

Parasitic infection patterns in *Coleodactylus meridionalis* (Squamata: Sphaerodactylidae) from Atlantic Forest fragments, northeast of the Neotropical Region

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Summary

Studies that seek to understand which factors influence the patterns of infection in the lizard *Coleodactylus meridionalis* are non-existent. In this way, we recorded the infection rates for these lizards from three different sized fragments of the Atlantic rainforest and investigated if there is influence of the size, mass and sex of the host regarding the endoparasite abundance. In addition, we investigated if there were possible associations between the host's diet and parasitism, by calculating the diet niche breadth (based on the prey number of pooled stomachs) for each host population and comparing the diet between fragments and sexes (from the three sampled fragments). We collected 38 lizards across the three sampled forest fragments. We only found parasites in the lizards sampled from the smallest of the three forest fragments which included: *Physaloptera lutzi*, *Haplometroides odhneri* and *Oligacanthorhynchus* sp. For this population, lizards with greater length and body mass have a greater endoparasite abundance. In addition, based on the results obtained in our study, the diet of the lizards varies in food composition between sampled populations. Finally, knowing that among the infected lizards there is no sexual dimorphism in relation to size and body mass and that the stomach sampling for this population is insufficient to calculate the indexes related to the food niche; we used the number of prey lizards from all populations, thus we were able to associate the fact that female lizards have a greater endoparasite abundance due to variations in food composition between sexes.

Keywords: Helminths; Gecko; Diet; Neotropical Region; Brazil.

Introduction

Belonging to the family Sphaerodactylidae, lizards of the *Coleodactylus* genus Parker, 1926 are very small, with some species reaching an average of 32 mm in total length (Goncalves *et al.*, 2012). They can be found in areas of the Amazon rainforest (Avila-Pires, 1995), Atlantic forest (Teixeira *et al.*, 2020), including mesic regions located in the Caatinga and the Cerrado (Colli *et al.*, 2002; Ribeiro *et al.*, 2013). However, most studies on *Cole-*

odactylus are aimed to test systematic and phylogenetic hypotheses (Correia *et al.*, 2016), are related with surveys about their geographic distributions (Ribeiro *et al.*, 2013), followed by scarce records on food composition and spatial niche (Werneck *et al.*, 2009). In addition, only two studies present simple surveys on endoparasites infecting these lizards (Ávila & Silva, 2013; Teixeira *et al.*, 2020), but without investigating, if life history traits, such as sex, body mass, size, and host diet, influence their infection patterns.

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Parasites are one of the main regulators of their host populations, and can cause simple infections with low prevalence (Teixeira *et al.*, 2018a), reduced fitness, organ damage and death (Almeida *et al.*, 2008). Also, due to the aggregate distribution character adopted by most parasite species, environmental changes accelerated by human action, such as habitat fragmentation and loss of biodiversity (Lafferty & Kuris, 2005), can cause parasite species to disappear before the actual extinction of their hosts (Lyles & Dobson, 1993), harming the meticulous balance present in this relationship. *Coleodactylus meridionalis* (Boulenger, 1988) is considered one of the smallest lizards in South America, reaching less than 30 mm in length; however, it has a wide distribution throughout northeastern Brazil, being found mainly in Atlantic Forest habitats (Ribeiro *et al.*, 2013). In addition, they have restricted spatial and food niches, occurring mainly in leaf litter (Oliveira *et al.*, 2015), with isopods being considered their primary food source (Dias *et al.*, 2003). Despite their wide distribution (Ribeiro *et al.*, 2013), these lizards are considered relictual due to their small size and their incredible ability to camouflage among the leaves and other fragments of trees in the forests, thus making the collection of sufficient

samples for statistical analysis purposes difficult (Werneck *et al.*, 2009), which in turn is reflected in the scarcity of existing studies. For the purpose of our study we focused on describing the parasitic infection patterns in *C. meridionalis* between fragments of Atlantic rainforest with different sizes. We recorded their infection rates and investigated if there is a relationship between SVL, body mass, host sex and endoparasite abundance. In addition, to identify possible associations between the host diet and parasitism, we calculated the diet niche breadth (based on the prey number of pooled stomachs) for each host population compared the diet similarity (through niche overlap) between fragments and sexes.

