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Very low prevalence of infection by *Physaloptera lutzi* (Nematoda: Physalopteridae) parasitizing *Kentropyx calcarata* (Squamata: Teiidae), from fragments of Atlantic Forest in Northeast Brazil with a summary of nematodes infecting congeneric species

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Abstract. We analysed the patterns of infection by helminthes in populations of the teiid lizard *Kentropyx calcarata* from two Atlantic Forest fragments in Northeast Brazil. From 303 lizards, only two specimens from a single population were infected by *Physaloptera lutzi* (*P%*: 0.66 and *II*: range 2-38). Our study showed a very low prevalence of infection in *K. calcarata* from the Atlantic Forest when comparing with congeners lizards from the Amazon Forest infected by helminthes. In addition, our study represents the first record of *P. lutzi* for *K. calcarata* lizards.

Keywords. Parasitism, Encounter Filter, Compatibility Filter, Lizards, Amazon Forest

Introduction

Kentropyx calcarata is a teiid lizard distributed east of the Andes from 25º latitude in South America (Gallagher and Dixon, 1992). Currently, eight species are known for the genus: *K. calcarata*, *K. altamazonica*, *K. pelviceps*, *K. paulensis*, *K. viridistriga*, *K. vanzoi*, *K. striata* and *K. borckiana* (Gallagher et al. 1986; Werneck et al. 2009). The lizards of this genus are characterized by the presence of keeled ventral scales (Gallagher and Dixon, 1992), are heliothermic, and active foragers that feed mainly on spiders and orthopterans (Abrams, 1977; Vitt, 1991; Vitt and Carvalho, 1992; Vitt et al. 2001; Vitt et al. 1995; Vitt et al. 1997; Vitt et al. 2001).

The most widely distributed species of this genus is *Kentropyx calcarata*, occurring from the eastern Andes in Amazonian regions of Venezuela, Guyana and Brazil and also in Brazilian Amazon Cerrado Transitions and Atlantic Forest regions (Gallagher et al. 1986; Roberto et al. 2012). They are frequently found in forest sites with direct incidence of sunlight, such as clearings and forest edges, where they are frequently observed basking to regulate their body temperature (Magnusson, 1993; Vitt, 1991; Vitt et al. 1997). Individuals of this species actively forage the substrate, usually on the ground, feeding on prey below the leaf litter, but they occasionally also climb stems and trunks of low vegetation (Vitt, 1991).

Currently, ecological (Costa et al. 2013; Magnusson and Lima, 1984; Vitt, 1991; Vitt et al. 1997) and parasitological investigations (Ávila and Silva, 2013; 2009; Bain, 1974; Baker, 1982; Baker, 1987; Goldberg

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et al. 2007; Macedo et al. 2017) for this species of lizard are much more frequent for Amazon populations. Considering Atlantic Forest, there are a few publications of natural history based on observations of isolated events (Lantyer-Silva et al. 2012; Filadelfo et al. 2013, Franzini et al. 2017) and small populations (Roberto et al. 2012). Studies with their endoparasite fauna are nonexistent for Atlantic Forest populations.

According to Lafferty and Kuris (2005), environmental stressors are responsible for the increased mortality of host, by weakening of their immune system and / or increase their rates of infection. On the other hand, they may also decrease parasitism, by direct death of parasites and / or reduction of intermediate hosts and vectors, thus preventing their transmission process (Lafferty and Kuris, 1999). The Brazilian Atlantic Forest has been explored since the beginning of the colonization of the country, and the sum of its fragments corresponds to only 8.1% (11610 km^2) of its original area (Pinto et al. 2006). However, when we focus on the extension of forest located in northeastern Brazil, the scenario is even more worrisome, since it maintains less than 2% of its original vegetation cover (Tabarelli et al. 2006).

In this way, knowing that the fragmentation results in the reduction of natural habitats, being one of the main causes of the loss of biodiversity (Pimm and Raven, 2000) and that due to the majority of the parasites adopt the mode of aggregate distribution (many hosts harbouring few parasites and few hosts harbouring many parasites) (Poulin, 2007) we can assume that the diversity of parasites that compose the Brazilian Atlantic Forest is very threatened, since they need a minimum threshold population of hosts to be able to establish themselves (Lyles and Dobson, 1993).

