ISSN Versión Impresa 1816-0719 | ISSN Versión en linea 1994-9073 | ISSN Versión CD ROM 1994-9081

The Biologist (Lima), 2017, 15(2), jul-dec: 459-468

The Biologist (Lima)

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

HELMINTH PARASITES OF *RHINELLA MARINA* (LINNAEUS, 1758) (ANURA: BUFONIDAE) FROM TARAPOTO, PERU

HELMINTOS PARÁSITOS DE *RHINELLA MARINA* (LINNAEUS, 1758) (ANURA: BUFONIDAE) DE TARAPOTO, PERÚ

Gislayne Melo Toledo[1]; Mariluce Gonçalves Fonseca[2]; Jose Iannacone[3,4]; Jorge Manuel Cárdenas Callirgos[5]; Carlos Urbano Mendoza Vidaurre[6] & Reinaldo José da Silva[1]

[1]UNESP - Univ Estadual Paulista, Campus de Botucatu, Instituto de Biociências, Departamento de Parasitologia, Botucatu, São Paulo, Brazil.
[2]UFPI - Universidade Federal do Piauí, Piauí, Brazil.
[3]URP - Universidad Ricardo Palma, Facultad de Ciencias Biológicas, Lima, Perú.
[4]UNFV – Universidad Nacional Federico Villarreal, Facultad de Ciencias Naturales y Matemática, Lima, Perú.
[5]Wabash College, Lima, Perú.
[6]UAP - Universidad Alas Peruanas, Tarapoto, Perú.
Correspondence author: gisatoledo@hotmail.com

ABSTRACT

Rhinella marina is a large bufonid with nocturnal and terrestrial habit, which inhabits humid areas with adequate cover, with a preference for open or disturbed habitat such as tracks, roads, low grassland, and areas that are near human settlement. Aiming to expand the knowledge on the amphibian parasites in Neotropical region, the helminth fauna associated with *R. marina* from two different environments at the municipality of Tarapoto, Peru was reported. A total of 30 *R. marina* specimens was surveyed for helminth parasites: 10 specimens from the Urku – Amazonian Studies and Cordillera Escalera Reserve (preserved area) and 20 from the Santa Rosa (anthropogenic area), both in the municipality of Tarapoto, Peru. The helminth component community included eight species (seven nematodes and one cestode). A total of 1,799 helminths was recovered, with a mean intensity of infection (MII) in animals from the preserved area (MII = 57.9 ± 23.8) not differing from those of the anthropogenic area (MII = 61.0 ± 11.5). The nematode *Oswaldocruzia urubambaensis* Guerrero, 2013 was the dominant species (d = 0.26) for the preserved area and *Cylindrotaenia americana* Jewell, 1916 was the dominant species (d = 0.46) for the anthropogenic area. The richness of parasites between two areas was similar and the diversity of helminth was also not different. The helminth component communities showed no differences in relation the richness and composition. However, the helminth community structure shows a slight difference in the infracommunities, suggesting that environmental characteristics can influence the structuration of helminth community parasites of this anuran species, since some species of parasites presented differences in prevalence, abundance, and relative importance values between the two infracommunities.

Keywords: amphibians – anura – infracommunity – Nematoda – Peruvian Amazon

RESUMEN

Rhinella marina es un bufónido grande con hábito nocturno y terrestre, que habita áreas húmedas con cobertura adecuada, con preferencia por hábitat abierto o perturbado como vías, caminos, pastizales bajos y áreas cercanas al asentamiento humano. Con el objetivo de ampliar el conocimiento sobre los anfibios parásitos en la región Neotropical, se registró la fauna de helmintos asociada a *R. marina* de dos ambientes diferentes en la municipalidad de Tarapoto, Perú fue reportado. Un total de 30 ejemplares de *R. marina* fueron estudiados para detectar helmintos parásitos: 10 especímenes de Urku - Estudios Amazónicos y de la Reserva Cordillera Escalera (área preservada) y 20 de Santa Rosa (área antropogénica), ambos en la municipalidad de Tarapoto, Perú. El componente de la comunidad de helmintos incluyó ocho especies (siete nematodos y un céstode). Se recuperó un total de 1.799 helmintos, con una intensidad media de infección (IMI) en animales del área preservada (IMI = 57,9 ± 23,8) que no difiere de los del área antropogénica (IMI = 61,0 ± 11,5). El nematodo *Oswaldocruzia urubambaensis* Guerrero, 2013 fue la especie dominante (d = 0,26) para el área preservada y *Cylindrotaenia americana* Jewell, 1916 fue la especie dominante (d = 0,46) para el área antropogénica. La riqueza de parásitos entre las dos áreas fue similar y la diversidad de helmintos tampoco fue diferente. El componente de la comunidad de helmintos no mostró diferencias en relación con la riqueza y composición. Sin embargo, la estructura de la comunidad de helmintos muestra una leve diferencia en las infracomunidades, lo que sugiere que las características ambientales pueden influir en la estructuración de helmintos parásitos comunitarios de esta especie de anuro, ya que algunas especies de parásitos presentan diferentes prevalencias, abundancias, y valor de importancia relativa entre las dos infracomunidades.

