

Research Paper

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Can differences between continental and insular habitats influence the parasites communities associated with the endemic frog *Haddadus binotatus*?

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Abstract

Habitats characterized by geographic isolation such as islands have been studied using different organisms as models for understanding the dynamic and insular patterns of biodiversity. Determinants of parasite richness in insular host populations have been conducted mainly with mammals and birds, showing that parasite richness decreases in insular areas. In the present study, we predicted that the type of environment (insular or continental) can influence the richness, diversity and abundance of parasites associated with the endemic frog *Haddadus binotatus* (Spix, 1824). We sampled frogs in two insular and two mainland fragments to survey their helminth parasites. The total richness was composed of 15 taxa of Nematoda and two of Acanthocephala, and the community composition of the two islands had more similarities between them than the two mainland localities. The insular effect was positive for richness and abundance of helminths, and no significant effect was observed on helminth diversity – even the mean diversity presented high numbers for the islands. We presumed that insular hosts could have lost some parasites in the colonization process when these continental islands were separated from the mainland, approximately 11,000 years ago. However, the high richness and abundance on islands can be explained by an epidemiological argument, which considers high population density due to insularity and other features of the host as factors that increase parasite transmission success among individuals.

Introduction

Studies comparing islands and mainland communities have been mostly conducted using organisms such as plants and animals (e.g. mammals, birds and reptiles) for understanding insular patterns of biodiversity (Brown, 1978; Mueller-Dombois, 2001; Helmus *et al.*, 2014; Medina *et al.*, 2015). These studies provide insights into the biogeography process where an island can act as a natural laboratory due to its isolation and dynamic (Lomolino, 2000a).

According to the biogeography of islands theory, the insular dynamic is characterized by its reduced area, geographic isolation and age, which select species and diversity (MacArthur & Wilson, 1967). The equilibrium of these populations is adapted and efficient in their environment, but they are very sensitive due to isolation and reduced size, which can induce some extinctions (MacArthur & Wilson, 1967; Vitousek *et al.*, 1995). In light of this theory, some studies (e.g. Lomolino, 2000b; Losos & Schluter, 2000) have corroborated that small areas (such as islands) harbour low richness when compared to large areas (such as mainland), while other studies have found the opposite or no substantial effect in the relation of size to richness (e.g. Dunn & Loehle, 1988; Lomolino, 2000b).

Although insular research that use attributes of parasites are still scarce, some studies have demonstrated that prevalence and intensity of infection might be higher on islands, with the highest infection values on the smallest island (Casanova *et al.*, 1996; Miquel *et al.*, 1996) when compared to those of the mainland (Lewis, 1968; Gregory & Munday, 1976). Thus, more studies are needed on the ecology of parasites, which investigate the response between the effects of environmental and host features (Poulin, 2004). The parasites represent a hidden diversity (Poulin & Morand, 2000), and some research seeks to understand how this diversity and other

parasitological parameters can be influenced by environmental conditions (Poulin, 2004). For example, host characters such as feeding, body size, sex, age, evolutionary history and the type of environment where the host lives can influence the parasite communities (Aho, 1990; Barton, 1999; Muzzall *et al.*, 2001; Bolek & Coggins, 2003; Brooks *et al.*, 2006; Hamann *et al.*, 2006; Campião *et al.*, 2015).

In this sense, amphibians are considered as good models for studying parasite communities due to their lifestyle, inhabiting both aquatic and terrestrial ecosystems, which allows contact with different parasite species. Numerous reproductive modes can be highlighted by the great ability of these vertebrates to exploit several types of microhabitats, especially those from the Atlantic Rain Forest (Myers *et al.*, 2000; Haddad & Prado, 2005). *Haddadus binotatus* (Spix, 1824) (Anura, Craugastoridae) is an endemic frog from the Brazilian Atlantic Forest biome. It lives on the forest floor and its distribution is from southern Bahia to Rio Grande do Sul States, Brazil (Frost, 2020). This anuran species has an average size, with the female (46–63 mm) generally larger than the male (32–44 mm) (Heyer *et al.*, 1990; Canedo & Rickli, 2006). In the breeding season, the eggs are released as foam on land, and there is no tadpole stage in the development of these frogs (Hedges *et al.*, 2008; Frost, 2020). Furthermore, they may prey on a variety of items of different sizes, representing an important component of the local food web as well as getting parasites by trophic transmission (Coco *et al.*, 2014). Concerning the records of parasites, there are only two studies reporting the helminth fauna associated with *H. binotatus* (Travassos, 1925; Aguiar *et al.*, 2014), but no study has compared parasite communities between host populations from mainland and islands.

