Trabajo

Parasitic helminths in *Boana pulchella* (Duméril & Bibron, 1841) (Anura: Hylidae) and their relation with host diet, body size, and habitat

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ABSTRACT

We analyzed the diet and helminthological fauna of the frog *Boana pulchella* from the extreme south of Brazil. A total of 100 males were collected from two wetland areas in the state of Rio Grande do Sul: Ilha dos Marinheiros (n = 50) in the Rio Grande municipality, and the UFPel/Embrapa (n = 50) in the Capão do Leão municipality. *Boana pulchella* food items and helminths found in different organs were identified and quantified. We analyzed the relationship between helminth assemblage and host diet, body size, and sampling sites. *Boana pulchella* presented a generalist diet, composed mainly of terrestrial insects, with diet richness higher at Ilha dos Marinheiros. Helminth fauna was composed of Nematoda, Cestoda, Digenea, and Acanthocephala, with no difference in helminth richness and abundance between sampling sites. However, the abundance of helminths presented a significant correlation with the volume of items found in the gastrointestinal contents of the anurans from UFPel/Embrapa-CL. Although for some helminth taxa there were significant differences in prevalence and mean intensity of infection among host size classes, the GLM (generalized linear model) between helminth abundance and anuran SVL (snout-vent length) was not significant. *Oxyascaris oxyascaris*, Cosmocercinae gen. spp., *Ochoterenella* sp., Diplostomidae gen. spp., *Pseudoacanthocephalus* sp., and *Centrorhynchus* sp. were the main taxa constituting the helminth assemblage associated to *B. pulchella* males at the sampling sites. The occurrence of helminths at larval and adult stages suggests that *B. pulchella* may occupy different trophic levels in the biological cycles of those helminths. This helminth parasitic fauna associated with *B. pulchella* is mainly composed of taxa with heteroxenous cycles involving several intermediate and paratenic hosts, which agrees with the observations of a typical generalist anuran diet in this species.

Key words: Anurans; Diet; Helminths; Nematoda; Cestoda; Digenea; Acanthocephala; Swamps; Brazil.

Introduction

Parasitic infections can be influenced by several factors related to the host including diet, body size or mass, age, behavior, hormones, as well as immunity and genetic diversity (Wegner *et al.*, 2003; Blanchet *et al.*, 2009). The complex life cycle of several helminth species may involve prey-predator interactions, and consequently, the analysis of host diet composition may provide hints of which helminth groups it can harbors (Brooks & Hoberg, 2000). Some studies on amphibian parasites suggest that they may become infected by gastrointestinal helminths through the ingestion of arthropods acting as intermediate or

paratenic hosts, which are the main items of the diet in most species (Duré *et al.*, 2004; Van *et al.*, 2006; Akani *et al.*, 2011; Klaion, 2011). Anuran body size is a relevant feature for parasitological studies, as it was observed that larger hosts usually present less nematode load, maybe due to enhanced resistance and/or more efficient physical protection mechanisms (Santos *et al.*, 2013).

Parasitological studies can provide indirect information about the environment since parasites can be viewed as indicators of environmental impacts (Sures, 2004). According to Mackenzie (2007), landscape alterations caused by anthropic actions can induce changes in parasite transmission, increasing or decreasing parasitism depending on the magnitude of the impact as well as the life history of the parasites and hosts. For example, eutrophication of aquatic environments can result in increased parasite populations (Spalding *et al.*, 1993; Coyner *et al.*, 2002).

Amphibians are important environmental regulators, contributing to the decomposition of organic matter and nutrient cycling, in addition to serving as bioindicators of pollution (Hocking & Babbit, 2014). Host and helminth community responses to environmental impacts may vary depending on the type and intensity of stressors, the life cycle of the parasite, and time of exposure to the stressors (Marcogliese, 2004). For amphibians, a global decline of many species and/or populations has been recorded in the past decades (Kelehear *et al.*, 2017; Guerrero & Yanez-Muñoz, 2018), which enhances the need for basic biological baseline information to be gathered.

Brazil harbors the highest anuran diversity worldwide with 1,155 known species distributed across 20 families (Frost, 2022). *Boana pulchella* (Duméril & Bibron, 1841) (Hylidae) is a frog commonly found in southern Brazil, inhabiting vegetation near water bodies in open areas, and is also present in Argentina, Uruguay, and Paraguay (Maneyro *et al.*, 2017; Frost, 2022). Its diet is composed mainly of insects such as dipterans and coleopterans, as well as spiders (Maneyro & Rosa, 2004; Solé & Pelz, 2007; Rosa *et al.*, 2011; Antoniazzi *et al.*, 2013). Studies on the interactions between endo/ectoparasites and *B. pulchella* are limited, with records of Monogenea (Vaucher, 1987), Cestoda larvae (Borteiro *et al.*, 2015) and intradermal mite larvae (Silveira *et al.*, 2019). Additionally, Draghi *et al.* (2020) studied the helmintofauna of this species

from two agroecosystem areas in Argentina. The goals of our study were to identify the parasitic helminth assemblage of *B. pulchella* in southern Brazil, and analyze its relationships with host diet, size, and sampling site.

Materials and methods

2.1 Study area

Fieldwork was carried out in two areas in the extreme south of Brazil, the municipalities of Capão do Leão (CL) (31°48'23" S and 52°25'07" W) and Rio Grande (RG) (32°00'00" S and 52°09'00" W). Both are in the coastal plains of the state of Rio Grande do Sul (Fig. 1). The regional climate is classified as humid subtropical with average temperatures ranging from 14.6°C in winter to 22°C in summer. Annual rainfall varies from 1,150 to 1,450 mm, and precipitation occurs all year round (Seeliger *et al.*, 1998).

Sampling in the CL was carried out in an area of Universidade Federal de Pelotas, and Empresa Brasileira de Pesquisa Agropecuária (UFPel/Embrapa-CL) (Fig. 1A). This area is characterized by temporary ponds with a predominance of herbaceous vegetation as well as aquatic macrophyte species (*e.g. Eichornia crassipes* and *Salvina herzogii*). The vegetation cover is mostly of native grasslands and there are patches of *Eucalyptus* spp. trees, as those bordering the studied pond. Sampling in the RG was carried out in Ilha dos Marinheiros (IM-RG) (Fig. 1B), a continental island located in the estuarine region of Lagoa dos Patos. The study site is characterized by the presence of ephemeral and permanent ponds which are formed on sandy terrain, with partially exposed dunes where grass and shrub vegetation are predominant. Macrophytes like those in CL can be found near the water bodies in this area.