Materials and Methods

We collected lizards from three Atlantic forest fragments of different sizes (Fig. 1). The first fragment presents the largest forest area, about 1,058.62 ha – The Private Reserve of Natural Heritage Engenho Gargaú (PRNHEG; 16 specimens collected in September 2016), which is an area belonging to the Japungu Agroindustrial S/A, in the Santa Rita municipality (06° 59' 52" S; 34° 57' 30" W).

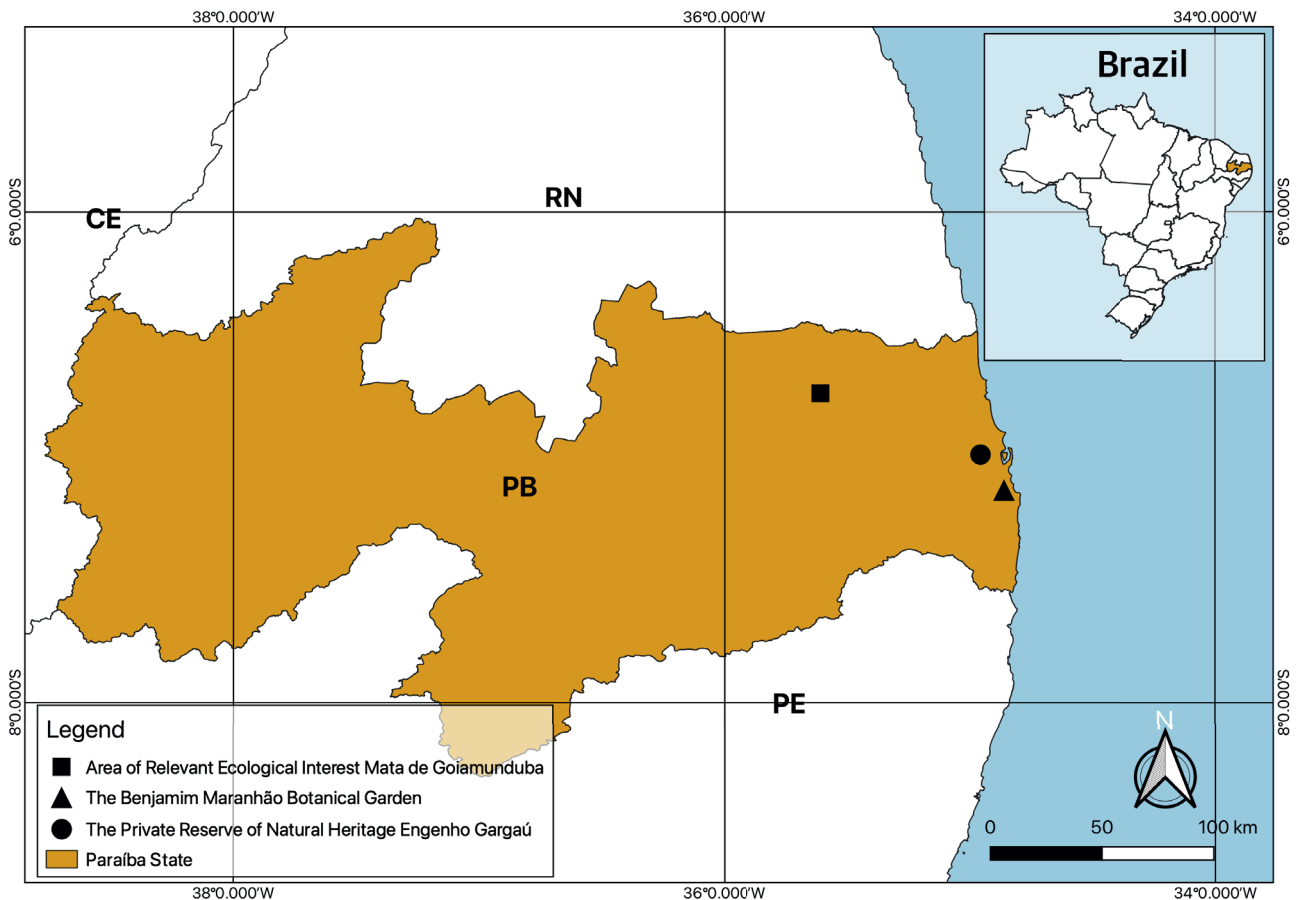


Fig. 1. Atlantic Forest fragments sampled in this study, located in the Paraiba state, Brazil.