Palabras clave: Amazonía peruana – anfibios – anura – infracomunidad – Nematoda

INTRODUCTION

The family Bufonidae Gray, 1825 currently includes 592 species distributed in 52 genera and has a wide geographic distribution, occurring on all continents except Antarctica, Madagascar, and oceanic regions (Lima *et al.*, 2006; Frost, 2017). The genus *Rhinella* Fitzinger, 1826 is represented by 93 species, of which 29 occur in Peru (Frost, 2017), including terrestrial anurans, with oviposition in gelatinous cords, inside or the margin of water bodies (Lima *et al.*, 2006).

Rhinella marina (Linnaeus, 1758), commonly known as cane toad, is a large buffoon, occurring naturally from southern Texas, USA, through tropical Mexico and Central America to the north of South America (Solís *et al*., 2009). This nocturnal and terrestrial toad that inhabits humid areas with adequate cover, with a preference for open or disturbed habitat such as trails, roads, low pastures and areas that are close to human settlements (Solís *et al*., 2009). Their feeding is composed mainly of arthropods (especially ants and termites) and small vertebrates; and has great flexibility regarding the breeding site (Solís *et al*., 2009).

In Peru, the first study of helminths in the genus *Rhinella* was done by Tantaleán & García (1989). However, in recent years, studies on anuran parasites infecting species of this genus have increased in the region of South America (Table 1).

Tantaleán & García (1989) and Bursey *et al*. (2001) have reported in previous studies the composition of the parasitic fauna of *R. marina* in Peru. However, we analyzed in this study the implications of two contrasting areas on the helminth fauna of these anurans. The first is an area of Amazon Forest, that is highly preserved representing a more stable environment, and the second is an urban area known as Santa Rosa, with greater human influence representing a more unstable environment.

The aims of this study were: (1) to determine the structure of the parasite helminth community; 2) to determine the richness and diversity of parasite communities; and (3) to analyze the relationship between the composition of the helminth communities and the characteristics of the environment.

Table 1. Richness of helminth species at the level of community component of *Rhinella* species of South America.

Amphibians	Sample number	Total Richness	Locality	Reference
Rhinella marina	30	8	Peru	Present study
Rhinella spinulosa (Wiegmann, 1834)	-	4	Peru	Tantaleán & García (1989)
Rhinella arequipensis (Vellard, 1959)	-	3	Peru	Tantaleán & García (1989)
Rhinella limensis (Werner, 1901)	-	5	Peru	Tantaleán & García (1989)
Rhinella marina	-	3	Peru	Tantaleán & García (1989)
Rhinella marina	5	5	Peru	Bursey *et al.* (2001)
Rhinella poeppigii (Tschudi 1845)	32	5	Peru	Chero *et al.* (2015a)
Rhinella limensis	30	4	Peru	Chero *et al.* (2015b)
Rhinella spinulosa	90	7	Peru	Chero *et al.* (2016)
Rhinella icterica (Spix, 1824)	32	15	Brazil	Luque *et al.* (2005)
Rhinella icterica	58	12	Brazil	Lux Hoppe *et al.* (2008)
Rhinella schneideri (Werner, 1894)	42	6	Brazil	Lux Hoppe *et al.* (2008)
Rhinella icterica	15	5	Brazil	Pinhão *et al.* (2009)
Rhinella fernandezae (Gallardo, 1957)	90	13	Brazil	Santos & Amato (2010)
Rhinella icterica	60	12	Brazil	Santos *et al.* (2013)
Rhinella major (Müller and Hellmich, 1936)	19	4	Argentina	González & Hamann (2006)
Rhinella fernandezae	25	4	Argentina	González & Hamann (2007)
Rhinella bergi (Céspedez, 2000)	20	4	Argentina	González & Hamann (2007)
Rhinella schneideri	11	8	Argentina	González & Hamann (2008)
Rhinella fernandezae	65	22	Argentina	Hamann *et al.* (2013)
Rhinella major	85	15	Argentina	Hamann & González (2015)

MATERIALS AND METHODS

Study area

Anurans were collected in the municipality of Tarapoto, Peru (Figure 1). The city is located near the line of Ecuador, with a mean altitude of 250 m (Google Earth) and is characterized by a tropical rainy climate, with temperatures ranging from 18 °C to 33 °C.