2.2 Collection and morphometric characterization of anurans

Field trips were carried out from August to October 2016, and from March to July 2017, since no individuals were found during summer. Sampling at each site was made every thirty days, during the first hours after sunset. We searched for *B. pulchella* for up to 3 hours or until we had captured 10 individuals, detected mostly by their advertisement call. We collected a total of 100 male specimens (n = 50 at CL, and n = 50 at RG), that once captured were stored in plastic containers and euthanized in
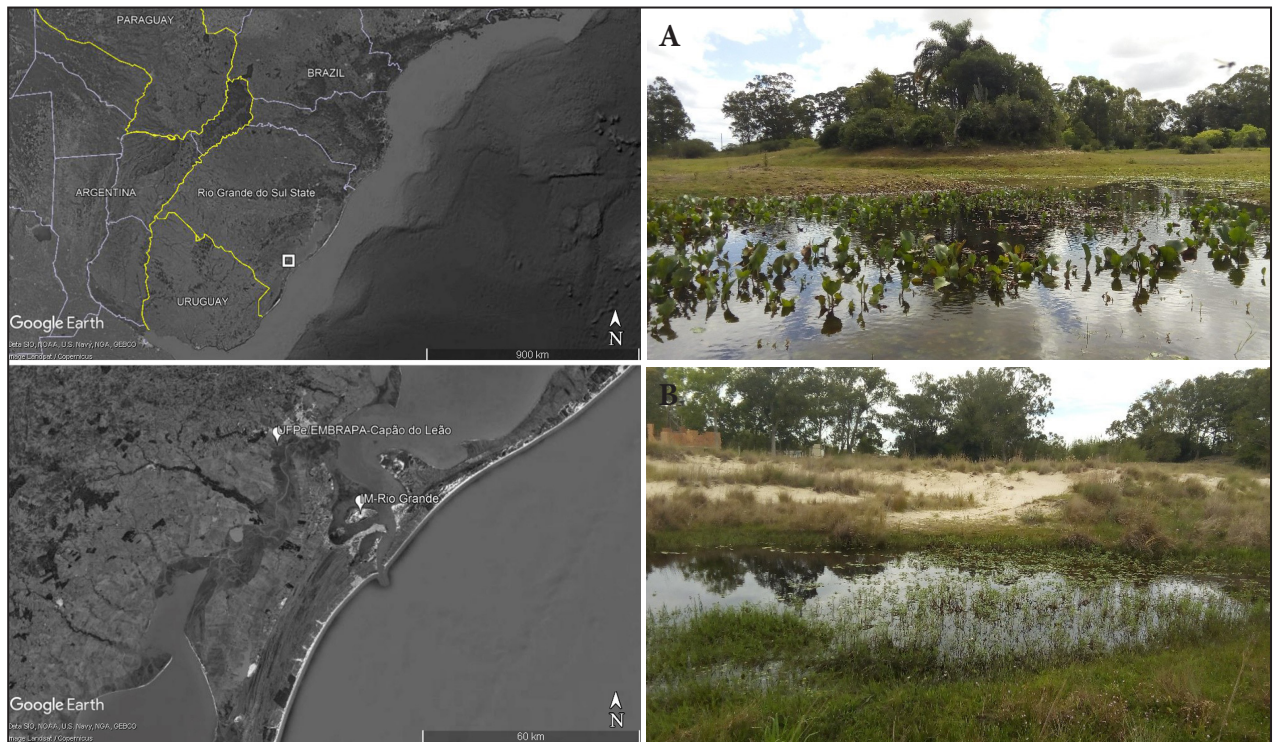
Figure 1. Collection sites of *Boana pulchella* (Duméril & Bribon, 1841) (Hylidae) in Universidade Federal de Pelotas, and Empresa Brasileira de Pesquisa Agropecuária (UFPel/Embrapa-CL), municipality of Capão do Leão (**A**) and Ilha dos Marinheiros (IM-RG), municipality of Rio Grande (**B**), Rio Grande do Sul State, southern of Brazil.

agreement with Resolution No. 1000 of the Federal Council of Veterinary Medicine (CFMV, 2012). The frogs were weighed, measured (snout-vent length), and refrigerated or frozen before necropsy. The study was licensed and approved by the Instituto Chico Mendes for Biodiversity Conservation (ICMBio no 43658-1), and approved by the Animal Ethics and Experimentation Commission (CEEA/UFPel no 6387 - 2016).

2.3 Qualitative and quantitative analysis of the diet
Host diet was investigated by analyzing the stomach and intestinal contents of 82 specimens (n = 50 from IM-RG, n = 32 from UFPel/Embrapa-CL). Food items were identified to the lowest taxonomic level using a stereomicroscope, based on Needham & Needham (1978) and Mugnai *et al.* (2010). The volume of each item, presented in ml, was estimated by measuring length, width, and height (Huckembeck *et al.*, 2014).

Frequency of occurrence (FO) and volume percentage (V) of food items were calculated respectively, as the percentage of digestive tracts in which the food item was found, and the relative volume (%) of a given item to the total volume of all food items found in the digestive tract. The alimentary index (AI) of each item was calculated as: AI = FO x V/Σ(FO x V) x 100, where Σ(FO x V) is the sum of the products of all food items (Kawakami & Vazzoler, 1980).

Feeding strategy and the importance of items in the diet of *B. pulchella* males were analyzed using a graphic method by Costello (1990). This method allowed us to identify the food ecology of predators through the relationship between the volume percentage (V) of a specific prey and its frequency of occurrence (FO). Food items were classified into broad groups, that is, Gastropoda, Arachnida, Crustacea, Entognatha, Insecta, and vegetable remains.

To compare the richness of the diet between the sampling sites, we used the rarefaction curve through incidence-type (Chao & Jost, 2012). This analysis was performed using the Inext package, in the R program (Hsieh *et al.*, 2016).

2.4 Collection, preparation, identification of helminths and infection parameters
All frogs were dissected searching for helminths, with particular attention to the oral cavity, esophagus, stomach, intestines, bladder, kidneys, testicles,

cloaca, lungs, heart, liver, and celomic cavity.

Helminths were fixed in AFA (70° ethanol, 37% formaldehyde, and acetic acid) for 24 hours and subsequently preserved in 70° ethanol. Some specimens of Digenea, Cestoda, and Acanthocephala were stained with Langeron's carmine or Delafield's hematoxylin and mounted in Canada balsam. Nematodes were clarified in Amann's lactophenol on semi-permanent slides for taxonomic identification (Amato & Amato, 2010).

Initial parasite identification was done according to Kiewiadomska (2002), Jones (2005) and Tkach (2008) for Digenea; Petrochenko (1971) for Acantocephala; Schmidt (1986) and Khalil *et al.* (1994) for Cestoda; and Chabaud (2009) and Anderson & Bain (2009) for Nematoda. Specific identification followed Bacher & Vaucher (1985), Freitas (1958) and Travassos (1920) for Nematoda, and Travassos *et al.* (1969) for Digenea. Voucher specimens were deposited in the Coleção de Helmintos do Laboratório de Parasitologia de Animais Silvestres (714 – 757 CHLAPASIL/UFPel), Rio Grande do Sul, Brazil, and Coleção Helmintológica do Instituto Oswaldo Cruz (39321, 39322, 39746a, 39746b, 39746c, 39747 CHIOC), Rio de Janeiro, Brazil. Prevalence (P), mean intensity of infection (MII), and mean abundance (MA) were estimated according to Bush *et al.* (1997).

2.5 Analysis of helminth assemblage relative to host diet, size, and sampling sites

Spearman correlation tests were performed to assess whether diet composition and volume are related to helminth abundance, using the Past software (Hammer *et al.*, 2001) and STATA v. 15.