The second fragment has an area of almost 500.00 ha – The Benjamin Maranhão Botanical Garden (BMBG; six specimens collected in November 2016) is a remnant of forest in the urban area of the João Pessoa municipality (07° 08' 08" S; 34° 51' 37" W). Finally, the last fragment is an area of about 47.50 ha – The Area of Relevant Ecological Interest Mata de Goiamunduba (AREIMG; 16 specimens collected in October 2016) is located in the Bananeiras municipality (06° 45' 03.78" S; 35° 38' 00.06" W). All the above forest fragments are located in the State of Paraíba, Northeastern Brazil and have an average annual rainfall of 1,521 mm and an average annual temperature of 24.2 °C.

We sampled each fragment during 20 consecutive days, between 8:00 a.m. to 4:00 p.m., due to the lack of visibility inside the forest (about 200m from the edge). We captured lizards manually or using pitfall traps (25 traps per study location, mounted in microhabitats more conducive to capturing specimens). Subsequently, we euthanized lizards with a lethal injection of 2 % lidocaine hydrochloride, and measured the snout-vent length (SVL) with a precision calliper to the nearest 0.01 mm and the mass using a decimal precision digital scale, fixed with 10 % formaldehyde, stored in 70 % alcohol and housed in the Coleção Herpetológica da Universidade Federal da Paraíba - CHUFPB.

The lizards were dissected under magnifying glass, and their respiratory and gastrointestinal tracts were examined for the presence of endoparasites. Endoparasites found were counted and their sites of infection recorded. For identification, nematodes were mounted on temporary slides with lactophenol, while trematodes were serially dehydrated through increasing concentrations of alcohol and stained with acetic carmine and later mounted on slides with eugenol (Kritsky *et al.*, 1986). Acanthocephalans, on the other hand, were mounted on slides in a glycerol medium (MacAllister & Bursey, 2007). Then, we stored all helminths in 99.7 % alcohol and housed in Coleção de Invertebrados Paulo Young, in Universidade Federal da Paraíba, Brazil (UFPB-NEM: 03, 04; UFPB-DIG: 03, 04, 05; UFPB-ACA: 01).

We calculated prevalence indices (% of infected hosts) and mean intensity of infection using the methods described in Bush *et al.* (1997). To check if the SVL and body mass influence the endoparasite abundance, we used generalised linear mixed models (GLMMs) (Bates *et al.*, 2014). In the first model, the endoparasite abundance corresponds to the response variable and, consequently, the SVL corresponds to the independent variable. In addition, host gender was included as a random effect. In the following model, the endoparasite abundance corresponds to the response variable, body mass corresponds to the independent variable, and host gender is a random effect. In both models, we used the Poisson distribution and log link function.

Posteriorly, we create four more models using the generalised linear model (GLM). In this case, the first model was used to verify if endoparasite abundance varies between male and female adult (using only the lizards from AREIMG). The second model was used to verify if the SVL varies between the sampled locations.

Finally, the third and fourth models were used to verify the existence of sexual dimorphism related to SVL and body mass (using only the lizards from AREIMG). In the first GLM model, we used the Poisson distribution and log link function; in the other GLM models, we used the Gamma distribution and inverse link function (Bolker *et al.*, 2009).

We analysed the stomach contents using a magnifying glass and identified the prey to the lowest possible taxonomic category. The niche breadth was based on the prey number, since prey items were too fragmented to accurately calculate volume estimates. We calculated the percentages of each prey category per species, from which we obtained the numerical niche breadths using the inverse of the Simpson diversity index (1949):

$$B = \frac{1}{\sum_{i=1}^n P_i^2}$$

Where i is the prey category, P is the proportion of prey category i , and n is the number of categories. We compared the diet niche breadth of lizards between males and females and sampled areas, based on the prey number of pooled stomachs.