In the municipality of Tarapoto, the animals were collected in two different landscapes. The first was represented by the preserved area of the Amazon Forest located at URKU – Amazonian Studies (6 ° 27'52.42 "S, 76 ° 21'7.73" W) and Cordillera Escalera Reserve (6 ° 27'41.30 "S, 76 ° 18'42.56 " O). In these localities, the specimens were collected in trials of closed forest area, where there is no human presence (Figure 1A). The second landscape was represented by an anthropogenic area, a district known as Santa Rosa (6 ° 25'39.84 "S, 76 ° 18'32.28" W), that is near of a highway, where there are several human residences, with many domestic animals (dogs, cats, horses, cattle, pig, among others) and great amount of garbage, characterizing a high degree of environmental degradation (Figure 1B).

Collection and examination of amphibians

Thirty specimens of *R. marina* were collected in March 2014 in the municipality of Tarapoto, Peru. Amphibians were sampled by active search at night. Toads were transported live to the laboratory and then euthanized with sodium thiopental solution, in accordance with Resolution No. 301 and Ordinance No 148/2012 of the Conselho Federal de Biologia – CFBio. The snout–vent length (SVL) and body mass were recorded. Then they were necropsied, sexed and organs of the gastrointestinal tract, lungs, heart, kidneys, liver, urinary bladder, gall bladder, and body cavity were surveyed for the presence of helminths. The collection was authorized by Director's Resolution

