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Helminths infecting *Dryadosaura nordestina* (Squamata: Gymnophthalmidae) from Atlantic Forest, northeastern Brazil

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Summary

We analyzed the patterns of infection by helminths in populations of the Gymnophthalmidae lizard *Dryadosaura nordestina* from three Atlantic Forest fragments in Northeast Brazil. Prevalence and mean intensity of infection by location showed the following results: ARIE Mata de Goiamunduba (60.8 % and 10.4 ± 8), RPPN Engenho Gargaú (83.3 % and 20.8 ± 19.7) and Benjamim Maranhão Botanical Garden (70.4 % and 7.78 ± 5.8). We provide the first records of helminth infection for the lizard *D. nordestina*, in which three species of nematodes, *Aplectana* sp., *Cosmocerca* sp. and *Physaloptera lutzi* and one trematode *Haplometroides odhneri* were recovered. Trematodes of the genus *Haplometroides* were previously known as parasites only in snake and amphisbaenian hosts in South America. Now, our study provides the first record of a species belonging to this genus parasitizing lizards. In conclusion, our study shows that *D. nordestina* have a depleted helminth fauna (three species at maximum), similar to other studies with lizards of this family in Brazil and that its parasite abundance is related to host snout-vent length, but not to the sex.

Keywords: nematode, trematode, lizard, parasitism

Introduction

Habitat fragmentation processes are identified as the main causes of extinction of animal and plant species (Pimm & Raven, 2000). This occurs because many species belonging to a particular habitat are restricted to small portions of the same. Thus, as areas are being lost, extinction rates tend to rise dramatically (Pimm & Raven, 2000; Rocha *et al.*, 2006). Considering parasite communities, environmental changes have a faster and devastating effect, compared to free-living organisms, and they may be extinct before their hosts (Lyles & Dobson, 1993). According to Pinto *et al.* (2006) the main way to ensure the preser-

vation of the biodiversity of the Brazilian Atlantic Forest will be with ecosystem management activities. However, the strategies, actions, and necessary interventions are hampered by the lack of knowledge about the ecology of the species and the functioning of the ecosystems where they are inserted (Pinto *et al.*, 2006). In addition, knowing that parasites play an important role in the balance of ecosystems (Freeland & Boulton, 1992; Lafferty & Kuris, 2005), studies addressing the host-parasite relationships are also important to propose and develop strategies for management and conservation of biodiversity (Marcogliese, 2004). Parasitological studies involving Gymnophthalmidae lizards in Brazil still remain scarce and mostly focused on records of new

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hosts (Ávila & Silva, 2010). Only 14 Gymnophthalmidae of the 93 species occurring in Brazil have some kind of investigation on the host-parasite relationship (Ávila & Silva, 2011; Ávila *et al.*, 2010b; Brito *et al.*, 2014; Burseley & Goldberg, 2004; Goldberg *et al.*, 2007a; Goldberg *et al.*, 2007b; Oliveira *et al.*, 2017; Neta & Ávila, 2018; Ribeiro *et al.*, 2018).

Dryadosaura Rodrigues, Xavier Freire, Machado Pellegrino & Sites, 2005 is a genus composed of only one species that occurs in the Northeast part of the Atlantic Forest of Brazil, distributed in the states of Rio Grande do Norte to Bahia (Oliveira & Pessanha, 2013). *Dryadosaura nordestina* Rodrigues, Xavier Freire, Machado Pellegrino & Sites, 2005 is a small lizard, with body size (Snout-vent length) ranging from 45.4 ± 5.7 mm for males and 42.7 ± 7.1 mm for females, it is an active forager with generalist diet, composed mainly of arthropods, being ants and insect larvae the main food items (Garda *et al.*, 2014). However, there are no records on parasites associated with *D. nordestina*, possibly because it is an uncommon lizard, the difficult to its collection, and because there are still few specimens housed in Herpetological Collections. Thus, the main objective of our study is to know the composition of helminth species associated with *D. nordestina*, evaluating effects of sex and host body size, using samples from the Atlantic Forest, northeastern Brazil.