Boana pulchella individuals were grouped into two size classes based on snout-vent length (SVL). Class I was composed of 46 individuals with SVL = 27-37 mm (33.02 ± 2.70), and Class II was composed of 54 individuals with SVL = 38-49 mm (41.55 ± 2.36). The relationships of helminth assemblage composition with host size classes and sampling sites were assessed using P and MII values tested through chi-square (X^2) and t-test (p≤0.05), respectively. The analyses were performed in the Quantitative Parasitology 3.0 software (Reiczigel *et al.*, 2019). The chi-square and t-test were done for cases in which helminths prevalence was ≥10% (Bush *et al.*, 1990).

A generalized linear model (GLM) analysis with a Poisson distribution and log link function was used to assess whether the abundance of parasites varied according to host body size and sampling sites, using the Stata software (StataCorp, 2007). Finally, a rarefaction curve was employed to compare helminth richness between sites (Chao & Jost, 2012; Hsieh *et al.*, 2016).

Results

3.1 Diet

Substantial gastrointestinal content was found in seventy anurans (85.4%). Animal and plant items, and also some of anthropic origin were identified (Table 1). The sampled males of *B. pulchella* displayed a generalist feeding strategy, mainly preying upon Insecta (Fig. 2). Coleoptera and Lepidoptera stood out among the insects with the highest alimentary indices (AI) being 20.9% and 5.1%. Diptera was present in 14.6% of anurans with an AI of 1.8%, and unidentified insects showed an AI of 26.4%. Araneae was the most frequent group among the Arachnida food items (14.6%), displaying the highest alimentary index (10.1%). Plant remains were recorded with a frequency of 35.4% and an AI of 28% (Table 1).

Through the rarefaction curve it was observed that the diet richness was higher in the IM-RG population because their confidence intervals did not overlap (Fig. 3). Coleoptera was the group with the highest FO (32%) and AI (28.2%) among prey items. Diptera and Isopoda were also significant food items. Araneae was also important, with a FO of 18% and AI of 8.6%. Plant remains were found in 30% of the anurans, with an AI of 24.1% (Table 1).
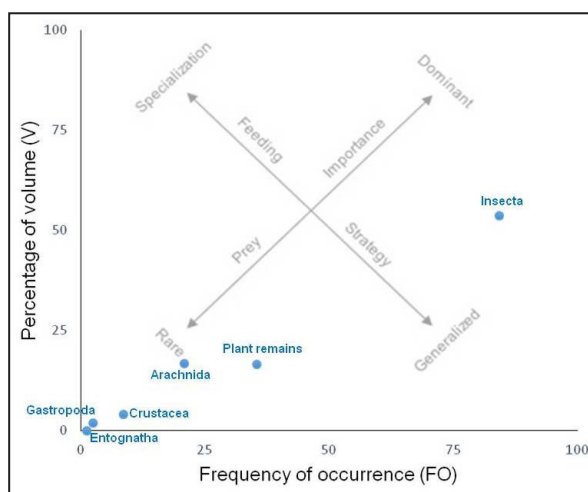

Figure 2. Graphic representation of Costello (1990) of the frequency of occurrence (FO) and percentage of volume (V) of the groups of food items found in the diet of *Boana pulchella* (Anura: Hylidae) males in southern Brazil.

Table 1. Frequency of occurrence (FO), volume (V) and alimentary index (AI) for the items found in the gastrointestinal contents of *Boana pulchella* (Anura: Hylidae) males from two sampling sites in southern Brazil. The values are presented as percentage.

ITEMS	TOTAL SAMPLE (n=82)			SAMPLING SITES					
				IM-RG (n=50)			UFPel/Embrapa-CL (n=32)		
	FO	V	AI	FO	V	AI	FO	V	AI
GASTROPODA	2.4	1.9	0.2	2	2.6	0.2	3.1	0.6	0.1
ARACHNIDA									
Acari	4.9	0.1	0.02	8	0.1	0.04	-	-	-
Araneae	14.6	14.4	10.1	18	10.9	8.6	9.4	20	9.8
Opiliones	1.2	2.6	0.2	2	4.3	0.4	-	-	-
CRUSTACEA									
Isopoda	8.5	4.2	1.7	14	6.8	4.2	-	-	-
ENTOGNATHA									
Collembola	1.2	0.03	<0.00	2	0.04	<0.00	-	-	-
INSECTA									
Hymenoptera	2.4	0.1	0.02	4	0.2	0.04	-	-	-
Hemiptera	4.9	3.1	0.7	2	0.4	0.03	9.4	7.4	3.6
Coleoptera	23.2	18.2	20.9	32	20.1	28.2	18.8	15.2	14.8
Diptera	14.6	2.6	1.8	24	4.2	4.4	-	-	-
Lepidoptera (larvae)	9.8	10.8	5.1	8	7.5	2.6	12.5	16.2	10.5
Unidentified insects	29.3	18.8	26.4	32	16.9	23.7	25	21.9	28.5
Unidentified arthropods	9.8	4.3	2	12	6.3	3.3	6.3	1.2	0.4
Plant remains	35.4	16.5	28	30	18.3	24.1	43.6	13.7	31.1
Anthropic material (synthetic)	1.2	0.2	0.01	2	0.4	0.03	-	-	-
Unidentified items	3.7	2.1	0.4	4	1.0	0.2	6.3	3.9	1.3
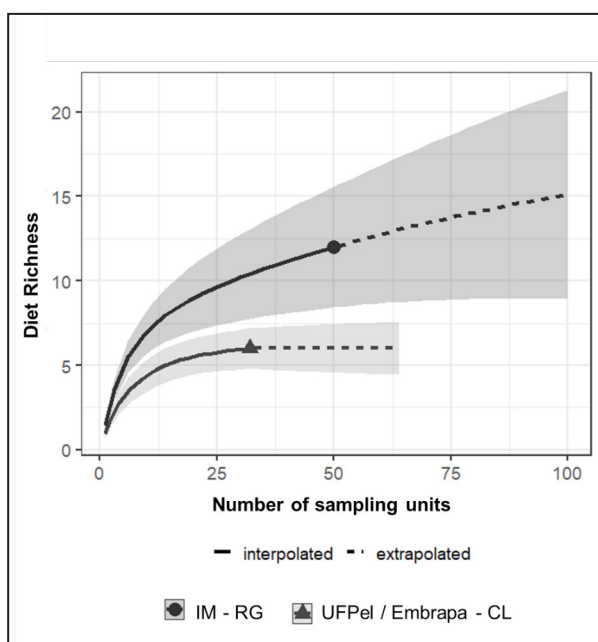
Figure 3. Rarefaction curve of the diet richness of *Boana pulchella* (Anura: Hylidae) males in two sampling sites (IM-RG, and UFPel/Embrapa-CL) in southern Brazil

The diet of UFPel/Embrapa-CL anurans included plant remains with the highest FO (43.6%) and AI (31.1%). Coleoptera at this site (FO of 18.8% and AI of 14.8%) and Lepidoptera (FO of 12.5% and AI of 10.5%) were the most important items of animal origin. Araneae was found with low FO (9.4%) and AI (9.8%), however it displayed a relatively high V (20%) (Table 1).