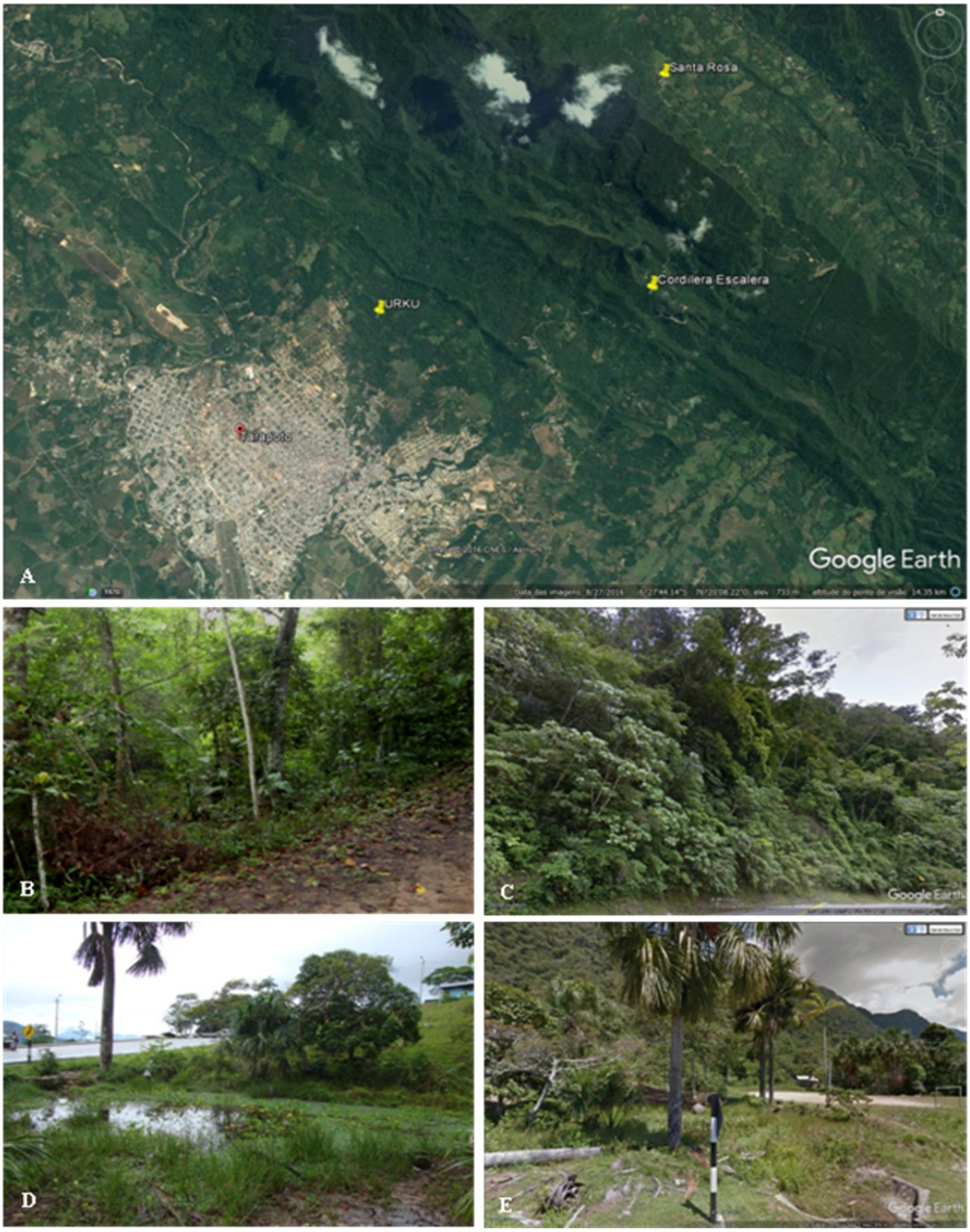

Figure 1. Geographical location of anuran sampling landscapes in the municipality of Tarapoto, Peru. A) Visualization of host sampling landscapes; B) Area of trail in the reserve URKU – Amazonian Studies (preserved environment); C) Forest of the Reserve Cordillera Escalera (preserved environment); D-E) Pond in Santa Rosa (anthropized environment). A, C and E, source Google Earth.

N°024-2014-SERFOR-DGGSPFFS.

Collection, preparation, and identification of helminths

The helminths were collected and processed following the methodologies used by Amato *et al.* (1991) and preserved in 70% ethyl alcohol until the preparation of the temporary slides for species identification. Cestodes were stained with hydrochloric carmine and cleared with creosote. Nematodes were cleared in lactophenol and examined as temporary mounts. Morphometric data and photomicrographs of helminths were obtained using a computerized LAS V3 image analysis system (Leica Application Suite, Leica Microsystems, Wetzlar, Germany) coupled to a DM 5000B microscope with differential interference contrast (Leica Microsystems, Wetzlar, Germany).

The voucher species will be deposited at the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB), Department of Parasitology, at the Universidade Estadual Paulista (UNESP), São Paulo State, Brazil and at the Colección Helmintológica y de Invertebrados Relacionados del Museo de Historia Natural (MUS - UNMSM) at the Universidad Nacional Mayor de San Marcos, Lima, Peru.

Statistical analysis

Quantitative descriptors of parasitism as prevalence, mean abundance, mean intensity, parasite richness, and amplitude were calculated as infection patterns for all infrapopulations of parasites found, according to Bush *et al.* (1997). The richness is described as the total number of helminth species. The mean helminth species richness is the sum of parasite species per individual host, divided by the total sample size. Measurements of community richness and diversity included the 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 helminth infracommunities (Pielou, 1966). The diversity indices were used with decimal logarithms (log10). The Student t-test was used to compare differences in intensity and mean diversity of infection between the two environments. The Mann-Whitney U test was used to test differences in helminth species abundances between the two locations. The Berger-Parker index of dominance (*d*) was used to determine the most dominant species (Magurran, 2004).

The helminth community structure was examined according to the methodology outlined by Thul *et al.* (1985), where helminth species are classified into 4 groups (dominant, codominant, subordinate, and unsuccessful colonizer) by taking into account the prevalence, intensity, and maturation factor, which is related to the degree of host specificity. The importance value (*I*) was calculated for each helminth species as follows:

$$I_j = \frac{(M_j)\, A_j B_j}{\Sigma A_i B_i}\; 100$$

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

The analyses were performed in the BioEstat 5.0 program (Ayres *et al.*, 2007).

RESULTS

The 30 amphibians analyzed were infected with at least one helminth species (overall prevalence = 100%). Results of the parasitological descriptors of the two localities are presented in Table 2. Eight helminth species, totaling 1,799 individuals, were found in *R. marina* in the two localities (Table 3). Nematoda was the predominant parasite group in this sample (seven species), while Cestoda was represented by only one species. About the species found, five have a direct life cycle (*Aplectana hylambatis* (Baylis, 1927), *Aplectana vellardi* Travassos, 1926, *Oswaldocruzia urubambaensis* Guerrero, 2013, *Rhabdias pseudosphaerocephala* Kuzmin, 2007, and *Cylindrotaenia americana* Jewel, 1916) and three have an indirect life cycle (*Falcaustra concordaqui* Ibañez & Córdova, 1976, *Ochoterenella vellardi* (Travassos, 1929) Esslinger, 1986, and larvae *Physaloptera* Rudolphi, 1819).

The helminth component communities of the animals from preserved and anthropic areas shared the same helminth species (Table 3), differentiating in the abundances of each species. In the preserved habitat the diversity of helminths was 0.77 and the equitability was 0.86. The nematode *O. urubambensis* was the dominant species (d = 0.26). At the infracomunity level, mean helminth richness was 2.9 ± 0.4 (maximum = 5) species per infected host. Multiple infections were common with 1, 2, 3, 4, and 5 species occurring in 1, 4, 2, 1, and 2 hosts, respectively (Figure 2).

Table 2. Parameters of infection of the helminth community associated with *Rhinella marina* from the municipality of Tarapoto, Peru. Preserved Areas - Urku and Reserve Cordillera Escalera; Anthropogenic Areas - Santa Rosa.