Materials and Methods

We collected 56 specimens of *D. nordestina* during expeditions in three localities. We collected 23 lizards in the ARIE Mata de Goiamunduba (ARIE-MG), area of 67.5 ha, located in the Bananeiras municipality ($-06^{\circ}45'03.78''S$ – $-35^{\circ}38'00.06''W$) in October 2016; six in the RPPN Engenho Gargaú (RPPN-EG) area of 1058.62 ha, in the Santa Rita municipality ($-06^{\circ}59'52''S$ – $-34^{\circ}57'30''W$) in September 2016 and 27 lizards at the Benjamim Maranhão Botanical Garden (BMBG) area of 471ha, in the João Pessoa municipality ($-07^{\circ}08'08''S$ – $-34^{\circ}51'37''W$) in November 2016 (Dias *et al.*, 2006). All areas are remnants of Atlantic Forest located in the Paraíba state, Brazil. The average annual rainfall of the areas is 1490 mm and the average annual temperature of 24.6° C. 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).

Lizards were killed with a lethal injection of 2 % lidocaine hydrochloride and measured snout-vent length (SVL) with digital calipers. Subsequently, they were sexed, preserved in 10 % formalin, and stored in 70 % alcohol. In the laboratory, we removed the respiratory and gastrointestinal tracts that were surveyed for endoparasites under a stereomicroscope. The endoparasites were cleared in Hoyer's solution (Everhart, 1957), counted, registered the site of infection, and subsequently identified in accordance to Anderson (2009) for nematodes and (Silva *et al.*, 2007) for trematode species. Subsequently, they were preserved in 70 % alcohol and deposited in “Coleção de Invertebrados Paulo Young”, in the

Universidade Federal da Paraíba, Brazil (UFPB-NEM: 03, 04; UFPB-DIG: 03, 04, 05).

The infection rates were calculated according to Bush *et al.* (1997), where prevalence of infection corresponds to the number of infected hosts divided by the total number of hosts in the sample $\times 100$ (it appears in percentages throughout the text) and mean intensity of infection (*MII*) is the total number of parasites found in a sample, divided by the number of hosts infected with that parasite; finally, parasite abundance is defined as the total number of parasites found in a sample (individual host, host population and/or host community) Throughout the text, means appear ± 1 SD.

To verify the influence of sex and host size (SVL) on the abundance of endoparasites Generalized linear mixed models – GLMM were used. This model was chosen due to the possibility of introducing into the equation random factors, thus removing possible effects that they may exert on the fixed variables (Bates, 2014).

Knowing that our sampling corresponds to three distinct component communities, to test the hypotheses suggested above, we adopted the study areas as random factors, in order to remove any influence of the locality on our dataset.

In order to verify if the abundance of endoparasites varies between the sexes of the hosts, we performed a GLMM adopting the locality and the body size (SVL) of the hosts as random factors. On the other hand, to verify the relationship between the abundance of endoparasites and the body size of the hosts (SVL), we adopted the locality and sex of the hosts as random factors.

Also, GLMM's were performed in R software with the help of packages “lme4” (Bates, 2014) and “MuMIn” (Barton, 2009) adopting the Poisson (link:log) distribution family (Wilson & Grenfell, 1997).

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 analyzing 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 Benjamim Maranhão Botanical Garden-BMBG (no: 003/2016/JBBM/SUDEMA).

Results

We examined 56 *D. nordestina* specimens, of which 33 were adult males (SVL: 40.4 ± 10.6), 14 adult females (SVL: 39.4 ± 10.5), and nine juveniles (SVL: 20.8 ± 10.2). Prevalence and mean intensity of infection in lizards by location, respectively, were: ARIE-MG (60.8 % and 10.4 ± 8), RPPN-EG (83.3 % and 20.8 ± 19.7), and BMBG (70.4 % and 7.78 ± 5.8).