3.2 Helminth assemblage
Eighty-seven anurans (87%) presented helminths in the following sites of infection: lungs, liver, kidneys, stomach, intestines, and free or encysted in the celomatic cavity. Helminths belonging to Nematoda, Cestoda, Digenea, and Acanthocephala were identified, represented by an adult and larval forms (Table 2).

Nematoda was the taxonomic group with the highest richness in the parasitic assemblage of *B. pulchella*. Cosmocercinae gen. spp. had the highest prevalence (P) and mean intensity of infection (MII)

Table 2. Helminths of *Boana pulchella* (Anura: Hylidae) males in southern Brazil, and their respective sites of infestation and parasitological parameters (prevalence – P, as %, mean intensity of infection - MII, mean abundance – MA, and range - R).

HELMINTHS	SITES OF INFESTATION	P	MII	MA	R
NEMATODA					
Oxyascaris oxyascaris	Intestine	18	1.50	0.27	1-3
Falcaustra sp.	Intestine	1	1.00	0.01	1
Cosmocercinae gen. spp.	Intestine	33	5.88	1.94	1-31
Rhabdias sp.	Lung	2	1.00	0.02	1
Ochoterenella sp.	Intestine and coelomic cavity	10	3.80	0.38	1-12
Physaloptera sp. (larva)	Stomach	1	1.00	0.01	1
Acuariinae gen. sp. (larvae)	Liver, stomach, intestine and coelomic cavity	8	1.38	0.11	1-4
Unidentified larva	Intestine	1	1.00	0.01	1
CESTODA					
Cyclophyllidea gen. sp.	Intestine	1	1.00	0.01	1
Unidentified larva	Coelomic cavity	1	1.00	0.01	1
DIGENEA					
Haematoloechus ozorioi	Lung	1	1.00	0.01	1
Catadiscus sp.	Intestine	5	1.60	0.08	1-2
Diplostomidae gen. spp. (metacercariae)	Kidney	32	4.19	1.34	1-21
Crassiphialinae gen. sp. (metacercaria)	Coelomic cavity	1	1.00	0.01	1
ACANTHOCEPHALA					
Pseudoacanthocephalus sp.	Intestine	27	2.22	0.60	1-7
Centrorhynchus sp. (cystacanths)	Stomach, intestine and coelomic cavity	59	6.68	3.94	1-41

(33%; 5.88 helminths/host), followed by *Oxyacaris oxyascaris* Travassos, 1920 (18%; 1.50 helminths/host) and *Ochoterenella* sp. (10%; 3.80 helminths/host). *Rhabdias* sp. and *Falcaustra* sp. were found with P of 2% and 1%, respectively. Acuariinae stood out among the larval forms with a prevalence of 8% and an MII of 1.38 larvae/host, contrasting with the other taxa, which displayed an MII of 1 larva/host (Table 2).

Regarding Digenea, metacercariae of Diplostomidae parasitized the kidney of 32% of anurans with MII of 4.19 helminth/host, followed by *Catadiscus* sp. whose prevalence was 5% and MII of 1.60 helminth/host, while metacercaria of Crassiphialinae gen. sp. and *Haematoloechus ozorioi* Freitas & Lent, 1939 were found in 1% of anurans with MII of 1 helminth/host (Table 2). Acanthocephala and Cestoda were represented by two taxa each (Table 2). *Centrorhynchus* sp. (cystacanths) infected 59% of anurans with MII of 6.68 helminths/host, while *Pseudoacanthocephalus* sp. was found with a prevalence of 27% and MII of 2.22 helminths/host. Cestoda was represented by a specimen of Cyclophyllidea gen. sp. and an unidentified larva (Table 2).

3.3 *Boana pulchella* helminth assemblage: aspects related to diet and host size, and sampling sites

The abundance of helminths presented a significant correlation only with the volume of items found in the gastrointestinal contents of the anurans from UFPel/Embrapa-CL ($r^2 = 0.50$, p <0.01).

Analyses on helminth assemblage and host size revealed that *O. oxyascaris* and Cosmocercinae were significantly more prevalent in the anurans belonging to Class I, with P of 26.1% (X^2, p = 0.026) and 39.1% (X^2, p = 0.022) respectively. Acuarinae larvae and *Pseudoacanthocephalus* sp. had higher prevalences in Class II hosts, of 13% (X^2, p = 0.047) and 35.2% (X^2, p = 0.046) respectively (Table 3). Only the Cosmocercinae showed significant differences in the mean intensity of infection between the two size classes with greater MII for Class I (9.50 helminths/host) (t-test, p = 0.0275) (Table 3). No significant differences were found in P and MII between host size classes infected by Digeneans belonging to Diplostomidae and *Centrorhynchus* sp. cystacanths (Table 3). Infections by the other helminths could not be compared as they were not detected in both classes. It is interesting to highlight that *Ochoterene-*

Table 3. Prevalence (P, as %), mean intensity of infestation (MII), mean abundance (MA) and range (R) of parasitic helminths of *Boana pulchella* (Anura: Hylidae) males in relation to the size classes (snout-vent length, SVL) in southern of Brazil.

HELMINTHS	SVL							
	Class I (27- 37mm) (n=46)				Class II (38 - 49mm) (n=54)			
	P	MII	MA	R	P	MII	MA	R
NEMATODA								
Oxyascaris oxyascaris	26.1[a]	1.50	0.41	1-3	9.3[a]	1.60	0.19	1-4
Falcaustra sp.	2.2	1.00	0.02	1	-	-	-	-
Cosmocercinae gen. spp.	39.1[b]	9.50[bb]	3.83	1-31	18.5[b]	1.80[bb]	0.33	1-5
Rhabdias sp.	-	-	0	-	3.7	1.00	0.04	1
Ochoterenella sp.	-	-	0	-	18.5	3.80	0.70	1-12
Physaloptera sp. (larva)	-	-	0	-	1.9	1.00	0.02	1
Acuariinae gen. sp. (larvae)	2.2[c]	1.00	0.02	1	13[c]	1.43	0.19	1-4
Unidentified larva	2.2	1.00	0.02	1	-	-	0	-
CESTODA								
Ciclophyllidea gen. sp.	2.2	1.00	0.02	1	-	-	0	-
Unidentified larva	-	-	0	-	1.9	1.00	0.02	1
DIGENEA								
Haematoloechus ozorioi	2.2	1.00	0.02	1	-	-	0	-
Catadiscus sp.	10.9	1.60	0.17	1-2	-	-	0	-
Diplostomidae gen. spp. (metacercariae)	37	5.12	1.89	1-14	27.8	3.20	0.89	1-21
Crassiphialinae gen. sp. (metacercaria)	-	-	0	-	1.9	1.00	0.02	1
ACANTHOCEPHALA								
Pseudoacanthocephalus sp.	17.4[d]	1.63	0.28	1-2	35.2[d]	2.47	0.87	1-7
Centrorhynchus sp. (cystacanths)	56.5	6.73	3.80	1-41	61.1	6.64	4.06	1-39
Centrorhynchus sp. (cystacanths)	56.5	6.73	3.80	1-41	61.1	6.64	4.06	1-39

a - X^2, p = 0.026; b - X^2, p = 0.022; c - X^2, p = 0.047; d - X^2, p = 0.046; bb - t-test, p = 0.0275

lla sp. was found only in Class II anurans, with a P of 18.5% and an MII of 3.8 helminths/host. Besides, *Catadiscus* sp. was present in only 10.9% of Class I hosts, with an MII of 1.60 (Table 3). Although there were significant differences in the prevalence and mean intensity of infection for taxa among host size classes, the GLM between helminth abundance and anuran SVL was not significant (z = -1.55, p = 0.120).