Parameters	Area	
	Preserved	Anthropogenic
Number of hosts	10	20
Prevalence	100%	100%
Mean intensity of infection	57.9 ± 23.8	61.0 ± 11.5
Range	3 – 240	4 - 245
Number of helminths	579	1220
Richness of species	8	8
Mean richness of species	2.9 ± 0.4	3.2 ± 0.3
Maximum number of species/host	5	5
Diversity (H') / Evenness (J)	0.77/0.86	0.64/0.71

In the anthropogenic habitat, the diversity of helminths was 0.64 and the equitability was 0.71. The cestode *C. americana* was the dominant species (d = 0.46). At the infracommunity level, mean helminth richness was 3.2 ± 0.3 (maximum = 5) species per infected host. Multiple infections were common with 1, 2, 3, 4, and 5 species occurring in 1, 6, 4, 7, and 2 hosts, respectively (Figure 2).

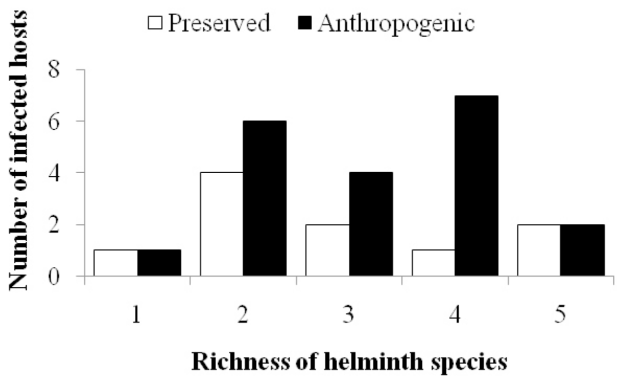

Figure 2. Number of *Rhinella marina* infected with 1, 2, 3, 4 and 5 helminth species in Tarapoto, Peru.

The helminth diversity between the two localities was similar (t = -1.25, GL = 28, p = 0.21). As well as, there was no significant variance between the two habitats in relation to the mean intensity of infection (t = -0.13, GL = 28, p = 0.89). When parasitic abundance between the localities was compared, only *C. americana* was found in higher abundance in relation to the preserved area (U = 37; $p < 0.05$).

The helminth species were classified according to importance value (Table 4). In the anthropogenic area, *C. americana* was the species with the highest value of importance, followed by *O. urubambaensis*; in the preserved area the nematodes *O. urubambaensis*, *A. hylambatis,* and *A. velardi* were the most important species of the community (highest values of importance).

Table 3. Helminths recorded in *Rhinella marina* from Tarapoto, Peru. Berger-Parked index (*d*), prevalence (P, in %), mean abundance and mean intensity infection (MII), followed by the standard error (SE) and the site of infection (SI).

Helminths	*d*	%	MA ± SE	MII ± SE	SI*	Area							
						Preserved				Anthropogenic			
						d	P	MA ± SE	MII ± SE	*d*	P	MA ± SE	MII ± SE
Cestoda													
Cylindrotaenia americana	0.37	56.7	22.4 ± 6.2	39.6 ± 8.9	SI, LI	0.2	10	11.4 ± 11.4[a]	114	0.46	80	28.0 ± 7.2[a]	34.9 ± 8.1
Nematoda													
Aplectana hylambatis	0.12	33.3	7.4 ± 4.3	22.1 ± 11.9	SI, LI	0.22	30	12.7 ± 11.5	42.3 ± 36.8	0.08	35	4.7 ± 3.1	13.4 ± 8.3
Aplectana vellardi	0.07	26.7	4.3 ± 2.4	16.3 ± 7.7	S, SI, LI	0.16	30	9.5 ± 6.8	31.7 ± 18.4	0.03	25	1.8 ± 0.9	7.0 ± 2.5
Falcaustra concordaqui	0.07	23.3	4.3 ± 2.2	18.3 ± 7.4	SI, LI	0.06	40	3.4 ± 2.6	8.5 ± 6.0	0.08	15	4.7 ± 3.0	31.3 ± 12.9
Ochoterenella vellardi	0.03	23.3	1.9 ± 1.1	8.3 ± 3.8	BC	0.07	50	3.8 ± 2.8	7.6 ± 5.4	0.02	10	1.0 ± 0.7	10.0 ± 3.0
Oswaldocruzia urubambaensis	0.26	70	15.8 ± 3.5	22.5 ± 4.2	SI, LI	0.26	60	14.9 ± 5.5	24.8 ± 6.5	0.27	75	16.2 ± 4.6	21.6 ± 5.4
Physaloptera sp.	0	10	0.2 ± 0.1	1.7 ± 0.7	S	0.01	10	0.3 ± 0.3	3	0	10	0.1 ± 0.1	1
Rhabdias pseudosphaerocephala	0.06	63.3	3.7 ± 1.2	5.8 ± 1.8	L	0.03	60	1.9 ± 0.7	3.2 ± 0.8	0.08	65	4.6 ± 1.8	7.1 ± 2.5

*Site of infection: BC = body cavity corporal; S = stomach; SI = small intestine; LI = large intestine; L = lung. [a] $p < 0.05$.