Several Brazilian islands of the Atlantic Forest, such as those from São Paulo State, can present similar characteristics to the mainland due to their continental origin, which occurred in the last glaciation during the Pleistocene (Martin *et al.*, 1986). These characteristics of the continental island can result in similar fauna and phytophysiognomies as those of the continent, allowing comparisons between patterns of abundance and biodiversity from each locality.

Here, considering the ecology of insular environments and the features of parasites and the host species, we predicted that the type of environment (insular or continental) can influence the richness and abundance of parasites associated with the frog *H. binotatus*. To test this effect, we predicted that: (1) insular environments will be more similar to each other in respect to parasite community, as well as continental forest fragments sharing more similarities; (2) the fragments from the mainland will have higher species richness and diversity of parasites due to their wide area compared to the limited area of islands; and (3) the islands will present a higher abundance of parasites due to their limited area, which can promote more aggregation of the insular hosts and, thus, more reinfection and higher intensity of infection. Based on these predictions, we aimed to describe an effect of the environment (i.e. insular and continental) in the richness, diversity and abundance of the parasite community associated with populations of *H. binotatus* collected in continental and insular regions from the Atlantic Forest, south-eastern Brazil.

Materials and methods

Study areas and collection of amphibian hosts

Eighty-six specimens of *H. binotatus* were collected using pitfall traps or visual encounter surveys in four localities from the

Atlantic Forest during the breeding seasons of the years 2004, 2005, 2006, 2009 and 2010: Núcleo Santa Virginia (NSV), Serra do Mar State Park ($n=23$); municipality of São Luis do Paraitinga (SLP) ($n=30$); Anchieta Island (ILA) ($n=9$); and Moela Island (ILM) ($n=24$). The localities from the continent are NSV (23°24'S, 45°03'W), which is located within a preserved area, and SLP (23°13'S, 45°18'W), which is located in a non-preserved area of a mountainous region of the Serra do Mar, São Paulo State. ILA (23°45'S, 45°033'W), belonging to the municipality of Ubatuba, is an insular protected area (Anchieta Island State Park), despite the intense influence of tourists and its short distance from the north coast of São Paulo State (approximately 800 m) (Guillaoumon *et al.*, 1989; Cicchi *et al.*, 2009). ILM (24° 03'S, 46°16'W) is an insular concession of the Brazilian Navy due to the maintenance of a lighthouse there, and the island is 2.5 km from the southern coast of São Paulo State, in the municipality of Guarujá (fig. 1). Currently, both islands are considered protected areas, despite their past environmental impacts (Secretaria de Estado de Infraestrutura e Meio Ambiente, 2019).

The collected anurans were killed with thiopental sodic and then, after the necropsy and helminth survey were carried out, they were fixed with formaldehyde (10%), preserved in alcohol 70% and deposited at the Museu de Zoologia da Universidade Estadual de Campinas (ZUEC 24874 and 24875) and Coleção Herpetológica da Universidade Regional do Cariri (URCA-H 10688–10700, 10946–10968).

Collection of helminths and procedures in the laboratory

All organs and body cavity were examined for helminths with the aid of a stereomicroscope. Recovered parasites were counted and site of infection registered. Nematodes were fixed using a hot solution with 93 parts of alcohol 70° GL, five parts of formalin, and two parts of acetic acid (AFA), while acanthocephalans were first kept on cold water for the exposition of proboscis and then fixed with AFA solution. Subsequently, all helminths were maintained in labelled bottles with alcohol 70%. In the laboratory, helminths were mounted in temporary slides for observation of taxonomic structures using a computerized system of image analysis (LAS DIC, Leica Microsystems, Wetzlar, Germany). The nematodes were cleared with lactic acid or lactophenol, and acanthocephalans were stained with chloridric carmine, dehydrated with alcoholic series and then cleared with eugenol (Amato *et al.*, 1991). Voucher specimens were deposited in the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB 8861–8887) of the Universidade Estadual Paulista/UNESP. The identification of parasite taxa was performed using Yamaguti (1961, 1963), Vicente *et al.* (1991), Anderson *et al.* (2009), Gibbons (2010) and papers with species descriptions. In the morphological analyses, the presence of juveniles (larvae of Nematoda) and the absence of males (e.g. nematodes of Cosmocercidae) did not enable us to make any specific identifications since these helminths present conservative characters. Thus, we separated the cosmocercids into morphospecies based on the main morphological differences.