The richness of helminths was not different between the sampling sites (Fig. 4). However, there was a significant difference in the prevalence of *O. oxyascaris*, Cosmocercinae gen. spp. and *Ochoterenella* sp. between the two sampled sites. A higher prevalence of the first two taxa was observed in anurans from IM-RG (28%; X^2, p = 0.009, and 52%; X^2, p < 0.000, respectively), while *Ochoterenella* sp. was found with a higher prevalence (18%; X^2, p < 0.008) in anurans from UFPel/Embrapa-CL. The mean intensity of Cosmocercinae gen. spp. was significantly higher in anurans from IM-RG (7.08 helminth/host; t-test, p = 0.0205) (Table 4). *Catadiscus* sp. occurred only in IM-RG parasitizing 10% of the hosts with an MII of 1.6 helminths/host. In general, although there is a variation in the prevalence of some taxa between sampled sites, the abundance of helminths did not vary significantly between the two localities (z = 1.76, p = 0.079).

Discussion

The study of the helminth fauna of *B. pulchella* in southern Brazil showed a greater number of taxa than a previous study in Buenos Aires province, Argentina, by Draghi *et al.* (2020). The prevalence of parasitism found by these authors was also lower as they recorded 35% of 150 hosts parasitized by at least one helminth species. Those differences may be associated with the characteristics of the sampling
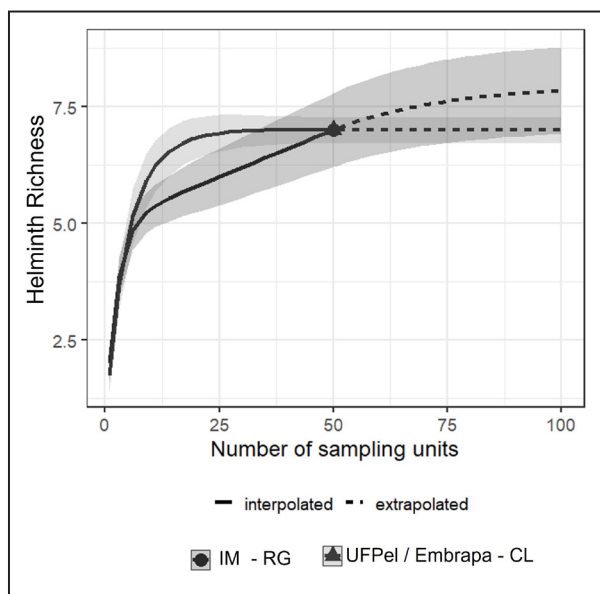
Figure 4. Rarefaction curve of the parasitic helminth richness of *Boana pulchella* (Anura: Hylidae) males in two sampling sites (IM-RG, and UFPel/Embrapa-CL) in southern Brazil.

sites, since Draghi *et al.* (2020) sampled cultivated and pasture areas.

Most parasitic helminths of *B. pulchella* have heteroxenous cycles that involve animals of several groups as intermediate and paratenic hosts, including anurans (Table 5). The generalist diet of *B. pulchella* observed in this study (insects, crustaceans, mollusks, and vegetation) is similar to previous ones (Maneyro & Rosa, 2004; Solé & Pelz, 2007; Rosa *et al.*, 2011; Antoniazzi *et al.*, 2013), and may provide the link between free-living forms of helminths and anurans. In this sense, studies on the diet and helminth fauna of anurans provide complementary information to improve our understanding of host-parasite relationships.

Some of the observed parasitic infections may be considered occasional since the prevalence of most helminths was less than 10%, and the intensity of infection of 11 taxa ranged from 1 to 7 helminths. The low rates of *O. oxyascaris*, *Falcaustra* sp., *Physaloptera* sp., Acuariinae gen. sp., *H. ozorioi*, and Cestoda may be related to the low consumption of intermediate and paratenic hosts that probably do not constitute prey commonly ingested by *B. pulchella* due to availability or preference bias for certain items. On the other hand, the infection rates of acanthocephalans may be related to the ingestion of arthropods, which were well represented in the diet of this species at both sampling sites. According to the literature, other taxa that compose the

helminth parasitic assemblage of *B. pulchella* (Cosmocercinae gen. spp., *Rhabdias* sp., *Catadiscus* sp. and Diplostomidae gen. spp.) can infect the anuran through the ingestion of immature forms during the tadpole phase or through active penetration of the skin (Table 5).

The infections by metacercariae of Diplostomidae and by cystacanths of *Centrorhynchus* sp. suggest that *B. pulchella* acts as an intermediate host for digeneans and as a paratenic host for the acanthocephalans since the above mentioned larval forms were found with expressive P% and MII values. This anuran act as a trophic bridge between the hosts at the lower level (invertebrates) and the hosts at the top of the trophic chain (birds and mammals, for example). The latter may acquire infections by ingesting anurans, thus ensuring the continuity of the parasitic cycle (Table 5). *Centrorhynchus* species, for example, were reported in natural predators of anurans, such as *Rupornis magnirostris* (Gmelin, 1788) (Accipitriformes) (Machado, 1940; Moura *et al.*, 2017), and *Guira guira* (Gmelin, 1788) (Cuculiformes) (Lunaschi & Drago, 2010). On the other hand, a study carried out in agroecosystems in Argentina reported neither cystacanths nor metacercariae associated with *B. pulchella* (Draghi *et al.*, 2020). The different land use in the sampling sites of Brazil (present study) and Argentina (Draghi *et al.*, 2020) may have influenced the parasitic infections in *B. pulchella*, since in the firstwe had no crops or livestock, as in Argentina. The sampling sites of the present study consist of temporary ponds with similar vegetation, to which anurans were closely associated, which may have contributed to the similarity in the richness and abundance of parasitic helminths.

It is important to highlight that parasite-host relationships can be influenced by several other factors. Intrinsic characteristics related to hosts (e.g. maturity, gender, reproductive behaviour, size, diet) (Poulin, 1996; Von Zuben, 1997; Wilson *et al.*, 2002; Klein, 2004) and parasites (e.g. size, quantity, fecundity rate, reproductive modes, dispersal ability) (Crofton, 1971; Von Zuben, 1997; Wilson et al, 2002; Khokhlova et al, 2010), as well as extrinsic factors (e.g. temperature, humidity, anthropogenic changes) (Marcogliese, 2005; Hudson *et al.* 2006; Koprivnikar *et al.*, 2012), can act on the complex network of parasite-host-environment interactions.

Similarly to the present study, Draghi *et al.* (2020) found no relationship between host body

Table 4. Prevalence (P, as %), mean intensity of infestation (MII), mean abundance (MA) and range (R) of parasitic helminths of *Boana pulchella* (Anura: Hylidae) males in two sampling sites from southern Brazil.