Table 4. Values of importance (I) and classification of the species of helminths in *Rhinella marina* from Tarapoto, Peru.

Helminth species	Area			
	Preserved		Anthropogenic	
	I	Classification	*I*	Classification
Cylindrotaenia americana	5.39	Dominant	55.33	Dominant
Aplectana hylambatis	18.02	Dominant	4.11	Dominant
Aplectana vellardi	13.34	Dominant	1.08	Dominant
Falcaustra concordaqui	6.43	Dominant	1.74	Dominant
Ochoterenella vellardi	8.99	Dominant	0.25	Codominant
Oswaldocruzia urubambaensis	42.29	Dominant	30.06	Dominant
Physaloptera sp.	0.0	Unsuccessful colonizer	0.0	Unsuccessful colonizer
Rhabdias pseudosphaerocephala	5.39	Dominant	7.4	Dominant

DISCUSSION

The results of the present study indicate that *R. marina* is a host with relative high helminth species richness (Espinola- Novelo *et al*., 2017), which was similar to previous studies with other *Rhinella* species in South America (Table 1). The parasitic fauna of *R. marina* included preferably nematode species. The high richness of this parasite may be related to the terrestrial habit of the host, since adult toads enter the water for a short period, only for breeding (Lima *et al*., 2006). Anurans with terrestrial habits generally present low prevalence and mean intensity of infection by digenetic trematode, which involves the ingestion of aquatic arthropods and snails (Bolek & Coggins 2000, 2003; Espinola- Novelo *et al*., 2017). According to Aho (1990), the time that the hosts remain in the terrestrial or aquatic environment during the larval phase and the period of reproduction is determinant for the parasitic richness. Tadpoles are more exposed to helminths with aquatic life cycles; on land, anurans are more exposed to nematodes with monoxenic life cycles (eg, *Oswaldocruzia* spp., *Rhabdias* spp., and cosmocercids), because most nematodes infect anurans by skin penetration or egg ingestion (Barton, 1999; Bolek & Coggins, 2000, 2001, 2003; Muzzal *et al.,* 2001; Yoder & Coggins, 2007).

Rhinella marina acted as the definitive host for more than 80% of the helminth species found to parasite this anuran. This anuran participated as an intermediate host of *Physaloptera* sp., a nematode that remains in the gastric mucosa for variable periods of time without reaching the adult stage (Anderson, 2000).

The helminth species recorded in this toad have low host specificity since their presence has been reported in species of amphibians belonging to several families. *Cylindrotaenia americana*, *A. hylambatis*, and *Physaloptera* sp. found parasitizing *R. marina* in the present study had also been recorded in other studies of helminth fauna with the same host (Tantaleán & García, 1989; Bursey *et al*., 2001). However, *Cosmocerca brasiliense* and *Cosmocerca parva* recorded by Bursey *et al*. (2001) were not found in this study.

In general, for *R. marina* there was no difference between the localities in relation to the parasitological descriptors. The populations of analyzed hosts presented parasitic fauna with the same parasite species. However, some species of parasites presented differences in prevalence, abundance, and relative importance values between the two infracommunities. Of the dominant species recorded for the preserved area, only *O. vellardi* was not dominant in the anthropogenic area, but the importance values of the other nematodes were smaller, whereas for the cestode *C. americana* the values was significantly larger. This is important since *O. vellardi* has an indirect life cycle involving hematophagous arthropods as a possible vector agent (Wong & Bundy, 1985) and the instability of the environment (eg, pollution) may have influenced the permanence of this intermediate host, inducing the lowest abundance and occurrence of this parasite.

In conclusion, the community of helminth parasites in *R. marina*, in this region of Peru, has the following characteristics: parasitic community composed predominantly of adult nematodes with monoxenic cycles; the helminth infracommunities did not show differences in relation to helminth richness and composition; and the relative importance of helminth species differed in the two habitats.

ACKNOWLEDGMENTS

The authors are grateful to colleagues from the Laboratório de Parasitologia de Animais Silvestres (LAPAS) - UNESP, for help with the fieldwork. G. M. Toledo thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for an doctoral scholarship (140301/2013-5). Silva is grateful to Pro-Reitoria de Pós-graduação da UNESP (PROPG-UNESP), CNPq (307808/2014-9) and CNPq-PROTAX (440496/2015-2) / FAPESP 2016/50377-1.