Thus, knowing that parasites play an important role in the regulation of their hosts (Freeland and Boulton, 1992), our study seeks to identify the composition of nematodes associated *Kentropyx calcarata* in Atlantic Forest fragments located in northeastern Brazil, thus enabling comparisons to other populations of congeners (Table 1), expanding nematode fauna knowledge records for this environment.

Materials and methods

The specimens of *Kentropyx calcarata* were collected during expeditions in two fragments of Atlantic Forest located in Northeast Brazil. The Reserva Particular do Patrimônio Natural Engenho Gargaú (RPPN-EG) located in the Santa Rita municipality (-6.75105° S/ -35.633335°W; area of 1058.62 ha) between September 2016 and August 2017 and at the Benjamim Maranhão Botanical Garden (BMBG), in the João Pessoa municipality (-7.1355555555556° S/ -34.860277777778° W; area of 471 ha) between November and December of 2016, both areas in Paraíba state, Brazil, with annual rainfall of 1490 mm and an annual average temperature of 24.6 ° C (Climate-Date, 2017).

All specimens were captured manually or using pitfall-traps (25 sets in each area), constructed with four buckets (20L) arranged in "Y" shape, totalizing 100 buckets per area (Oliveira et al. 2017).

We killed the lizards with a lethal injection of 2% lidocaine hydrochloridec and measured snout-vent length (SVL) with digital callipers. Subsequently, we sexed them, preserved in 10% formalin and stored in 70% alcohol. In the laboratory, we removed the respiratory and gastrointestinal tracts and analysed them in a stereomicroscope to search for endoparasites. The endoparasites were cleared in Hoyer's solution (Everhart, 1957), counted, registered the site of infection, and subsequently identified in accordance to Vicente et al. (1993). Subsequently, they were preserved in 70% alcohol and deposited in Coleção de Invertebrados Paulo Young, in Universidade Federal da Paraíba, Brazil.

The infection rates were calculated according to Bush et al. (1997), where prevalence of infection (*P%*) corresponds to the number of infected hosts divided by the total number of hosts in the sample x 100 and intensity of infection (*II*) corresponds to the number of parasites found in each infected host. Throughout the text, means appear ± 1 SD.

Results

An amount of 246 lizards was collected in RPPN-EG, where the mean size (SVL) of females is 67.60 ± 20.07 mm and the mean size (SVL) of males is 56.12 ± 20.15 mm. Another 57 lizards were collected in BMBG, where the mean size (SVL) of females is 53.18 ± 10.74 mm and the mean size (SVL) of males is 49.64 ± 9.35 mm. Despite the large sampling, of the 303 *Kentropyx calcarata* lizards examined, only two specimens (Female: SVL 97.24 mm and a Male: SVL: 89.34 mm) collected respectively in June and August 2017 in the RPPN-EG were infected by *Physaloptera lutzi* Cristofaro, Guimarães and Rodrigues, 1976 (*P%*: 0.66 and *II*: range 2-38).

Table 1. Summary based on studies and reports of Nematode parasites of *Kentropyx* lizards from South America.

Host Species	N°	Parasite Family	Nematode Species	Locality, State - Country	Source
Kentropyx altamazonica (Cope, 1876)	11	Physalopteridae	*Physaloptera retusa*	Cuzco - Peru	Bursey et al. 2005
		Physalopteridae	*Physalopteroides venancioi*		
Kentropyx calcarata Spix, 1825	-	Molineidae	*Kentropyxia sauria*	Belém, Pará - Brazil	Baker, 1987
	-	Onchocercidae	*Piratuba shawi*	Belém, Pará - Brazil	Baker, 1987
	-	Onchocercidae	*Piratuba shawi*	Belém, Pará - Brazil	Bain, 1974
	17	Molineidae	*Kentropyxia sauria*	Novo Progresso, Pará - Brazil	Goldberg et al. 2007
		Physalopteridae	*Physaloptera retusa*		
	7	Molineidae	*Oswaldocruzia* sp.	Juara, Mato Grosso - Brazil	Ávila and Silva, 2009
		Onchocercidae	*Piratuba digiticauda*		
		Physalopteridae	*Physaloptera retusa*		
		Physalopteridae	*Physalopteroides venancioi*		
	10	Molineidae	*Kentropyxia sauria*	Aripuanã, Mato Grosso - Brazil	Ávila and Silva, 2013
		Physalopteridae	*Physaloptera retusa*		
	13	Heterakidae	*Spinicauda spinicauda*	Caxiuanã, Pará - Brazil	Macedo et al. 2017
		Pharyngodonidae	*Parapharyngodon alvarengai*		
		Physalopteridae	*Physaloptera* sp.		
	246	Physalopteridae	*Physaloptera lutzi* *	Santa Rita, Paraíba - Brazil	This study
Kentropyx pelviceps Cope, 1868	15	Ascarididae	*Dujardinascaris* sp.	Cuzco - Peru	Bursey et al. 2005
		Pharyngodonidae	*Parapharyngodon sceleratus*		
		Physalopteridae	*Physaloptera retusa*		
		Physalopteridae	*Physalopteroides venancioi*		
	2	Molineidae	*Kentropyxia sauria*	Ucayali - Peru	Mcallister et al. 2010
		Pharyngodonidae	*Parapharyngodon sceleratus*		
		Physalopteridae	*Physaloptera obtusíssima*		
	4	Physalopteridae	*Physaloptera retusa*	Cruzeiro do Sul, Acre - Brazil	Albuquerque et al. 2012