Data analysis

To characterize the helminth communities of each anuran population among the continents and islands localities, we analysed the prevalence, mean abundance, mean intensity of infection and mean richness according to Bush *et al.* (1997). To describe the

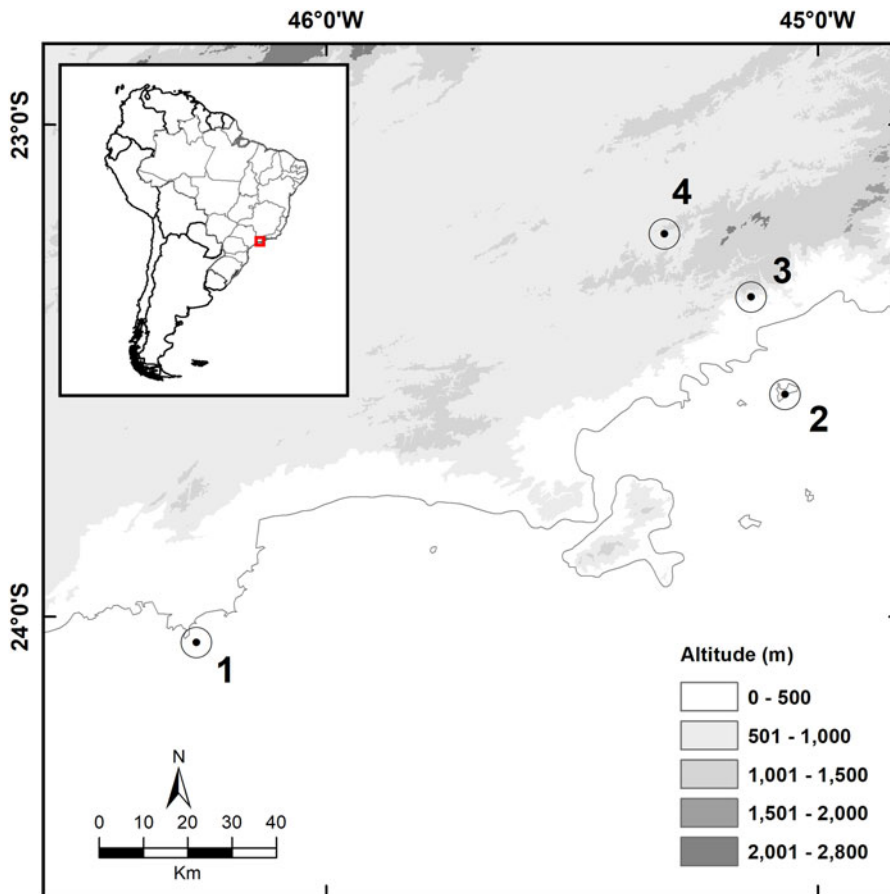


Fig. 1. Map of the São Paulo coast, indicating the four localities where the anurans *Haddadus binotatus* were collected. Insular: (1) Moela Island and (2) Anchieta Island. Continental: (3) Núcleo Santa Virginia and (4) São Luis do Paraitinga.

diversity for each infracommunity, we used the Brillouin diversity index, and then we provided the mean diversity for the component community (bootstrap 95%). We performed a cluster analysis with the Bray–Curtis index (B) to explore potential similarities among helminth communities from each host population, taking into account the mean intensity of infection of each helminth taxa. These descriptive analyses were performed using Sigma-Stat version 3.1 (SigmaStat, 2005) and PAST (Hammer *et al.*, 2001), adopting a significance level at the value of $P \leq 0.05$.

