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Trophic analysis and parasitological aspects of Liolaemus parvus (Iguania: Liolaemidae) in the Central Andes of Argentina

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Abstract: The objective of this study was to describe temporal variations in the diet and parasitological aspects in *Liolaemus parvus*. In order to examine the diet, we determined the volume, numerousness, and frequency of occurrence for each prey item and calculated the relative importance index. We removed nematodes from the stomach and estimated parasitic indicators. *Liolaemus parvus* presents an active searching mode. It is a predominantly insectivorous species with low intake of plant material and a specialist in feeding on prey items of the family Formicidae, although it also feeds on other arthropods like coleopterans, hemipterans, and spiders. We found temporal variations in its diet. The first record of *Parapharyngodon riojensis* nematodes is reported herein. Males showed higher nematode prevalence than females. We have expanded the number of host species and the distribution range of *Parapharyngodon riojensis*. The information provided about trophic ecology and parasitism is the first contribution to this lizard species' biology.

Keywords: Diet, endoparasites, foraging strategy, helminth, *Parapharyngodon riojensis*

1. Introduction

Currently there are two strategies for obtaining food: active searching and sit-and-wait, also called passive. Lizards may explore either of these two strategies, and many times they may use both modes, thus constituting a continuum between them (Roca, 1999; Castillo et al., 2017). The "active searching" strategy is practiced by species specialized in consuming small and locally numerous prey. The "sit-and-wait" strategy is employed by opportunist species that consume a smaller number of larger solitary prey (Pianka, 1966; Schoener, 1968, 1969; Huey and Pianka, 1981; Pianka, 1982; Cox et al., 2007; Vidal and Labra, 2008; Vitt and Caldwell, 2009). Specialists have narrow tolerance limits (Bunnell, 1978; Pianka, 1982) and are considered to be rare, whereas generalists are abundant (Pianka, 1982). Background data have shown that populations (lizards of the same species occupying a particular geographic area) are not strictly herbivorous, omnivorous, or insectivorous (carnivorous), and that they vary on occasion depending on factors such as season, size, or resource availability (Aun et al., 1999; Martori et al., 2002; Astudillo et al., 2015; Castillo et al., 2017).

The main studies addressing nematodes in the genus *Liolaemus* were contributed by Ramallo and Díaz (1998), Ramallo et al. (2002, 2017), Goldberg et al. (2004), O'Grady and Dearing (2006), and Castillo et al. (2017, 2018). No parasitological features of *L. parvus* are known thus far, nor is there information about its trophic ecology.

The mountain lizard *Liolaemus parvus* is distributed across the central-west Argentina, between 2700 and 3500 m elevation, in San Juan, Mendoza, and La Rioja provinces. It is a viviparous species with a litter size of 2 to 4 young (Acosta, pers. comm.), an active and efficient thermoregulator with body temperature close to the preferred one and higher than that of its habitat (Gómez-Alés et al., 2017). It is a saxicolous species, able to live in sympatry with *Liolaemus olongasta*, *L. ruibali*, *L. uspallatensis*, and *Phymaturus palluma* (Quinteros et al., 2008). With regard to the conservation status of lizards and amphisbaena of Argentina, this species is categorized as nonthreatened (Abdala et al., 2012).

In this study we investigated dietary composition and parasitism in *L. parvus* in a temporally varying environment, addressing the following objectives: 1)

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describe the qualitative (types) and quantitative (number) prey composition; 2) define trophic breadth and type of diet (insectivorous/omnivorous/herbivorous); 3) establish the food-searching strategy (active/sit-and-wait); 4) determine the helminth species recorded in *L. parvus*; 5) estimate parasitic parameters.

2. Materials and methods

2.1. Study site

Samplings were performed in Vallecito Ravine (31.216654°S, 69.681305°W; 3000 m a.s.l.), Calingasta district, San Juan, Argentina. This area is embedded in the Andes Cordillera, on the west border of the Iglesia-Calingasta-Uspallata Valley. The study area encompasses cordilleran foothills and lies between 2500 and 3000 m in elevation (Suvires et al., 1999). From the phytogeographic viewpoint, it corresponds to the Puna province, an area dominated by low and medium-height shrubland of *Ephedra breana*, *Lycium tenuispinosum*, cacti like *Maihuenopsis glomerata* and *Lobivia formosa*, and grasses such as *Stipa ichu* and *Aristida mendozana* isolated in lower layers (Cabrera and Willink, 1973).