HELMINTHS	SAMPLING SITES							
	IM-RG (n=50)				UFPel/Embrapa-CL (n=50)			
	P	MII	MA	R	P	MII	MA	R
NEMATODA								
Oxyascaris oxyascaris	28[a]	1.43	0.40	1-3	8[a]	1.75	0.14	1-3
Falcaustra sp.	2	1.00	0.02	1	-	-	0	-
Cosmocercinae gen. spp.	52[b]	7.08[bb]	3.68	1-31	14[b]	1.43[bb]	0.20	1-3
Rhabdias sp.	4	1.00	0.04	1	-	-	0	-
Ochoterenella sp.	2[c]	1.00	0.04	1	18[c]	4.11	0.74	1-12
Physaloptera sp. (larva)	2	1.00	0.02	1	-	-	0	-
Acuariinae gen. sp. (larvae)	2	1.00	0.02	1	14	1.43	0.20	1-4
Unidentified larva	2	1.00	0.02	1	-	-	0	-
CESTODA								
Ciclophyllidea gen. sp.	2	1.00	0.02	1	-	-	0	-
Unidentified larva	-	-	0	-	2	1.00	0.02	1
DIGENEA								
Haematoloechus ozorioi	2	1.00	0.02	1	-	-	0	-
Catadiscus sp.	10	1.60	0.16	1-2	-	-	0	-
Diplostomidae gen. spp. (metacercariae)	36	5.17	1.86	1-14	28	3.00	0.82	1-21
Crassiphialinae gen. sp. (metacercaria)	-	-	0	-	2	1	0.02	1
ACANTHOCEPHALA								
Pseudoacanthocephalus sp.	22	2.09	0.46	1-5	32	2.31	0.74	1-7
Centrorhynchus sp. (cystacanths)	58	6.69	3.88	1-41	60	6.67	4.00	1-39

a - X^2, p = 0.009; b - X^2, p = < 0.000; c - X^2, p = 0.008; bb - t-test, p = 0.0205

Table 5. Helminth parasites of *Boana pulchella* (Anura: Hylidae) males in southern Brazil and their respective hosts and possible means of infestation, considering the information available for life cycles of similar species or species of the same family.

Helminths	Definitive Host	Intermediate host (IH)/ paratenic host (PH)	Possible means of infestation in anurans	References
Nematoda				
Oxyascaris oxyascaris	Amphibians	Direct cycle – Invertebrates (PH)	Ingestion of PH	Anderson (2000)
Falcaustra sp.	Amphibians, and reptiles	Direct cycle – Freshwater mollusk (PH)	Ingestion of PH	Anderson (2000)
Cosmocercinae	Amphibians	Direct cycle	Ingestion of larvae by tadpoles or skin penetration	Anderson (2000)
Rhabdias sp.	Amphibians, and reptiles	Direct cycle	Penetration of the larvae across the skin	Anderson (2000)
Ochoterenella sp.	Anurans	Mosquito (IH)	Ingestion of IH	Anderson (2000)

Physaloptera sp.	Birds, mammals, reptiles, fish and amphibians	Insects (crickets, cockroaches) (IH)	Ingestion of IH	Anderson (2000)
Acuariinae	Birds, and mammals	Insects (Coleoptera, Orthoptera) (IH)/Amphibians and Reptiles (PH)	Ingestion of IH	Anderson (2000)
Cestoda	Vertebrates	Vertebrates and Invertebrates (IH)	Ingestion of IH	Olsen (1974)
Digenea				
Haematoloechus ozorioi	Anurans	Freshwater mollusk (1st IH), and Odonata (2nd IH)	Ingestion of 2nd IH	Olsen (1974)
Catadiscus sp.	Anurans	Mollusk Planorbidae (IH)	Tadpole ingests metaceraria present in aquatic vegetation	Kehr & Hamann (2003)
Diplostomatidae	Birds, and mammals	Freshwater mollusk (1st IH), fish, amphibians and occasionally mammals (2nd IH)	Penetration of the cercaria in the tadpole	Travassos et al. (1969), Hamann & Gonzáles (2009)
Acanthocephala				
Pseudoacanthocephalus sp.	Amphibians and reptiles	Aquatic arthropod (IH)	Ingestion of IH	Barger & Nickol (1998)
Centrorhynchus sp.	Birds, and mammals	Crustaceans or Insects (IH), Amphibians and reptiles (PH)	Ingestion of IH	Travassos (1926), Petrochenko (1971), Schmidt (1985), Amato et al. (2003)

1st IH=first intermediate host, 2nd IH=second intermediate host

size and helminth abundance. Host body size has also been considered a good indicator of parasite species richness since larger hosts may provide more space and other resources, possibly widening niche diversity for parasites (Campião *et al.*, 2016). In addition, larger hosts live longer, establishing in less ephemeral habitats than smaller species and may have enhanced exposure to parasites (Poulin, 1997).

According to our findings is possible to conclude that *Boana pulchella* males present a generalist feeding strategy with a diet composed of a wide variety of arthropods, mainly terrestrial insects. Their diverse helminth fauna was composed of larval and adult forms, indicating that at least males of this anuran may act as definitive, intermediate, as well as paratenic hosts. The generalist diet and the association of the species with the vegetation close to aquatic environments are important factors for the persistence and the life cycle of its parasitic

helminths. *Oxyascaris oxyascaris*, Cosmocercinae gen. spp., *Ochoterenella* sp., Diplostomidae gen. spp., *Pseudoacanthocephalus* sp., and *Centrorhynchus* sp. are the main taxa of this assemblage.

Literature cited

Aho, J.M. 1990. Helminth communities of amphibians and

reptiles: comparative approaches to understanding patterns and processes, p.157-195. *In*: Esch GW, Bush 276 AO, Aho JM (eds.) Parasite communities: patterns and processes. Chapman and Hall, London.

Akani, G.C.; Luiselli, L.; Amuzie, C.C. & Wokem, G.N. 2011. Helminth community structure and diet of three Afrotropical anuran species: a test of the interactive-versus-isolationist parasite communities hypothesis. *Web Ecology* 11: 11–19.

Amato, J.F.R.; Amato, S.B.; Araujo, P.B. & Quadros, A.F. 2003. First report of pigmentation dystrophy in terrestrial isopods, *Atlantoscia floridana* (van Name) (Isopoda, Oniscidea), induced by larval acanthocephalans. *Revista Brasileira de Zoologia* 20: 711–716.

Amato, J.F.R. & Amato, S.B. 2010. Técnicas gerais para coleta e preparação de helmintos endoparasitos de aves: 367–393. *In*: Von Matter S., F. C. Straube, I. Accordi, V. Piacentini, J. F. Cândido-Jr (ed.), Ornitologia e Conservação: Ciência Aplicada, Técnicas de Pesquisa e Levantamento. Technical Books Editora, Rio de Janeiro.

Anderson, R. C. 2000. Nematode parasites of vertebrates: their development and transmission. 2ª Ed. CABI International.

Anderson, R.C. & Bain, O. 2009. Capítulo 3: Rhaditida. *In*: Key to the Nematode Parasites of Vertebrates. CAB International, Massachusetts.