Accumulation curves for helminth species were generated to evaluate sampling effectiveness by randomizing samples 1000 times using EstimateS 9.1.0 (Colwell, 2006). According to Poulin (1998), this estimator is the most invariable and it present reduced susceptibility for overestimation in the real richness, independently of how frequent species appear in the observed community. For constructing curves of observed and estimated richness, we used Statistica software version 7.1 (Statsoft, 2005).

We used generalized linear mixed models to investigate whether richness and abundance of parasites (response variables) are affected by the predictor variables ‘environment’ (insular or continental) and ‘locality’ (NSV, SLP, ILA and ILM) for each individual frog. These predictor variables were also used in linear mixed models to investigate the diversity as response variable. In each model set, we considered the ‘environment’ as a fixed effect and the ‘locality’ as random effect. For richness and abundance data, our models ran with negative binomial distribution using the ‘glmer.nb’ function in the lme4 package (Bates *et al.*, 2014) in R (R Development Core Team 2020, version 4.0.0). For diversity, a Gaussian distribution was most appropriate for

the model, and we used the ‘lmer’ function in the lmerTest package (Kuznetsova *et al.*, 2017) in R (R Development Core Team 2020, version 4.0.0).

Results

A total of 86 specimens of *H. binotatus* were analysed from the following localities: NSV ($n = 23$), SLP ($n = 30$), ILA ($n = 9$) and ILM ($n = 24$). The sampling efforts were representative according to richness estimators (fig. 2). The overall richness of helminths (number of *taxa*) in each locality was as follows: eight (NSV), four (SLP), seven (ILA) and eight (ILM). Helminths identified up to the genus level (e.g. *Ochoterenella* sp., *Oxyascaris* sp., *Rhabdias* sp. and *Physaloptera* sp.) were considered the same morphospecies in the host populations, whereas *Cosmocercidae* sp. 1, *Cosmocercidae* sp. 2, *Cosmocercidae* sp. 3 and *Cosmocercidae* sp. 4 were treated as distinct species.

In NSV, we recorded 258 helminths associated with 22 of 23 sampled frogs (table 1); the mean diversity for the component community was 0.17 ± 0.05 (0–0.68) and the mean richness was 1.54 ± 0.16 (1–3). In SLP, 170 helminths were recorded in 24 of 30 sampled frogs (table 1). The mean diversity and the mean richness for the component community from SLP were 0.16 ± 0.04 (0–0.49) and 1.42 ± 0.10 (1–2), respectively. In this way, the samples from continental localities (NSV and SLP) numbered ten helminth taxa and shared two species (*Oxyascaris* sp. and *Rhabdias* sp.) (table 1).

In ILA, we recorded 695 helminths associated with eight of nine sampled frogs (table 1); the mean diversity for the