2.2. Fieldwork

Temporal samples were collected through a random survey of shrubs and areas bare of vegetation (Tellería, 1986). A noose was used as the capture method. A total of 84 adult individuals, 44 males and 40 females, were trapped and analyzed over four years, 2008, 2009, 2010, and 2011 (Table 1).

2.3. Laboratory work and data analysis

Captured individuals were euthanized by administering intraperitoneal sodium thiopental, fixed with 10% formaldehyde and preserved in 70% alcohol. In the laboratory, stomach contents were dissected and analyzed under a stereoscopic binocular magnifying glass. All specimens are housed in the Herpetological Collection, Biology Department, School of Exact, Physical, and Natural Sciences, National University of San Juan, UNSJ 264-443. All applicable national and/or institutional guidelines for the care and use of animals were followed.

In order to determine the diet (insectivorous/omnivorous/herbivorous), the plant material content in stomachs was analyzed by percentages. This content was quantified following the criteria of Espinoza et al. (2004), Astudillo et al. (2015), Cordoba et al. (2015), and Castillo et al. (2017). For plant quantification (stems, leaves, flowers, fruits, and seeds), we calculated the percentage that plant material occupied in the stomach as related to that occupied by arthropod prey.

Stomach contents were analyzed to describe the qualitative (type) and quantitative (number) prey composition. All variables recorded for each prey were systematically determined at the order, family, or genus level, following Brewer and Argüello (1980). Each prey item was measured for maximum length (L), maximum width (W), and body volume (V). For volume of prey consumed, we used the formula proposed by Dunham (1983), $V = 4/3\pi\ (½L)\ (½W)^2$, where: L is prey maximum length and W is prey maximum width. The importance of each feeding category in the diet was estimated with the relative importance index (RII; Pinkas et al., 1971): RII = %FO (%V + %N). To hierarchize the diet, we adopted the criterion of taking the highest index value and relativizing all other values to it in percentage terms. If the percentage of prey falls between 100% and 75%, it will be considered fundamental; if between 75% and 50%, it will be considered secondary; accessory if between 50% and 25%; and accidental if below 25% (Aun and Martori, 1998). Richness (R) and diversity (Shannon–Wiener) of prey items were determined on the different sampling dates.

For trophic breadth (specialist/opportunist) we considered the Levins index (Levins, 1968), where *i* is the prey category, *p* is the proportion of individuals associated with prey category *i*, and *n* is the total number of prey categories represented in the diet.

$$Nb = 1 / \sum_{i=1}^{n} Pi_{\ 2}$$

This was standardized by the index proposed by Hulbert: $B = (B - 1) / (n - 1)$, where B is the Levins index and n is the number of prey items consumed. This index value is maximal when resources are consumed in higher numbers, in which case species are considered opportunistic. A low index value denotes specialist-type species (González et al., 2006).

The Jaccard index was used to determine temporal and sex differences in food item composition (Moreno, 2001; González et al., 2006; Nieva et al., 2016).

For the food-searching strategy (active/sit-and-wait), we took into account the criterion of type of intake of prey items. Considering that trophic breadth (specialist/opportunist) corresponds with food-searching mode, specialists are related to active searching and generalists (opportunists) to sit-and-wait (Huey and Pianka, 1981).

In order to conduct parasitic analyses, we examined 53 *Liolaemus parvus* individuals (Table 2). The nematodes found were isolated and kept in 70% alcohol. For their identification, following Ramallo et al. (2002), they were made diaphanous using lactophenol and observed under light optical microscope. All identified nematodes were

Table 1. Date and number of adults of *Liolaemus parvus* analyzed for this study.

Period	Date	No. males	No. females
Spring	November 2008	5	9
	December 2008	6	3
	October 2009	3	2
	December 2009	5	4
	December 2010	9	6
Autumn	April 2009	5	9
Summer	March 2011	11	7

Table 2. Date and number of adults of *Liolaemus parvus* analyzed (prevalence, intensity and mean abundance) for this study.

Period	Date	No. males	No. females
Spring	November 2008	9	5
	December 2008	14	5
	December 2009	4	4
Autumn	April 2009	5	7

deposited in the parasitological collection of the Biology Department, National University of San Juan (UNSJPar 251).