BIBLIOGRAPHIC REFERENCES

Aho, J.M. 1990. *Helminthes communities of amphibians and reptiles: comparative approaches to understanding patterns and process*. In: Esch, G.W; Bush, A.O. & Aho, J.M. (Eds.). *Parasite Communities: patterns and process.* New York: Chapman and Hall. pp.157-195.

Amato, J.F.R.; Boeger, W. & Amato, S.B. 1991. *Protocolos para laboratório: coleta e processamento de parasitos de pescado.* Rio de Janeiro: Imprensa Universitária, UFRRJ, 81p.

Anderson, R.C. 2000. *Nematode parasites of vertebrates: their development and transmission*, 2nd ed. Wallingford: CABI Publishing. 650p.

Ayres, M.; Ayres-Júnior, M.; Ayres, D.L. & Santos, A.A.S. 2007. *Bioestat 5.0: aplicações estatísticas nas áreas das ciências biomédicas*. Belém: Sociedade Civil Mamirauá, CNPq. Disponível em: http://www.mamiraua.org.br/download.

Barton, D.P. 1999. Ecology of helminth communities in tropical Australian amphibians. International Journal for Parasitology, 29: 921-926.

Bolek, M.G. & Coggins, J.R. 2000. Seasonal occurrence and community structure of helminth parasites from the Eastern American toad *Bufo americanus americanus*, from Southeastern Wisconsin, U.S.A. Comparative Parasitology, 67: 202-209.

Bolek, M.G. & Coggins, J.R. 2001. Seasonal occurrence and community structure of helminth parasites from the green frog, *Rana clamitans melanota*, from southeastern Wisconsin, U.S.A. Comparative Parasitology, 68:164-172.

Bolek, M.G. & Coggins, J.R. 2003. Helminth community structure of sympatric eastern American toad, *Bufo americanus americanus*, northern leopard frog, *Rana pipiens*, and blue-spotted salamander, *Ambystoma laterale*, from southeastern Wisconsin. The Journal of Parasitology, 89, 673-680.

Bursey, C.R.; Goldberg, S.R. & Pamarlee, J.R. 2001. Gastrointestinal helminths of 51 species of anurans from Reserva Cuzco Amazónico, Peru. *Comparative* Parasitology, 68: 21–35.

Bush, A.O.; Lafferty, K.D.; Lotz, J.M. & Shostak, A.W. 1997. Parasitology meets ecology on its terms: Margolis *et al*. revisited. The Journal of Parasitology, 83: 575-583.

Chero, J.; Cruces, C.; Iannacone, J.; Sáez, G.; Alvariño, L.; Silva, R.J.; Morales, V.R.; Minaya, D. 2015a. Parasitofauna of the Neotropical amphibian *Rhinella limensis* Werner, 1901 (Anura: Bufonidae) from peruvian central coast. Neotropical Helminthology, 9: 87-102.

Chero, J.; Cruces, C.; Iannacone, J.; Sáez, G.; Alvariño, L.; Guabloche, A.; Romero, S.; Tuesta, E.; Morales, V.R. & Silva, R.J. 2015b. Ecological índices of helminth parasites of the Andean toad *Rhinella poeppigii* (Tschudi, 1845) (Anura: Bufonidae) from Peru. The Biologist (Lima), 13: 111-124.

Chero, J.; Cruces, C.; Iannacone, J.; Sáez, G.; Alvariño, L.; Luque, J. & Morales, V. 2016. Comunidad de helmintos parásitos del sapo espinoso *Rhinella spinulosa* (Wiegmann, 1834) (Anura: Bufonidae) de Perú. Revista de Investigaciones Veterinarias del Perú, 27:114-129.

Espínola-Novelo, J.F.; Guillén-Hernández, S.; González-Salas, C.F.; Canto, A. 2017. Helminth diversity of two anurans: *Rhinella marina* and *Incilius valliceps* (Anura: Bufonidae) from lagunas de Yalahau, Yucatán, Mexico. Revista Mexicana de Biodiversidad, 88: 365–371.

Frost, D.R. 2017. *Amphibian species of the world: an online reference.* Version 5.6. New York: American Museum of Natural History, 2017. Disponible en: <http://research.amnh.org/herpetology/amph

ibia/index.php.>. Acesso en: 04 de Noviembre del 2017 .

González, C.E. & Hamann, M.I. 2006. Nemátodes parásitos de *Chaunus granulosus major* (Müller & Hellmich, 1936) (Anura: Bufonidae) en Corrientes, Argentina. Cuadernos de Herpetología, 20: 43-49.

González, C.E. & Hamann, M.I. 2007. Nematode parasites of two species of *Chaunus* (Anura: Bufonidae) from Corrientes, Argentina. Zootaxa, 1393: 27-34.