Antoniazzi, C.E.; López, J.A.; Duré, M. & Falico, D.A. 2013. Alimentación de dos especies de anfibios (Anura: Hylidae) en la estación de bajas temperaturas y su relación con la acumulación de energía en Santa Fe, Argentina. *Revista de Biología Tropical* 61: 875–886.

Bacher, M.R. & Vaucher, C. 1985. Parasitic helminths from Paraguay VII: systematic position of *Oxyascaris* Travassos, 1920 (Nematoda: Cosmocercoidea). *Revue Suisse de Zoologie* 92: 303–310.

Barger, M.A. & Nicko, B.B. 1998. Structure of *Leptorhynchoides thecatus* and *Pomphorhynchus bulbocolli* (Acanthocephala) eggs in habitat partitioning and transmission. *Journal of Parasitology* 84: 534-537.

Blanchet, S.; Rey, O.; Berthier, P.; Lek, S. & Loot, G. 2009. Evidence of parasite-mediated disruptive selection on genetic diversity in a wild fish population. *Molecular Ecology* 18: 1112–1123.

Borteiro, C., Castro, O., Sabalsagaray, M.J., Kolenc, F.; Debat, C.M. & Ubilla, M. 2015. Spargana in the Neotropical frog *Hypsiboas pulchellus* (Hylidae) from Uruguay. *North-Western Journal of Zoology* 11: 171–173.

Brooks, D.R. & Hoberg, E.P. 2000. Triage for the biosphere: the need and rationale for taxonomic inventories and phylogenetic studies of parasites. *Comparative Parasitology* 67: 1–21.

Bush, A.O.; Aho, J.M. & Kennedy, C.R. 1990. Ecological versus phylogenetic determinants of helminth parasite community richness. *Evolutionary Ecology* 4: 1–20.

Bush, A.O.; Lafferty, K.D., Lotz, J.M. & Shostar, A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* Revisited. *Journal of Parasitology* 83: 575–583.

Campião, K.M.; Dias, O.T.; Silva, R.J.; Ferreira, V.L. & Tavares, L.E.R. 2016. Living apart and having similar trouble: frog helminth parasites determined by the host or by the habitat? *Canadian Journal of Zoology* 94: 761–765.

Chabaud, A.G. 2009. Capítulo 9: Ascaridida. *In*: Key to the Nematode Parasites of Vertebrates. CAB International, Massachusetts

Chao, A. & Jost, L. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology*, 93: 2533-2547.

Costello, M.J. 1990. Predator feeding strategy and prey importance: a new graphical analysis. *Journal of Fish Biology* 36: 261-263.

Coyner, D.F.; Spalding, M.G. & Forrester, D. J. 2002. Epizootiology of *Eustrongylides ignotus* in Florida: distribution, density, and natural infections in intermediate hosts. *Journal of Wildlife Diseases* 38: 483-499.

CRMV. 2012. Conselho Federal de Medicina Veterinária. Available at: http://portal.cfmv.gov.br/ Accessed in: 25 September 2015.

Crofton, H.D. 1971. A quantitative approach to parasitism. *Parasitology* 62: 179-194.

Draghi, R.; Drago, F.B.; Saibene, P.E. & Agostini, M.G. 2020. Helminth communities from amphibians inhabiting agroecosystems in the Pampean Region (Argentina) *Revue suisse de Zoologie* 127: 261-274.

Duré, M.I.; Schaefer, E.F; Hamann, M.I. & Kehr, A.I. 2004. Consideraciones ecológicas sobre la dieta, la reprodución y el parasitismo de *Psedopaludicola boliviana* (Anura, Leptodactylidae) de Corrientes, Argentina. *Phylomedusa* 3: 121-131.

Freitas, J.F.T. 1958. Estudos sobre "Oxyascarididae" (Travassos, 1920) (Nematoda, Subuluroidea). *Memórias do Instituto Oswaldo Cruz* 56: 489–515.

Frost, D.R. 2022. Amphibian Species of the World: an Online Reference. Version 6.1 (Accessed June 20, 2022). Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA.

Guerrero, R. & Yánez-Muñoz, M.H. 2018. Acarine biodiversity in Ecuador: two new species of endoparasitic chiggers (Acarina: Trombiculidae) from terrestrial andean anurans. *MANTER: Journal of Parasite Biodiversity* 9: 18.

Hamann, M.I. & González, C.E. 2009. Larval digenetic trematodes in tadpoles of six amphibian species from northeastern Argentina. *Journal of Parasitology* 95: 623-628.

Hammer Ø.;, Harper, D.A.T. & Ryan, P.D. 2001 PAST: Paleontological Statistics Software Package for Education and Data Analysis. Paleontological Statistics, PAST, 2.16. Available at: https://palaeo-electronica.org/2001_1/past/issue1_01.htm Accessed 10 March 2020.

Hocking, D.J. & Babbitt, K.J. 2014. Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology* 9: 1-17.

Hsieh, T. C., Ma, K. H. & Chao, A. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). *Methods in Ecology and Evolution* 7: 1451-1456.

Huckembeck, S.; Loebmann, D.; Albertoni, E.F.; Hefler, S.M.; Oliveira, M.C.L.M. & Garcia, A. 2014. Feeding ecology and basal food sources that sustain the Paradoxal frog *Pseudis minuta*: Multiple approaches combining stomach content, prey availability, and stable isotopes. *Hydrobiologia* 740: 253-264.

Hudson, P.J.; Dobson, A.P. & Lafferty, K.D. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution* 21: 38-385.

Jones, A. 2005. Capítulo 25: Diplodiscidae Cohn, 1904. *In*: Key to the Trematoda. Volume 2. CABI Publishing, Massachusetts.

Kawakami, E. & Vazzoler, C. 1980. Método gráfico e estimative de índice alimentar aplicado no estudo de alimentação de peixes. *Boletim do Instituto Oceanográfico* 29: 205-207.

Kehr, AI. & Hamann, M.I. 2003. Ecological aspects of parasitism in the tadpole of *Pseudis paradoxa* from Argentina. *Herpetological Review* 34: 336-341.

Kelehear, C.; Hudson, C.M.; Mertins, J.W. & Shine, R. 2017. First report of exotic ticks (*Amblyomma rotundatum*) parasitizing invasive cane toads (*Rhinella marina*) on the Island of Hawai'i. *Ticks and tick-borne diseases* 8: 330-333.

Khalil, L.F.; Jones, A. & Bray, R.A. 1994. Order Cyclophyllidea (diagnosis and key to families). *In*: Keys to the cestode parasites of vertebrates. CAB International, Wallingfors, U.K.

Khokhlova, I.S.; Serobyan, V.; Degen, A.A.; Krasnov, B.R. 2010. Host gender e offspring quality in a flea parasitic on a rodent. *The Journal of Experimental Biology* 213: 3299-3304.

Kiewiadomska, K. 2002. Família Diplostomidae. In: Gibson, D.I.; Jones, A. & Bray, R.A. Keys to the Trematoda – Volume I. CABI International e The Natural History Museum, Londres, UK.

Klaion, T. 2011. Diet and nematode infection in *Proceratoprhys boiei* (Anura: Cycloramphidae) from two Atlantic rainforest remnants in Southeastern Brazil. *Anais da Academia Brasileira de Ciências* 83: 1303-1312.