For parasitic analyses, we calculated indicators of parasitic infection (Bush et al., 1997) as follows: prevalence, number of infested hosts divided by number of hosts examined (expressed in percentage); intensity: total number of parasites affecting the host; mean intensity, total number of parasites of one particular species present in a sample divided by number of infested hosts; and mean abundance, total number of individuals of a parasite species divided by total number of hosts examined (including infested and noninfested individuals).

Chi-square analyses were conducted to compare prevalence between sexes. To compare nematode intensity between periods, we used Kruskal–Wallis analysis. The level of significance of analyses was 0.05, using Statistica 10.0 and the methods of Zar (1996) and Sokal and Rohlf (1999).

3. Results

3.1. Temporal effect on diet

A total of 23 prey items were determined in 84 individuals over 7 sampling dates. Overall, there was a preference for consuming prey items of the family Formicidae; however, there were variants, with the orders Coleoptera and Hemiptera being predominant in some periods (Table 3). Out of the total of analyzed individuals, 70% had between 0% and 10% of plant material content in the stomach and intestine.

The Levins index values were low, indicating that *L. parvus* has a specialist diet obtained through active searching. The highest prey-item diversity occurred in March 2011, denoting lack of uniformity between the studied periods (Table 4). No similarities were found in prey-item composition between periods, with Jaccard's index values being low (less than 50% similarity) (Table 5), and with temporal dietary variations being observed in the periods analyzed.

3.2. Parasitological aspects

Parapharyngodon riojensis nematodes were recorded in *Liolaemus parvus*, which widens their distribution range and number of host species. In total, 42 nematodes were recorded in 53 individuals over four periods. The specimens possessed the characteristic diagnosis. Male: Presence of seven caudal papillae (1 pair ventral, preanal; 1 pair sublateral, postanal; 1 pair on caudal appendage; 1 papilla median, postanal) and an echinate anal lip. Female: Ovaries that do not coil around the esophagus and oval eggs.

Values for prevalence, intensity, and mean abundance are shown in Table 6. No statistical differences in intensity were found between periods (Kruskal–Wallis; $H(2.34) = 4.01$; $n = 34$; $P = 0.13$). Significant differences in prevalence were recorded between sexes in April and December 2009. In all periods, *L. parvus* males showed higher prevalence than females.

Values for prevalence, intensity, mean abundance, and statistical results between sexes are given in Table 7. In all cases, we found adult-stage nematodes with location in the stomach. Table 8 shows a review of lizard species of the families Liolaemidae and Tropiduridae of Argentina parasitized by nematodes of the genus *Parapharyngodon*.

4. Discussion

From the general description, and based on the type of prey consumed by *L. parvus*, we can infer that this species is predominantly insectivorous, with low intake of plant material. Temporal variations were recorded in the diet, with March 2011 being the period of highest diversity and richness of prey items.

Previous studies on *L. ruibali* have shown it to be an omnivorous species (Villavicencio et al., 2005). In other species inhabiting environments similar to those of *L. parvus*, such as *Liolaemus eleodori* and *Liolaemus vallecurensis*, diet fluctuates among insectivory-omnivory-herbivory (Astudillo et al., 2015; Castillo et al., 2017). Background data on the genus *Liolaemus* show that the dietary habits of these lizards would depend on environmental, temporal, or population factors.

Table 3. Periodic composition of the diet of a population of *L. parvus* (n = 84) in an Andean area in central-west Argentina. %V = Volume, %N = numerousness, %FO= frequency of occurrence, and %IRI = index of relative importance. Cat. = Categories; Fund. = fundamental, Sec. = secondary, Acce. = accessory, Acc. = accidental.