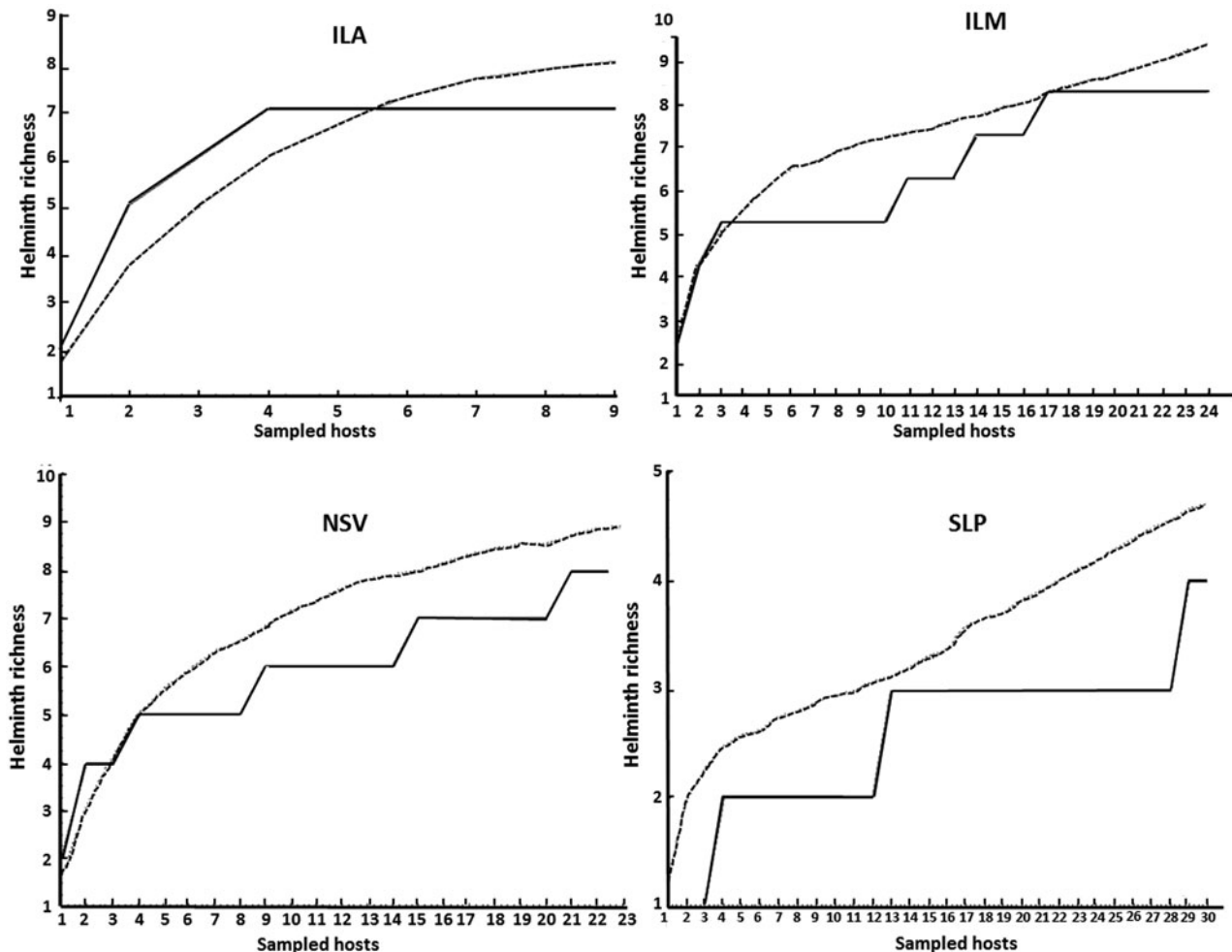


Fig. 2. Sampling efforts in observed (solid line) and expected (dashed line) richness within each community of helminths associated with *Haddadus binotatus* from ILA (Anchieta Island), ILM (Moela Island), NSV (Núcleo Santa Virginia) and SLP (São Luis do Paraitinga).

component community was 0.21 ± 0.07 (0–0.46) and the mean richness was 1.87 ± 0.35 (1–4). In ILM, 435 helminths were recorded in all 24 sampled frogs (table 1). The mean diversity and the mean richness for the component community from ILM were 0.45 ± 0.08 (0–1.27) and 2.29 ± 0.22 (1–5), respectively. Thus, the samples from islands (ILA and ILM) numbered 11 helminth taxa and shared four species (*Aplectana pintoi* Travassos, 1925, *Cosmocerca brasiliensis* Travassos, 1925, *Physaloptera* sp. and *Rhabdias* sp.) (table 1).

The four populations of anurans shared only *Rhabdias* sp., whereas the anurans from islands shared four taxa (*A. pintoi*, *C. brasiliensis*, *Physaloptera* sp. and *Rhabdias* sp.), and the continental ones shared two (*Oxyascaris* sp. and *Rhabdias* sp.). In addition to *Rhabdias* sp., the comparisons NSV–ILM and SLP–ILA both presented *Ochoterenella* sp. and *Oswaldocruzia subauricularis* (Rudolphi, 1819), respectively.

A similarity cluster analysis (Bray–Curtis index, co-phenetic coefficient of 0.93), using the mean intensity of infection of each helminth taxa, showed two principal groups regarding the four anuran populations: (1) continental populations (NSV and SLP) ($B = 0.06$) and (2) the insular population (ILA and ILM) ($B = 0.19$).

Our models showed a significant influence of the environment on richness and abundance of helminths associated with

H. binotatus (table 2), where the islands presented high richness and abundance of helminths. On the other hand, the effects of environment and locality were not significant for helminth diversity (table 2).