Klein, S.L. 2004. Hormonal e immunological mechanisms mediating sex differences in parasitic infection. *Parasite Immunology* 26: 247–264.

Koprivnikar, J.; Marcogliese, D.J.; Rohr, J.R.; Orlofske, S.A.; Raffel, T.R. & Johnson P.T.J. 2012. Macroparasite infections of amphibians: what can they tell us? *EcoHealth* 9: 342-360.

Lunaschi, L.I. & Drago, F.B. 2010. A new species of *Centrorhynchus* (Acanthocephala, Centrorhynchidae) endoparasite of *Guira guira* (Aves, Cuculidae) from Argentina. *Helminthologia* 41: 38-47.

Machado, D.A.F. 1940. Pesquisas helmintológicas realizadas no estado de Mato Grosso – Acanthocephala. *Memórias do Instituto Oswaldo Cruz* 35: 593-601.

Mackenzie, J.V. 2007. Human land use and patterns of parasitism in tropical anphibian host. *Biological Conservation* 137: 102-116.

Maneyro, R. & Rosa, I. 2004. Temporal and spatial changes in the diet of *Hyla pulchella* (Anura, Hylidae) in southern Uruguay. *Phyllomedusa* 3: 101-113.

Maneyro, R.; Loebmann, D.; Tozetti, A. & Fonte, L.F.M. 2017. Anfíbios das planícies costeiras do extremo sul do Brasil e Uruguai. Anolis Books Editora, 176 pp.

Marcogliese, D.J. 2004. Parasites: Small Players with Crucial Roles in the Ecological Theater. *EcoHealth* 1: 151-164.

Marcogliese D.J. 2005. Parasites of the superorganism: are they indicators of ecosystem health? *International Journal for Parasitology* 35: 705-716.

Moura, M.O.F.L.; Bregue, S.B. & Valente, A.L.S. 2017. Levantamento de helmintos de aves de rapina de Pelotas, Rio Grande do Sul. XXVI Congresso de Iniciação Científica, Pelotas, 4 pp.

Mugnai, R.; Nessimian, J.L & Baptista, D.F. 2010. Manual de identificação de macroinvertebrados aquáticos do estado do Rio de Janeiro. Technical Books Editora, 174 pp.

Needham, J.G. & Needham, P.R. 1978. Guía para el estudio de los seres vivos de las aguas dulces. Editora Reverté, S.A., 131 pp.

Olsen, O.W. 1974. Animal parasites: their life cycles and ecology. Third Edition.

Petrochenko, V.I. 1971. Acanthocephala of domestic and wild animals. Israel Program for Scientific Translations, 465 pp.

Poulin, R. 1996. Sexual inequalities in helminth infections: a cost of being male? *The American Naturalist* 147: 287-295.

Poulin, R. 1997. Species richness of parasite assemblages: evolution and patterns. *Annual Review of Ecology and Systematics* 28: 341-358.

Reiczigel, J.; Marozzi, M.; Fábián, I. & Rózsa, L. 2019. Biostatistics for parasitologists – a primer to Quantitative Parasitology. *Trends in Parasitology* 35: 277-281.

Rosa, A.C.; Maneyro, R. & Camargo, A. 2011. Trophic niche variation and individual specialization in *Hypsiboas pulchellus* (Duméril and Bibron, 1841) (Anura, Hylidae) from Uruguay. *South American Journal of Herpetology* 6: 98-106.

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

Seeliger, U.; Odebrecht, C. & Castello, J.P. 1998. Fluxo de energia e habitats no estuário da Lagoa dos Patos: 326. *In*: Seeliger *et al.* Os ecossistemas costeiro e marinho do extremo sul do Brasil. Rio Grande.

Schmidt, G.D. 1986. Handbook of tapeworm identification. *Florida* Constitution Revision Commission, 675 pp.

Silveira, E.C.; Mascarenhas, C.S.; Antunes, G.M.; Loebmann, D. 2019. Occurrence of *Hannemania* sp. (Acariformes: Leeuwenhoekiidae) larvae in males of *Boana pulchella* (Anura: Hylidae) from southern Brazil. *Revista Mexicana de Biodiversidad* 90: 1-5.

Solé, M. & Pelz, B. 2007. Do male tree frogs feed during the breeding season? Stomach flushing of five syntopic hylid species in Rio Grande do Sul, Brazil. *Journal of Natural History* 41: 2757-2763.

Spalding, M.G.; Bancroft, G.T. & Forrester, D.J. 1993. The epizootiology of eustrongylidosis in wading birds (Ciconiiformes) in Florida. *Journal of Wildlife Diseases* 29: 237-249.

StataCorp, L.P. 2007. Stata data analysis and statistical software. Special Edition Release (10.1 ed.), Stata, College Station. 733pp.

Sures, B. 2004. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology* 20: 170-177.

Tkach, V.V. 2008. Family Haematoloechidae Freitas & Lent, 1939: 361-365. *In*: Keys to the Trematoda. v. 3. – CABI International, London.

Travassos, L. 1920 Contribuições para o conhecimento da fauna helmintológica brasileira. *Memórias do Instituto Oswaldo Cruz* 67:1–886. *Archivos da Escola Superior de Agronomia e Medicina Veterinária* 4: 17-20.

Travassos, L. 1926. Contribuição para o conhecimento da fauna helmintológica brasileira. XX. Revisão dos acantocéfalos brasileiros. Parte II. Fam. Echinorhynchidae. Sf. Centrarchinae Travassos, 1919. *Memórias do Instituto Oswaldo Cruz* 19: 31-125.

Travassos, L.; Freita, J.F.T. & Kohn, A. 1969. Trematódeos do Brasil. *Memórias do Instituto Oswaldo Cruz* 67: 1-886.

Van, S.M.; Schittini, G.M.; Marra, R.V.; Azevedo, A.R.M.; Vicente, J.J. & Vrcibradic, D. 2006. Body size, diet and endoparasites of the microhylid frog *Chiasmocleis capixaba*

in an Atlantic Forest areaof southern Bahiastate, Brazil. *Brazilian Journal of Biology* 66: 167-173.

Vaucher, C. 1987. Polystomes d'Equateur, avec description de deux nouvelles especes. *Bulletin de la Societe Neuchfteloise des Sciences Naturelles* 110: 45-56.

Von Zuben, C.J. 1997. Implicações da agregação espacial de parasitas para a dinâmica populacional na interação hospedeiro-parasita. *Revista de Saúde Pública* 31: 523-530.

Wegner, K.M.; Kalbe, M.; Kurtz, J.; Reusch, T.B.H. & Milinski, M. 2003. Parasite selection for immunogenetic optimality.

Science 301: 1343.

Wilson, K.; Bjørnstad; O.; Dobson, N.A.P.; Merler, S.; Poglayen, G.; Randolph, S.E.; Read, A.F & Skorping, A. 2002. Heterogeneities in macroparasite infections: patterns e processes, p 6-44. *In:* Hudson, P. J.; Rizzoli, A.; Grenfell, B. T.; Heesterbeek, H.; Dobson, A. P. (eds) The Ecology of Wildlife Diseases, United Kingdom, Oxford Press.