The diet niche overlap among males and females and the similarity between the areas was calculated using the Pianka overlap index (Pianka, 1973):

$$\emptyset_{jk} = \frac{\sum_{i=1}^n p_{ij} p_{ik}}{\sqrt{\sum_{i=1}^n p_{ij}^2 \sum_{i=1}^n p_{ik}^2}}$$

where P_{ij} and P_{ik} are the consumption rate of the category of prey i , with j and k representing sexes and areas compared. The Pianka overlap index ranges from zero (no overlap/similarity) to one (total overlap/similarity). We performed all analyses in the Software "R", using the packages 'lme4' (Bates *et al.*, 2014) and "R commander". Throughout the text, means appear as ± 1 SD.

Ethical Approval and/or Informed Consent

The present research has complied with all the relevant national regulations and institutional policies for the care and use of animals. Permits for capturing of the lizards and analysing of the endoparasites used in this study were released by SISBIO-IBAMA (no: 54378/3, authentication code: 78752298; no: 56863-1, authentication code: 47783645), SUDEMA (no: 004/2016, process no. 5376/16), and Benjamin Maranhão Botanical Garden-BMBG (no: 003/2016/JBBM/SUDEMA).

Results and Discussion

Considering all areas together, we examined 38 *C. meridiona-*

Table 1. Basic morphometric data of *C. meridionalis* from three sampled areas.

	PRNHEG		BMBG		AREIMG	
	SVL	Mass	SVL	Mass	SVL	Mass
Male	22.21 ± 1.23	0.25 ± 0.06	21.59 ± 1.65	0.18 ± 0.06	25.02 ± 2.2	0.26 ± 0.06
Female	24.21 ± 1.02	0.29 ± 0.07	-	-	25.06 ± 2.13	0.28 ± 0.06
Juvenile	-	-	-	-	20.85 and 20.8	0.2 and 0.15

lis, including 11 adult females (SVL = 22.51 ± 2.0 mm), 25 adult males (SVL = 23.38 ± 1.9 mm) and two juveniles (SVL = 20.85 and 20.8 mm) (Table 1). Of the three populations analysed, only the AREIMG specimens were parasitised. We found 152 endoparasites, with 68 infecting eight adult males (Prevalence = 80 %), 82 infecting three adult females (Prevalence = 75 %) and two infecting one juvenile (Prevalence = 50 %). Twelve of the 16 lizards from AREIMG were parasitised by the nematode *Physaloptera lutzi* Cristofaro, Guimarães and Rodrigues, 1976 (prevalence of 75 %; mean intensity of infection of 12.5 ± 12.95), one lizard was infected with two trematodes *Haplometroides odhneri* Ruiz and Perez, 1959 (prevalence of 6.2 %) and one lizard was infected with one acanthocephalus *Oligacanthorhynchus* sp. Travassos, 1915 (prevalence of 6.2 %). The mean intensity of infection (OVERALL) was 12.66 ± 12.86.

We found that the endoparasite abundance is correlated with SVL ($R^2m= 0.06$; $R^2c= 0.9$; $z\text{-value}= 2.176$; $p\text{-value}= 0.0295$) and body mass of the hosts ($R^2m= 0.33$; $R^2c= 0.89$; $z\text{-value}= 5.335$; $p\text{-value} < 0.0001$). In both cases, the greater the body mass and SVL of the lizards, the greater the endoparasite abundance it can support (Fig. 2).

The results obtained by the GLM models revealed that female hosts harbor a greater endoparasite abundance compared to male hosts ($z\text{-value}= -6.821$; $p\text{-value} < 0.0001$) (Fig. 3); on the other hand, lizards from AREIMG are larger (SVL) than those of the