* = New records; N°= Represents the number of lizards used in each study.

Discussion

In theory, there are two filters that define how animals can be used as hosts by a parasite (Combes, 2001; Euzet and Combes, 1980; Poulin, 2007): the encounter filter, which excludes all animals which the parasites cannot encounter in nature, and the compatibility filter, which excludes the species where the parasite cannot establish itself (Combes, 2001). However, the infection already does not succeed if just one of them is closed (Combes, 2001).

Because populations of *Kentropyx* from the Amazon Forest present 11 documented nematode species (Ávila and Silva, 2013; 2009; Goldberg et al. 2007; Macedo et al. 2017), we have excluded the possibility of the compatibility filter being closed to the *K. calcarata* populations from the Atlantic Forest, since parasites can infect closely related hosts whose immunological defences tend to be similar (Poulin and Mouillot, 2004). In this way, it is more likely that the compatibility filter is open to the populations of lizards of the Amazonian and Atlantic Forest. However, it is possible that the encounter filter is closed to the second population, reflecting the very low prevalence of infection and the depleted helminth fauna. According to Combes (2001) the selection of foraging sites with low occurrence of parasites may be important to explain the low rates of infection found in *K. calcarata*. This explanation also helped Anjos et al. (2011) understand the causes of low diversity of helminths parasitizing the sympatric lizards *Hemidactylus agrius* and *Lygodactylus klugei* in an area of Caatinga, in the northeast of Brazil.

In addition to this, habitat fragmentation may also be responsible for the loss of parasitic fauna of the Atlantic Forest lizards (Lafferty and Kuris, 2005), since, as areas are being reduced, the extinction of parasites species may occur before their hosts (Lafferty and Kuris, 2005), since the infection requires a minimum population size (threshold) of hosts to be maintained (Lyles and Dobson, 1993). Thus, habitat loss may have closed the encounter filter, making it impossible to establish a richer parasitic fauna with higher infection rates, since fragmentation affects prey diversity and environmental heterogeneity, throughout the extinction of invertebrates and plants (Pimm and Raven, 2000). This hypothesis was supported by Brito et al. (2014), who verified that the microhabitat use and prey diversity consumed by the Caatinga lizards in northeastern Brazil has a direct relationship with the diversity of associated endoparasites.

Finally, the present study records for the first time the infection of *Physaloptera lutzi* in the lizard *Kentropyx calcarata*. Nematodes of the genus *Physaloptera* are generalists and infect stomachs of reptiles (Ávila and Silva, 2010) and amphibians (Campião et al. 2014) in South America. In Brazil, *P. lutzi* has been recorded parasitizing several families of lizards, with a greater emphasis on Teiidae (Aho, 1990; Ávila and Silva, 2011; Brito et al. 2014; Ribas et al. 1995; 1998; Teixeira et al. 2017) and Tropiduridae (Ávila et al. 2011; Van Sluys et al. 1997; Vrcibradic et al. 2000). Nevertheless, further studies need to be performed, enabling the comparison of autoecology (mainly prey and microhabitat use) among populations of *K. calcarata* occurring in the Amazon and Atlantic Forest, to understand what filters are truly responsible for explaining the patterns of infection presented here, in addition to trying to understand how fragmentation can alter these infection processes.

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