We identified three nematode species, *Aplectana* sp. Railliet and Henry, 1916; *Cosmocerca* sp. Diesing, 1861, and *Physaloptera lutzi* Cristofaro, Guimarães and Rodrigues, 1976; and also, one

Table 1. Parasitological data from populations of *Dryadosaura nordestina* from Atlantic Forest, northeastern Brazil, infected by helminths.

Locality	<i>Aplectana</i> sp.			<i>Cosmocerca</i> sp.			<i>Physaloptera lutzi</i>			<i>Haplometroides odhneri</i>		
	%	II (Range)	SI	%	II (Range)	SI	%	II (Range)	SI	%	II (Range)	SI
RPPN-EG	83.3	17.8 ± 5.7	S; S.I; L.I	16.7	1	S.I	-	-	-	33.3	(3 – 11)	S; S.I
ARIE-MG	56.5	5.2 ± 3.7	S; S.I; L.I	-	-	-	26.1	12 ± 4.8	S	13	6	S
BMBG	70.4	7 ± 5	S; S.I; L.I	-	-	-	-	-	-	14.8	4 ± 2.3	S.I

Prevalence (%), Mean intensity of infection – II, Site of infection – SI: Stomach – S, Small intestine – S.I and Large intestine – L.I.

trematode species, *Haplometroides odhneri* Ruiz and Perez, 1959 infecting *D. nordestina* (Table 1).

The GLMM did not find significant differences in relation to the sex of the hosts and the endoparasites abundance ($R^2 = 0.01235937$; $Z_{1,34} = 0.668$; $P = 0.504$), however, revealed a significant relationship between endoparasites abundance and SVL ($R^2 = 0.4674977$; $Z_{1,34} = 8.377$ $P < 2e-16$).

Discussion

Our results provide the first records of helminth infection for the lizard *D. nordestina*, in which three species of nematodes (*Aplectana* sp., *Cosmocerca* sp. and *P. lutzi*) and one trematode (*H. odhneri*) were recovered.

Nematodes belonging to the family Cosmocercidae infect amphisbaenians (Amorim *et al.*, 2017), lizards (Ávila & Silva, 2010), and frogs (Gomez *et al.*, 2017) and they have a monoxenic life cycle, in which their infective stages are transmitted directly via tegument or accidental ingestion (Anderson, 2000). This characteristic of transmission may explain the high prevalence of infection by *Aplectana* sp. in all sampled areas (Table 1), considering that the adaptations presented in Gymnophthalmidae lizards for fossorial and semi-fossorial life (Garda *et al.*, 2014; Grizante *et al.*, 2012; Oliveira *et al.*, 2018) may facilitate the process of infection with parasites that have a direct life cycle (Oliveira *et al.*, 2017). On the other hand, *Cosmocerca* sp. presented low infection rates, corroborating the results obtained by Bursey & Goldberg (2004) for Gymnophthalmidae lizards from Amazonia, which showed prevalence varying between 16-36 % and mean infection of intensity inferior to two. Thus, knowing that monoxenic nematodes can have their infection process favored by the ecology of Gymnophthalmidae lizards (Garda *et al.*, 2014; Grizante *et al.*, 2012; Oliveira *et al.*, 2018), in case of *Cosmocerca* sp., historical factors (phylogeny) can be pointed out as one of the main determinants that explain the low infection rates presented here (Poulin, 2007; Brito *et al.*, 2014).

Poulin (1998) states that there is a trade-off between the performance and range of host species that a parasite can exploit. This can easily be perceived for nematodes of the genus *Physaloptera*, since they infect a wide range of lizards in South America (Ávila & Silva, 2010; Teixeira *et al.*, 2017), however, almost always followed by low prevalence of infection: *P. lutzi* (0.9 %) parasitizing *Ameivula ocellifera* (Spix, 1825) (Ribas *et al.*, 1995); *P. lutzi*