other two populations ($t\text{-value}= -4.671$; $p\text{-value} < 0.0001$) (Fig. 4); in addition, the *C. meridionalis* population from AREIMG does not present sexual dimorphism in relation to the SVL ($t\text{-value}= 0.03$; $p\text{-value}= 0.977$) and body mass ($t\text{-value}= 0.471$; $p\text{-value}= 0.646$). Lizards collected in AREIMG present the greatest diet niche breadths (based on the prey number of pooled stomachs) among the three sampled locations. On the other hand, male lizards also have greater numerical niche breadths when compared to females (Table 2). Finally, we found a low numerical niche overlap among the three sampled areas (AREIMG/PRNHEG: 0.19; AREIMG/BMBG: 0.005; PRNHEG/BMBG: 0.00) and between male and female lizards (0.05), from the grouping of the food composition of all areas. Knowing that all species of helminths recorded in this study have a heteroxene life cycle, the differences in food composition between male and female lizards and the between the studied areas may help to understand the patterns of infection present in *C. meridionalis* lizard.

Habitat fragmentation is one of the processes that most threatens biodiversity (Fischer *et al.*, 2005; Pineda & Halffter, 2004). In these environments, dense host populations may face direct and increased parasitic pressures (Primack & Rodrigues, 2006). The AREIMG presents the smallest area among the studied fragments. In this way, the hosts can use specific sites more frequently (Leu *et al.*, 2010), providing a greater probability of a meeting between these parasites and their hosts (Kerr & Bull, 2006). In the case

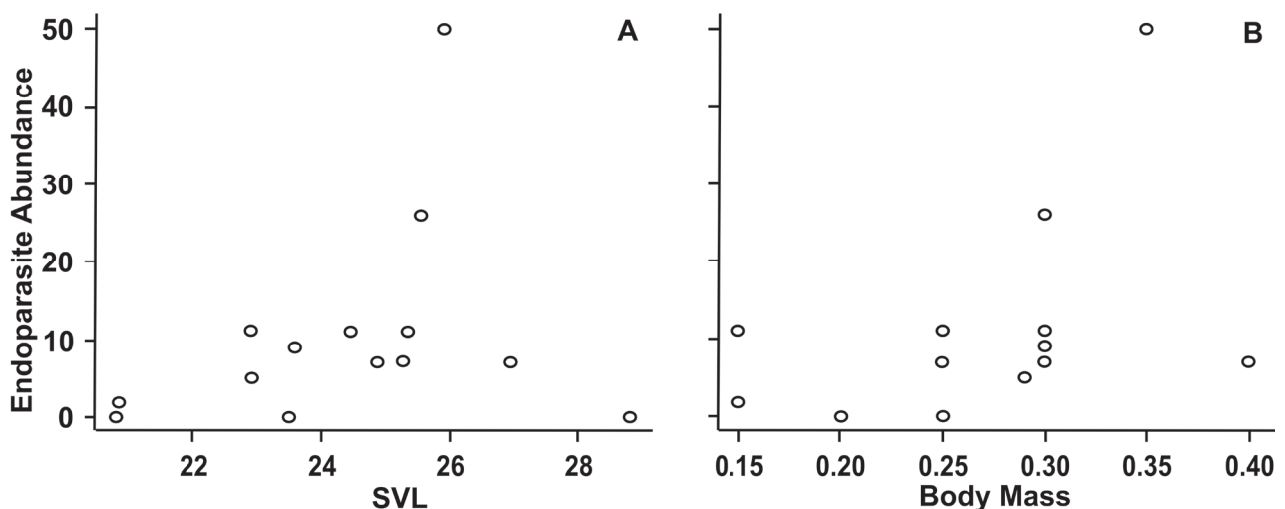


Fig. 2. Relationships between endoparasite abundance, SVL (A) and body mass (B), only for lizards from AREIMG.