	Spring (Nov.) 2008, n = 14					Spring (Dec.) 2008, n = 9					Spring (Oct.) 2009, n = 5					Spring (Dec.) 2009, n = 9				
Prey items	V	FO	N	IRI	Cat.	V	FO	N	IRI	Cat.	V	FO	N	IRI	Cat.	V	FO	N	IRI	Cat.
Insecta																				
Hymenoptera (Formicidae)	5.5	50	72.2	100	Fund.	0.5	17.3	25	35	Acce.	0.6	33.3	63.8	100	Fund.	5,7	33	75	33,8	
Coleoptera	2.26	8.3	18.5	4.4	Acc.	7.3	39.1	25	100	Fund.	4.2	16.6	11.1	12	Acc.	94	66	25	100	
Diptera	43.1	8.3	1.8	9.6	Acc.	21.5	4.3	2.5	8	Acc.	71.7	8.3	8.33	31	Acce.					
Hemiptera	40.7	8.3	1.8	9	Acc.	10.3	17.3	27.5	52	Sec.	2.4	8.3	2.7	2	Acc.					
Lepidoptera																				
Hymenoptera (No Formicidae)																				
Phasmatodea (*Anisomorpha* sp.)											3	8.3	2.7	2.23	Acc.					
Orthoptera																				
Homoptera																				
Dermaptera																				
Isoptera																				
Siphonaptera																				
Undetermined eggs																				
Undetermined larvae											9.1	8.3	5.5	5	Acc.					
Undetermined pupae	2.2	8.3	1.8	0.8	Acc.															
Colembolla																				
Arachnida																				
Araneae						18.7	8.6	5	16	Acc.	1.8	8.3	2.7	1.7	Acc.					
Trombidiforme																				
Annelida																				
Haplotaxida																				
Mollusca																				
Gastropoda																				
Plant matter																				
Flowers	6	16.6	3.7	4	Acc.	39.8	8.6	12.5	35	Acce.	6.8	8.3	2.7	3.7	Acc.					
Fruits						1.5	4.3	2.5	1.3	Acc.										
Seed																				

Table 3. Continued.

	Spring (Dec.) 2010, n = 15					Summer (Mar.) 2011, n = 18					Autumn (Apr.) 2009, n = 14				
Prey items	V	FO	N	IRI	Cat.	V	FO	N	IRI	Cat.	V	FO	N	IRI	Cat.
Insecta															
Hymenoptera (Formicidae)	0.6	22.8	20.3	32	Acce.	1.2	20.6	36	100	Fund.	0.7	22.5	61.9	100	Fund.
Coleoptera	3.7	14	3.9	7	Acc.	28.9	7.9	1.7	31	Acce.	42.8	19.3	15	79	Fund.
Diptera	10	3.5	1.3	2.6	Acc.	4.4	11.1	2.8	10	Acc.	12	6.4	1.5	6	Acc.
Hemiptera	9.4	24.5	50.4	100	Fund.	2.4	6.3	4	5	Acc.	0.08	3.2	0.7	0.2	Acc.
Lepidoptera						2.3	6.3	1.1	2	Acc.	9.74	9.67	3.1	8.7	Acc.
Hymenoptera (No Formicidae)	4.6	7	2.6	3.4	Acc.	1.2	4.7	1.7	1.8	Acc.	29.4	3.22	0.79	6.8	Acc.
Phasmatodea (*Anisomorpha* sp.)						0.3	1.5	0.2	0.1	Acc.					
Orthoptera															
Homoptera	1.5	1.7	0.4	0.2	Acc.										
Dermaptera	7.9	1.7	0.45	0.9	Acc.										
Isoptera						0.05	1.5	0.5	0.13	Acc.					
Siphonaptera						0.02	1.5	0.5	0.12	Acc.					
Undetermined Eggs						0.013	1.5	5.1	1	Acc.					
Undetermined larvae	14.6	8.7	2.2	10	Acc.	6.9	12.6	4	18	Acc.	0.25	3.22	0.79	0.2	Acc.
Undetermined pupae											0.33	3.22	0.79	0.2	Acc.
Colembolla						0.01	1.5	1.1	0.2	Acc.					
Arachnida															
Araneae	4.6	3.5	0.8	1.29	Acc.	27.6	3.1	0.5	11.5	Acc.	0.58	9.67	3.96	3	Acc.
Trombidiforme						0.7	9.5	38.3	48	Acce.					
Annelida															
Haplotaxida						9.8	1.5	0.2	2	Acc.					
Mollusca															
Gastropoda	13.5	3.5	1.3	3.5	Acc.										
Plant matter															
Flowers	29	8.7	15.9	26.8	Acce.						3.35	6.45	3.96	3	Acc.
Fruits						13.6	7.9	1.4	15.4	Acc.	0.61	9.67	2.38	2	Acc.
Seed											0.08	3.22	4.76	1	Acc.