Discussion

This is the first study to compare helminth fauna of *H. binotatus* from island and continent localities and to verify a significant influence of environment on richness and abundance of helminth communities. We found high richness and abundance of helminths in hosts from the insular environments studied, and the findings suggested a greater similarity between islands than that found between continental fragments.

We detected eight new records of helminth associated with *H. binotatus* (*Pseudoacanthocephalus lutzi* (Hamann, 1891), *C. brasiliensis*, *Cosmocerca parva*, *A. pintoi*, *Oxyascaris* sp., *Ochoterenella* sp., *O. subauricularis* and *Spiroxys* sp.). As expected, no one of these four anuran populations presented trematodes in the component community, probably due to the direct reproductive mode of this frog, as they do not enter water bodies to spawn (Haddad & Sazima, 1992; Canedo & Rickli, 2006). Consequently, the adult frogs and tadpoles are not infected with aquatic cercariae. Other studies (e.g. Bolek & Coggins, 2003; Kehr & Hamann, 2003;

Table 1. Helminths associated with populations of *Haddadus binotatus* from Núcleo Santa Virginia (NSV) ($n=23$), São Luis do Paraitinga (SLP) ($n=30$), Anchieta Island (ILA) ($n=9$) and Moela Island (ILM) ($n=24$), São Paulo State, Brazil.

Helminth parasites	NH	P%	MA \pm SE	MII \pm SE	Range	SI
NSV						
Acanthocephala						
<i>Pseudoacanthocephalus lutzi</i>	9	13.04	0.39 \pm 0.31	3.00 \pm 2.00	1–7	Cav, Sin
Nematoda						
<i>Cosmocerca parva</i>	135	60.87	5.87 \pm 1.29	9.64 \pm 1.37	2–19	Sin, Lin
Cosmocercidae sp. 1	27	4.35	1.17 \pm 1.17	27.00	27	Lin
Cosmocercidae sp. 2	63	17.39	2.74 \pm 1.44	15.75 \pm 4.38	3–22	Sin, Lin
Larvae of Nematoda	5	8.69	0.22 \pm 0.18	2.50 \pm 1.50	1–4	St, Sin
<i>Ochoterella</i> sp.	12	17.39	0.52 \pm 0.32	3.00 \pm 1.41	1–7	Cav, Sin, Lu
<i>Oxyascaris</i> sp.	6	21.74	0.26 \pm 0.11	1.20 \pm 0.20	1–2	Sin
<i>Rhabdias</i> sp.	1	4.35	0.04 \pm 0.04	1.00	1	Lu
Overall (NSV)	258	95.65	11.22 \pm 1.56	11.77 \pm 1.55	1–27	-
SLP						
Nematoda						
Cosmocercidae sp. 3	130	66.67	4.33 \pm 1.08	6.50 \pm 1.39	1–22	St, Sin, Lin
<i>Oswaldocruzia subauricularis</i>	37	40.00	1.23 \pm 0.52	3.08 \pm 1.12	1–15	St, Sin, Lin
<i>Oxyascaris</i> sp.	2	3.33	0.07 \pm 0.07	2.00	2	Sin
<i>Rhabdias</i> sp.	1	3.33	0.03 \pm 0.03	1.00	1	Lu
Overall (SLP)	170	80.00	5.67 \pm 1.19	7.08 \pm 1.34	1–23	-
ILA						
Nematoda						
<i>Aplectana pinto</i>	14	22.22	1.56 \pm 1.04	7.00 \pm 1.00	6–8	Sin, Lin
<i>Cosmocerca brasiliensis</i>	633	44.44	70.33 \pm 69.33	158.25 \pm 55.58	2–625	Sin, Lin
Larvae of Cosmocercidae	3	11.11	0.33 \pm 0.33	3.00	3	Sin, Lin
<i>Oswaldocruzia subauricularis</i>	2	22.22	0.22 \pm 0.15	1.00 \pm 0	1	Sin, Lin
<i>Physaloptera</i> sp.	36	33.33	4.00 \pm 3.17	12.00 \pm 8.50	3–29	St
<i>Rhabdias</i> sp.	5	22.22	0.56 \pm 0.44	2.50 \pm 1.50	1–4	Cav, Lu
<i>Spiroxys</i> sp.	2	11.11	0.22 \pm 0.22	2.00	2	Sin
Overall (ILA)	695	88.89	77.22 \pm 1.56	86.87 \pm 78.25	3–634	-
ILM						
Acanthocephala						
Unidentified cystacanth	244	87.50	10.17 \pm 2.86	11.62 \pm 3.15	1–58	Cav, St, Sin, Lin
Nematoda						
<i>Aplectana pinto</i>	9	4.17	0.37 \pm 0.37	9.00	9	Sin
<i>Cosmocerca brasiliensis</i>	105	58.33	4.37 \pm 1.08	7.50 \pm 1.33	1–17	St, Sin, Lin
Cosmocercidae sp. 4	14	25.00	0.58 \pm 0.30	2.33 \pm 0.95	1–7	St, Sin, Lin
Larvae of Nematoda	22	16.67	0.92 \pm 0.55	5.50 \pm 2.33	1–10	St, Lin
<i>Ochoterella</i> sp.	4	4.17	0.17 \pm 0.17	4.00	4	Lin
<i>Physaloptera</i> sp.	35	29.17	1.46 \pm 0.58	5.00 \pm 1.19	1–10	St, Sin, Lin
<i>Rhabdias</i> sp.	2	4.17	0.08 \pm 0.08	2.00	2	St
Overall (ILM)	435	100	18.12 \pm 3.00	18.12 \pm 3.00	1–61	-