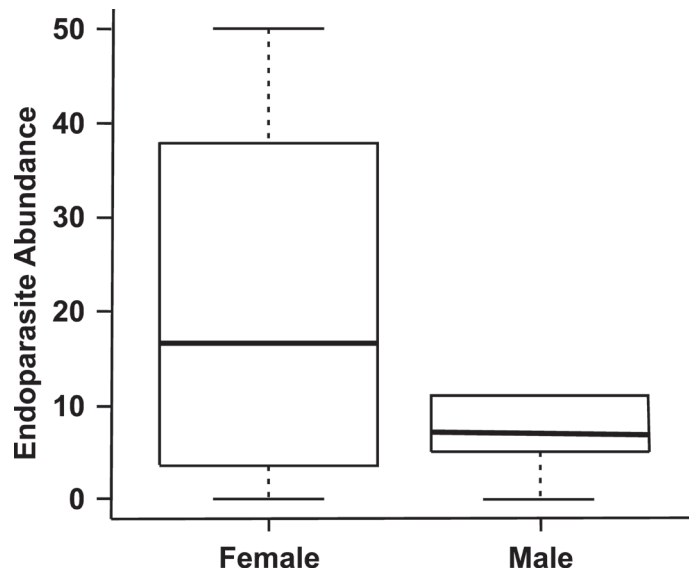


Fig. 3. Endoparasite abundance among females and males from AREIMG.

of endoparasites with heteroxenous life cycles, as in the present study, the higher the density of the hosts, the greater the chances of infection because of the limitations that prevent the transmission of the parasite between intermediate and final hosts are reduced (Buck *et al.*, 2017).

In addition, smaller hosts are less susceptible to harboring large parasite abundance (Kuris *et al.*, 1980). Size and body mass are factors that have been considered in an attempt to explain patterns of endoparasite abundance in vertebrates (George-Nascimento *et al.*, 2004; Poulin, 2007). The present study showed a significant positive relationship when we separately compare body mass and

SVL with the endoparasite abundance in *C. meridionalis*. Theoretically, larger hosts with greater body mass, have the capacity to offer more resources for the development and reproduction of parasites (George-Nascimento *et al.*, 2004; Poulin, 2007). Furthermore, the difference in *C. meridionalis* SVL measurements between the areas is also an aspect that may be associated with infection only in lizards of the AREIMG fragment, with the lizards in this population being larger than those in the other two areas. However, as mentioned by Patterson (2008), the mass and size of the host does not always correspond to greater endoparasite abundance, because ecological, behavioural and phylogenetic

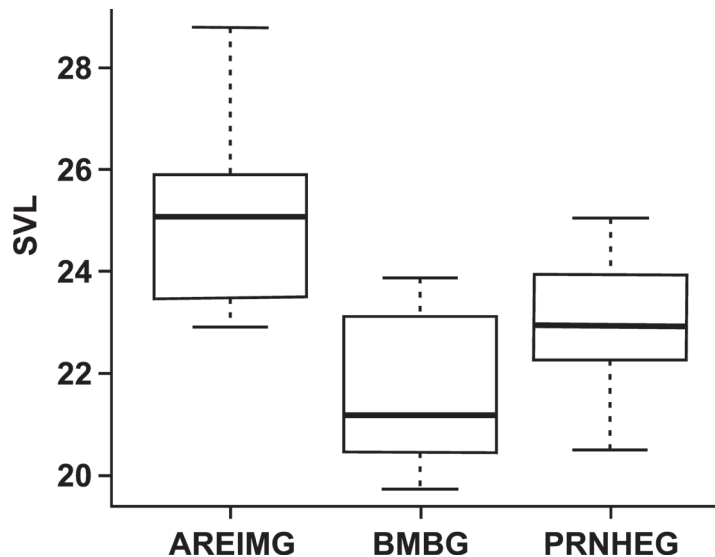


Fig. 4. Snout-vent length (SVL) of the three populations of *C. meridionalis* sampled in this study.

Table 2. Diet composition, prey number and IVI (importance value index) from three populations of *Coleodactylus meridionalis* including sex. Niche breadth based on the prey number of pooled stomachs "Niche N° A" for each site at the bottom of the table.