Table 4. Levins and Shannon–Wiener (H') indices for seven periods analyzed in a population of *L. parvus* in central-west Argentina. Maximum values are indicated in bold.

					Periods			
Indexes		Nov. 08	Dec. 08	Apr. 09	Oct. 09	Dec. 09	Dec. 010	Mar. 011
Levins	(Nb)	0.18	0.59	0.12	0.18	0.7	0.2	0.17
Shannon–Wiener	(H')	0.1	0.1	0.4	0.1	0.04	0.4	**0.8**
Prey richness	R	6	7	12	8	2	11	**16**

Table 5. Distance matrix measures of the Jaccard index for trophic similarity for seven periods of analysis in a population of *L. parvus*. Maximum value in bold.

Periods	Dec. 2008	Apr. 2009	Oct. 2009	Dec. 2009	Dec. 2010	Mar. 2011
Nov. 2008	0.6	0.3	0.4	0.3	0.3	0.2
Dec. 2008		0.3	0.5	0.28	0.3	0.2
Apr. 2009			0.5	0.1	0.5	0.4
Oct. 2009				0.25	**0.7**	0.4
Dec. 2009					0.1	0,1
Dec. 2010						0.5

Table 6. Parasitic indicators in *Liolaemus parvus* from the cordilleran sector of central-west Argentina.

Period	Prevalence	Medium intensity	Medium abundance	Stages	Localization
November 2008	42.80%	2.67 ± 2.3	1.14	Adult	Stomach
December 2008	-	-	-	-	-
April 2009	58.30%	3.29 ± 2.3	1.91	Adult	Stomach
December 2009	12.50%	3 ± 0.0	0.3	Adult	Stomach

Table 7. Parasitic indicators in *Liolaemus parvus* in males and females from the cordilleran sector of central-west Argentina. Chi-square analysis between sexes according to the periods studied.

Period	Sex	Medium intensity	Medium abundance	Prevalence	$\chi 2$	P	Gl
November 2008	Males	2 ± 1.15	0.88	44.4	0.22	0.89	2
	Females	4 ± 4.2	1.6	40			
April 2009	Males	3.75 ± 2.9	3	80	11.2	0.003	2
	Females	2.67 ± 1.5	1.14	42.8			
December 2009	Males	3 ± 0.00	0.75	25	25	0.000004	2
	Females	0	0	0			

Regarding the prey items found in the diet of *L. parvus*, this species has a preference for prey of the family Formicidae (RII). However, it also feeds on other arthropods like coleopterans, hemipterans, and spiders. In general, the largest volume of the diet was contributed by dipterans, coleopterans, larvae, and spiders, as well as by flowers and fruits. The most frequent prey were ants, coleopterans, and hemipterans. The most numerous items were eggs, seeds, acarids, and ants.

The larger number of Formicidae consumed by *L. parvus* could be due to the fact that ants are small and contain much indigestible chitin (Pianka, 1982). Their

Table 8. Nematodes of the genus *Parapharyngodon* in lizards of the families Liolaemidae and Tropiduridae for Argentina.

Nematodes	Host family	Host species	Prevalence	References
P. riojensis Ramallo, Bursey & Goldberg, 2002	Liolaemidae	*Phymaturus punae*	100%	[1]
		P. palluma	100%	[2]
		P. extrilidus	100%	[3, 4]
		P. antofagastensis	Unmentioned	[5]
		P. zapalensis	Unmentioned	[5]
		L. buergeri	Unmentioned	[2]
		L. ruibali	100%	[6]
		L. rothi	Unmentioned	[5]
		L. boulengeri	Unmentioned	[5]
		L. umbrifer	Unmentioned	[5]
		Liolaemus parvus	Nov. 08 = 42.8%; Apr. 09 = 58.3%; Dec. 09 = 12.5%	Current study
P. sanjuanensis Ramallo, Bursey, Castillo & Acosta, 2016	Liolaemidae	*Phymaturus punae*	100%	[7]
Parapharyngodon sp.	Tropiduridae	*Tropidurus torquatus*	Unmentioned	[8]
		T. etheridgei	Unmentioned	[9]