NH, number of helminths recorded; P%, prevalence; MA, mean abundance; MII, mean intensity of infection; min–max, range; SE, standard error; SI, sites of infection; Cav, cavity; Lin, large intestine; Lu, lungs; Sin, small intestine; St, stomach.

Table 2. Effects of environment and study site on response variables (richness, diversity and abundance of helminths associated with *Haddadus binotatus* from south-eastern Brazil).

Response variables		Estimate	Fixed effects			AIC
			Standard error	Test value	P-value	
Helminth richness	(Intercept)	0.249	0.119	2.098	0.036	245.9
	Insular environment	0.503	0.166	3.023	0.002	
Helminth abundance	(Intercept)	2.069	0.373	5.553	2.81×10^{-8}	628.6
	Insular environment	1.476	0.543	2.718	0.006	
Helminth diversity	(Intercept)	0.144	0.081	1.769	0.275	-
	Insular environment	0.193	0.120	1.606	0.282	

Predictor variables included environment with two levels (continent and insular) and the studied sites with four levels (NSV, SLP, ILA and ILM). The study site was considered as random effects in the three models. Akaike information criterion (AIC) values are presented in the models with Negative Binomial distribution.

González & Hamann, 2006) have correlated the composition of the parasite community with the habit of hosts, suggesting the predominance of monoxenic nematodes in terrestrial frogs and trematodes in aquatic or semiaquatic amphibians.

For each component community, a dominant species that presented the greatest prevalence and mean intensity of infection was observed (table 1). *Haddadus binotatus* from ILM presented a dominance by cysthacanth (Acanthocephala), which usually reach frogs by the trophic transmission of the first intermediate host (e.g. an arthropod) (Santos & Amato, 2010). Besides the availability of an arthropod as prey for frogs, acanthocephalans demand a bird as final hosts, as reported in other studies that point out the important role of birds in parasite exchange among ecosystems (e.g. Karvonen & Valtonen, 2004; Poulin & Leung, 2011). These parasites could finish their development in sea birds that usually nest or rest on coastal islands (Neves et al., 2006). In the other component communities, the nematodes *C. parva*, *Cosmocercidae* sp. 3 and *C. brasiliensis* were dominant in NSV, SLP and ILA, respectively. These nematodes are expected as dominant in this frog species, which occupies a terrestrial habit, thus enabling the contact between infective larvae from the soil and skin and eyes of the host (Anderson, 2000).

Despite the differences in the composition of parasite communities, some parasites were found in more than one host population (e.g. *Rhabdias* sp., *C. brasiliensis* and *O. subauricularis*), indicating a historic relationship between these hosts and parasites, which would have adapted to insular conditions since they were separated from the mainland by the ocean. When the islands were created, during the oscillations in the sea level approximately 11,000 years ago (Pleistocene) (Martin et al., 1986; Souza et al., 2005), insular hosts could have then retained these parasites.