González, C.E. & Hamann, M.I. 2008. Nematode parasites of two anuran species *Rhinella schneideri* (Bufonidae) and *Scinax acuminatus* (Hylidae) from Corrientes, Argentina. Revista de Biología Tropical, 56: 2147-2161.

Hamann, M.I. & González, C.E. 2015. Helminth parasites in the toad, *Rhinella major* (Bufonidae) from Chaco region, Argentina. Acta Herpetologica, 10: 93-101.

Hamann, M.I.; Kehr, A.I. & González, C.E. 2013. Helminth community in the burrowing toad, *Rhinella fernandezae* from Northeastern Argentina. Biologia, 68: 1155-1162.

Lima, A.P.; Magnusson, W.E.; Menin, M.; Erdtmann, L.K.; Rodrigues, D.J.; Keller, C. & Hödl, W. 2006. *Guia de Sapos da Reserva Adolpho Ducke: Amazônia Central.* Manaus: Attema Design Editorial Ltda. 168 p.

Luque, J.L.; Martins, A.N. & Tavares, L.E.R. 2005. Community structure of metazoan parasites of the yellow Cururu toad, *Bufo ictericus* (Anura, Bufonidae) from Rio de Janeiro, Brazil. Acta Parasitologica, 50: 215-220.

Lux Hoppe, E.G.; Pedrassani, D.; Hoffmann-Inocente, A.C.; Tebaldi, J.H.; Storti, L.F.; Zanuzzo, F.S.; Avancini, N. & Nascimento, A.A. 2008. Estudos ecológicos em taxocenoses helmínticas de *Chaunus ictericus* (Spix, 1824) e *C. schneideri* (Werner, 1894) (Anura: Bufonidae) simpátricos, capturados no distrito de São Cristóvão, município de Três Barras, Santa Catarina. Revista Brasileira de Parasitologia Veterinária, 17: 166-169.

Magurran, A.E. 2004. *Measuring biological diversity*. Oxford: Blackwell Science. 256p.

Muzzall, P.M.; Gillillant, M.G.; Summer, C.S. & Mehne, C.J. 2001. Helminth communities of green frogs *Rana clamitans* Latreille, from southwestern Michigan. The Journal of Parasitology, 87: 962-968.

Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. The Journal of Theoretical Biology, 13: 131-144.

Pinhão, R.; Wunderlich, A.C.; Anjos, L.A. & Silva, R.J. 2009. Helminths of toad *Rhinella icterica* (Bufonidae), from the municipality of Botucatu, São Paulo State, Brazil. Neotropical Helminthology, 3: 35-40.

Santos, V.G.T. & Amato, S.B. 2010. Helminth fauna of *Rhinella fernandezae* (Anura: Bufonidae) from the Rio Grande do Sul Coastland, Brazil: analysis of the parasite community. International Journal for Parasitology, 23: 937-944.

Santos, V.G.T., Amato, S.B. & Borges-Martins, M. 2013. Community structure of helminth parasites of the "Cururu" toad, *Rhinella icterica* (Anura: Bufonidae) from southern Brazil. Parasitology Research, 112: 1097-1103.

Solís, F.; Ibáñez, R.; Hammerson, G.; Hedges, B.; Diesmos, A.; Matsui, M.; Hero, J.; Richards, S.; Coloma, L.; Ron, S.; Marca, E.L.; Hardy, J.; Powell, R.; Bolaños, F.; Chaves, G. & Ponce, P. 2009. *Rhinella marina. The IUCN Red List of Threatened Species*. Disponible in: <http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T41065A1038242.en.>. Acesso in: 22 de November, 2016.

Tantaleán, M.V. & García, L. 1989. Contribución al estudio de los helmintos parásitos de anfibios del Perú. Boletín de Lima, 64: 69-77.

Thul, J.E.; Forrester, D.J. & Abercrombie, C.L. 1985. Ecology of parasitic helminthes of wood ducks, *Aix sponsa*, in the Atlantic Flyway. Proceedings of the Helminthological Society of Washington, 52: 297-310.

Wong, M.S.; Bundy, D.A.P. 1985. Population distribution of *Ochoterenella digiticauda* (Nematoda: Onchocercidae) and *Mesocoelium monas* (Digenea: Brachycoeliidae) in naturally infected *Bufo marinus* (Amphibia: Bufonidae) from Jamaica. Parasitology, 90: 457-461.

Yoder, H.R. & Coggins, 2007. J.R. Helminth communities in five species of sympatric amphibians from three adjacent ephemeral ponds in southeastern Wisconsin. The Journal of Parasitology, 93: 755-761.

Zar, J.H. 2010. *Biostatistical analysis*. 5th ed. Upper Saddle River: Prentice-Hall. 944p.

Received September 19, 2017.
Accepted November 17, 2017.