Diet	BMBG	PRNHEG	AREIMG	Males	Females
Araneae	-	5/36.44	1/7.38	5/23.96	1/8.43
Diptera	-	1/8.28	1/2.99	2/5.56	-
Formicidae	48/44.1	-	-	48/20.95	-
Homoptera	2/7.94	-	-	2/2.32	-
Hymenoptera	-	1/12.13	-	-	1/12.89
Insect larva	1/35.15	-	3/11.71	4/16.01	-
Insect egg	-	-	6/7.79	6/4.38	-
Isopoda	-	3.35.71	5/34.09	2/5.17	6/69.96
Isoptera	-	-	14/20.45	11/8.99	-
Orthoptera	1/12.78	-	2/5.87	3/6.82	-
Psocoptera	-	-	7/9.68	7/5.78	-
Scorpiones	-	1/7.42	-	-	1/8.67
Niche N° A	1.73	3.27	4.73	3.14	2.07

ic aspects can also be linked to the differences in endoparasite abundance and diversity. Furthermore, Price (1990) suggests that smaller hosts complete their life cycles in less time, also decreasing the time for the establishment of abundant parasite populations.

According to Kuris *et al.* (1980), the host body can be considered an island for parasites, and diversity may be correlated with size. *Coleodactylus meridionalis* is a small lizard, and in the present study obeys the standards already described in the literature, with low parasite richness; this is commonly represented by the following lizards: *Anotosaura vanzolinia* Dixon, 1974 (Squamata: Gymnophthalmidae) with an average SVL of 36 mm; *Liolaemus lutzae* Mertens, 1938 (Squamata: Liolaemidae), 50 mm of SVL; *Aspronema dorsivittatum* (Cope, 1862) (Squamata: Scincidae) 64 mm of SVL; *Phylllopezus lutzae* (Loveridge, 1941) (Squamata: Phyllodactylidae) 42 mm of SVL and *Dryadosaura nordestina* Rodrigues, Xavier Freire, Machado Pellegrine and Sites, 2005 (Squamata: Gymnophthalmidae) 40 mm of SVL, which harbor up to three parasite species (Ávila *et al.*, 2010; Oliveira *et al.*, 2017; Rocha, 1995; Rocha *et al.*, 2003; Teixeira *et al.*, 2018b).

This pattern may be explained by specific sites of infection only being available within larger hosts due to potential greater niche heterogeneity, which thus facilitates segregation of microhabitats (Kuris *et al.*, 1980).

Our results supports previous studies of parasitic fauna associated with small lizards, since we found three species of helminths infecting *C. meridionalis* from Atlantic Forest: *Physaloptera lutzi* (Nematoda: Physalopteridae), *Haplometroides odhneri* (Trematoda: Plagiorchiidae) and *Oligacanthorhynchus* sp. (Acanthocephala: Oligacanthorhynchidae). Heteroxenic species of the genus *Physaloptera* are reptiles (Ávila & Silva, 2010; Teixeira *et al.*, 2017; Teixeira *et al.*, 2018a) and amphibian parasites (Campaño *et al.*,

2014) in Brazil. The trematode *H. odhneri* has previously been recorded in snakes (Silva *et al.*, 2005; Silva & Barrella, 2002), and recently in lizards (Teixeira *et al.*, 2018b). In addition, *Oligacanthorhynchus* sp. has records infecting reptiles and amphibians in South America (Smales, 2007).

The hosts are mainly infected through diet, when the parasites present heteroxenous cycles (Martin *et al.*, 2005). Based on the results obtained in our study, the diet of the lizards vary in food composition between sampled populations, with low numerical niche overlap, and the numerical niche breadths being highest in the AREIMG population, which may also explain the parasitism only occurring in this area. The AREIMG lizards consumed eight categories of prey, with PRNHEG consuming five and BMBG four, suggesting that the hosts of the population in which endoparasites were found consumed a greater diversity of food items. This corroborated the results of Brito *et al.* (2014), which presented a higher parasite diversity in lizards with higher food diversity.

Finally, the endoparasite abundance is related to host sex, with females being more parasitised than males. Since the lizards from the AREIMG fragment do not show sexual dimorphism with respect to SVL and body mass, we understand that possibly the host diet may be related to the differences in the endoparasite abundance present between males and females of *C. meridionalis* (Aho, 1990; Fontes *et al.*, 2003; Pereira *et al.*, 2012; Zuk & McKean, 1996). However, we emphasize that this was an speculation on our part, because the patterns obtained for both indexes, niche breadth and food niche overlap between the sexes, were based on the stomach contents of all lizards from the three sampled fragments.

Conflict of Interest.

Authors state no conflict of interest.

Acknowledgments

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