In this context, the similarity in helminth fauna between ILM and ILA ($B = 0.19$) could also be explained by their continental origin, when they were considered as a continuous mainland. Moreover, comparable conditions imposed by this type of environment can contribute to the likeness on communities from these islands, even though they have a different history of human occupation and differences in the distance from the continent; it is possible that these recent factors were not sufficient to modify completely these two insular communities. In other words, ILM and ILA can share similar factors that modulate parasite communities. The proximity between ILA and the coast does not seem to contribute to the similarity with the communities from continent fragments since only *Rhabdias* sp. was verified in ILA and NSV,

and comparing ILA and SLP only two taxa were shared (*Rhabdias* sp. and *O. subauricularis*). These findings corroborate the two groups formed (ILA–ILM and NSV–SLP) by cluster similarity analysis.

ILA and ILM presented the highest mean richness and mean abundance compared to fragments from the continent. We confirmed these findings by generalized linear mixed models, which showed the influence of insularity on the increase of richness and abundance of parasite of *H. binotatus*. The positive influence of these islands on richness was different from the others studies, which found that insularity affects decreasing parasite richness (e.g. Fromont et al., 2001; Bellocoq et al., 2002). Some studies assume that high abundance could be an effect of the small competition caused by low richness in insular environments (Dobson, 1985; Fromont et al., 2001); however, our results do not corroborate with such studies, because in addition to the high richness we found high abundance in islands. In this context, two frameworks of determinants of parasite communities have been considered: the ‘founder effect’ and the ‘epidemiological argument’ (Poulin, 2004). The first derives from biogeography theory, and it assumes that insular hosts possess a subset of parasites, which was the result of a small number of migrants with few parasites species causing species loss during colonization (Miquel et al., 1996; Morand & Guégan, 2000). In other words, insular hosts should harbour lower parasites richness than those on the mainland. Unlike the ‘founder effect’, our results showed a high richness and abundance in islands, which can probably be explained by the ‘epidemiological argument’. This argument considers high population density due to insularity and other features of the host as factors that increase parasite transmission success among individuals (Morand & Guégan, 2000; Poulin, 2004). Also, we cannot disregard the possible impact levels of human interference in these areas from the Atlantic Rain Forest as factors which can influence the parasite communities. In the diversity model, we did not verify a significative effect of insular or continental environment. However, considering the mean diversity of helminths, the islands presented the highest numbers, and, in the same sense of richness, this finding does not corroborate with the previous studies (e.g. Fromont et al., 2001; Bellocoq et al., 2002).

The presence of a helminth species in a locality can depend on the probability of colonization from nearby localities and the habitat’s general suitability for parasite establishment (Poulin & Leung, 2011). In other words, speciation, extinction and

dispersion are the three main processes that could influence biological richness observed presently (Ricklefs, 1987; Poulin, 1995). This also explains the differences in the richness of helminth communities. According to Poulin (2004), there is no general rule as a key factor promoting rich parasite faunas; a prediction can be important in one study and unimportant in another. There are several factors related to parasite colonization and extinction, and, for this reason, multivariate approaches are essential for understanding at least part of the contributory determinants of parasites richness (Poulin, 2019).

According to our proposed hypotheses, we confirmed that insular environments are more similar to each other in respect to parasite community, and continental forest fragments can also share more similarities; furthermore, the similarity index was higher between islands than fragments of the mainland. Our findings did not corroborate with the second hypothesis, since we found a significant increase of insular environment in parasite richness and no significant influence of environment on diversity, although the islands showed the highest numbers of mean diversity. On the other hand, the third hypothesis was confirmed, with a significant influence of insularity in abundance.

Thus, our findings fill some knowledge gaps concerning parasite communities of insular host populations with the conclusion that richness and abundance of helminth parasites of the frog *H. binotatus* are positively affected by the island environment.

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Conflicts of interest. None.

Ethical standards. The authors assert that all activities contributing to this work comply with the ethical standards in this type of study (SISBIO collection permission number 31716-2; IBAMA permission number 567/05).

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