those that use a sit-and-wait strategy (e.g. *S. nasicus*). Furthermore, the lowest number of encounters between parasites and hosts take place in amphibians with a specialist diet (e.g. *P. santafecinus*).

The hypothesis that semi-aquatic amphibians harbour a greater richness of adult trematode parasites was supported by this study. In this sense, the habitat characteristics and the feeding strategies of amphibian hosts were important factors for the determination of larval and adult trematode parasite communities. Larval trematode parasites have a wide range of hosts, which allows them to adopt alternative environments in the event that the preferred host is not abundant or inaccessible by ecological circumstances. Also, the lack of specificity for prey (intermediate host) allows those parasites which have complex life cycles to increase the chance of encountering the predator (definitive host of the parasite). Finally, dispersion depends on the mobility of the definitive host, which assists in the spatial dispersion of the propagative parasite forms (e.g. metacercariae, eggs).

Table 9. Summary of trematode life cycles in amphibians from Corrientes Province, Argentina; the first intermediate hosts for the majority of the trematodes are aquatic snails, apart for aquatic clams in the case of the three species of *Gorgoderina*.

Trematodes	Hosts		
	Second intermediate	Definitive	References*
<i>Travtrema</i> aff. <i>stenocotyle</i>	Amphibian	Snake	Ostrowski de Núñez (1979b)
<i>Opisthogonimus</i> sp.	Amphibian	Snake	Grabda-Kazubska (1963)
<i>Styphlodora</i> sp.	Amphibian	Snake	Grabda-Kazubska (1963)
<i>Bursotrema tetracotyloides</i>	Amphibian	Mammal	Yamaguti (1975)
<i>Heterodiplostomum</i> sp.	Amphibian	Snake	Yamaguti (1975)
<i>Lophosicyadiplostomum</i> aff. <i>nephrocystis</i>	Amphibian	Bird	Pearson (1960)
Unknown strigeid species #1	Amphibian	Bird–Mammal <sup>a</sup>	Yamaguti (1975)
Unknown strigeid species #2	Amphibian	Bird–Mammal <sup>a</sup>	Yamaguti (1975)
Unknown echinostomatid sp.	Amphibian	Reptile–Bird–Mammal <sup>a</sup>	Ostrowski de Núñez (1974)
<i>Nephrostomum</i> sp.	Amphibian	Bird	Ostrowski de Núñez (1974)
<i>Petasiger</i> sp.	Fish–Amphibian	Bird	Ostrowski de Núñez <i>et al.</i> (1991)
<i>Haematoloechus longiplexus</i>	Aquatic insect larva	Amphibian	Smyth & Smyth (1980)
<i>Glypthelmins repandum</i>	Amphibian	Amphibian	Leigh (1946), Smyth & Smyth (1980)
<i>Glypthelmins palmipedis</i>	Amphibian	Amphibian	Leigh (1946), Smyth & Smyth (1980)
<i>Glypthelmins</i> sp.	Amphibian	Amphibian	Leigh (1946), Smyth & Smyth (1980)
<i>Catadiscus inopinatus</i>	Aquatic vegetation–Amphibian	Amphibian	Ostrowski de Núñez (1979a)
<i>Gorgoderina parvicava</i>	Aquatic insect larvae–Tadpole	Amphibian	Bolek <i>et al.</i> (2009)
<i>Gorgoderina rochalimai</i>	Aquatic insect larvae–Tadpole	Amphibian	Bolek <i>et al.</i> (2009)
<i>Gorgoderina criptorchis</i>	Aquatic insect larvae–Tadpole	Amphibian	Bolek <i>et al.</i> (2009)

<sup>a</sup>Possible definitive hosts. \* References: life cycles of congener species or species of the same family.



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