References: [1] Ramallo **et al.**, 2002; [2] Goldberg **et al.**, 2004; [3] Ramallo **et al.**, 2017; [4] Castillo **et al.**, 2018; [5] O'Grady and Dearing, 2006; [6] Castillo **et al.**, 2017; [7] Ramallo **et al.**, 2016; [8] Lamas and Zaracho, 2006; [9] Cruz **et al.**, 1998.

intake in large numbers could result in energy gain (Kozykariski, 2011). In addition, the prey-searching costs are lower because of ants being grouped in nests, columns, or aggregations (Roca, 1999). Consuming Formicidae is common in different lizards of the genera *Liolaemus* (De Viana et al., 1994; Aun and Martori, 1998; Aun et al., 1999; Azocar and Acosta, 2011; Kozykariski, 2011), *Homonota* (Nieva et al., 2015), and *Phrynosoma* (Pianka, 1982).

According to background studies, *L. ruibali* (Villavicencio et al., 2005) and *L. occipitalis* (Verrastro and Ely, 2015) display a sit-and-wait behavior. Species like *L. wiegmannii* (Aun et al., 1999) and *L. saxatilis* (Martori et al., 2002) exhibit a mixed strategy (between active mode and sit-and-wait). *L. vallecurensis* uses both modes; males show active searching and females and juveniles forage by sit-and-wait (Castillo et al., 2017). The specialist diet is related to active food-searching (Huey and Pianka, 1981). In *L. parvus*, the active food-searching mode is defined by exhibiting exploratory movements with the intake of small and locally numerous prey such as eggs, seeds, acarids, and ants. However, information about types of food-searching in lizards is currently scarce. In this respect, for the genus *Liolaemus* it has been found that its species vary from an active searching mode to a stalking mode.

From the parasitological analysis, we observed the occurrence of the nematode *Parapharyngodon riojensis* in *L. parvus*. Consistent with notions expressed about trophic ecology, the type of food-searching in lizards, active searching, would explain the high parasite prevalence likely to be recorded in different lizard species, due to their probability of encountering parasites (Ribas et al., 1998).

According to the points mentioned above, only two of the four periods analyzed showed high parasite prevalence in *L. parvus*, November 2008 (42.80%) and April 2009 (58.30%). At present, there is very little information for Argentina on nematode prevalence in reptile populations. Our results are not consistent with the suggestion that the genus *Parapharyngodon* parasitizes only herbivorous and omnivorous species (Anderson, 2000). In this sense, and according to our observations on trophic ecology and parasitism, we infer that species of the genus *Parapharyngodon* are parasites of herbivorous, omnivorous, and insectivorous lizards.

In *L. parvus*, *Parapharyngodon riojensis* was found to be associated with the stomach. However, for other species like *L. buergeri*, *L. ruibali*, and species of the genus *Phymaturus*, nematodes were located in the long intestine (Ramallo et al., 2002, 2016, 2017; Goldberg et al., 2004; Castillo et al., 2017, 2018).

Speaking in general terms about parasitism in reptiles of Argentina, two nematode species of *Parapharyngodon* have been recorded to date. *P. riojensis* and *P. sanjuanensis* nematodes (Ramallo et al., 2002, 2016) were recorded in three lizard genera, *Liolaemus*, *Phymaturus*, and

Tropidurus, corresponding to two families, Liolaemidae and Tropiduridae. A total of 13 lizard species parasitized by this genus are known for Argentina. We report the first record of *Parapharyngodon riojensis* nematodes in *L. parvus*, which is the thirteenth lizard species in Argentina parasitized by nematodes of the genus *Parapharyngodon*.

As final conclusions, and based on our observations, we suggest that a species' diet should be defined over several seasons, without settling for isolated estimates that may not reflect the real trophic spectrum. For *L. parvus*, the periods of analysis showed a trend toward consuming ants, with variations involving other arthropods, thus determining its insectivorous nature, and that this is a specialist species displaying an active food searching mode and with temporal variations in its diet. Specialist species like *L. parvus* are important to conservation because their populations could be at risk. Therefore, the presented data will help determine the conservation status for lizards in Argentina.

We expanded the number of host species and the distribution range of the parasite to the Calingasta district, Vallecito locality, San Juan Province, Argentina. The information afforded by this study represents an important first contribution to the knowledge of trophic and parasitological habits in *Liolaemus parvus*. Thus, we afford an advance in knowledge of this species' biology and ecology in cordilleran ecosystems.

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