(2 %) and *P. retusa* Rudolphi, 1819 (3.9 %) in *Ameiva ameiva* (Linnaeus, 1758) (Ribas *et al.*, 1998); *P. retusa* (5.3 %) and *P. lutzi* (19.3 %) in the lizard *Tropidurus hispidus* (Spix, 1825) (Anjos *et al.*, 2012); *P. retusa* (9 %) registered in *Polychrus acutirostris* Spix, 1825 (Araujo Filho *et al.*, 2014); *P. retusa* in the sympatric lizards *Hemidactylus mabouia* (Moreau de Jonnés, 1818) (3.94 %) and *Phylllopezus pollicaris* (Spix, 1825) (2.54 %) (Sousa *et al.*, 2014); *Physaloptera* sp. (5 %) in the stomach of *Iguana iguana* (Linnaeus, 1758) (Teles *et al.*, 2017) and more recently, *P. lutzi* registered by Lima (2017) parasitizing the gecko lizards *P. pollicaris* (3 %), *Hemidactylus brasiliensis* (Amara, 1935) (4.1 %), *Hemidactylus agrius* Vanzolini, 1978 (1.6 %), *Gymnodactylus geckooides* Spix, 1825 (12.6 %), and *Lygodactylus klugei* (Smith, Martin e Swain, 1977) (1.5 %), from northeastern Brazil. Thus, the low infection rates presented here by the generalist nematode *P. lutzi* apparently obey the standard cited above. However, further testing is needed to verify if this hypothesis truly fits the patterns of infection exhibited by the parasitic nematode species of lizards in the Neotropical region.

Trematodes of the genus *Haplometroides* Odhner, 1910 were previously known as parasites only in snake and amphisbaenian hosts in South America (Silva & Barrella, 2002; Silva *et al.*, 2005a,b; 2007; 2008; Santos *et al.*, 2008). Now, our study provides the first record of *H. odhneri* parasitizing lizards.

Our study did not reveal significant differences between the sexes of the lizards in relation to the abundance of endoparasites, similar to the results obtained for other Gymnophthalmidae lizards by Neta & Ávila (2018) for *Colobosauroides cearensis* Cunha, Lima-Verde e Lima, 1991 and Ribeiro *et al.* (2018) for *Nothobachia ablephara* Rodrigues, 1984. However, to populations of *Anotosaura vanzolinia* Dixon, 1974 from Caatinga, females were more infected than males, since they tend to use more humid habitats during their reproductive period, potentializing the process of infection by the monoxenic nematode *Oswaldocruzia brasiliensis* Lent et Freitas, 1935 (Oliveira *et al.*, 2017).

Usually, juvenile hosts tend not to be parasitized or harbor a reduced parasite load when compared to adult individuals, due to the shorter exposure time the sources of infection (Kuris *et al.*, 1980). In conformation with this theory, no juvenile lizard in our sample was parasitized.

According to Nascimento (2004), larger hosts harbor a larger parasitic load, because they present a larger area and possibly more resources for the establishment of parasite populations. Our study

corroborates this hypothesis since we observed a significant relationship between SVL and the abundance of parasites in the lizard *D. nordestina*, similar to Neta & Ávila (2018) for *C. cearensis* in a Caatinga area, northeast of Brazil.

Small lizards may restrict the diversity of associated endoparasites, since niche differentiation and microhabitat segregation by competing parasite species may be impaired by the reduced size of the host (Ávila *et al.*, 2010a; Kuris *et al.*, 1980; Oliveira *et al.*, 2017). For lizards, this hypothesis is supported mainly by Gymnophthalmidae: *Cercosaura eigenmanni* (Griffin, 1917) and *C. oshaughnessyi* (Boulenger, 1885) both parasitized by three taxa of parasites (Burseley & Goldberg, 2004); *Micrablepharus maximiliani* (Reinhardt e Luetken, 1862) also parasitized by three taxa of parasites (Brito *et al.*, 2014); *A. vanzolinia* parasitized by only one species of nematode (Oliveira *et al.*, 2017); *N. ablephara* with three gastrointestinal helminth taxa identified (Ribeiro *et al.*, 2018) and *C. cearensis* infected with five gastrointestinal helminth taxa (Neta & Ávila, 2018).

In conclusion, our study shows that *D. nordestina* have a depleted helminth fauna (three species at maximum) composed of nematodes and trematodes and that its parasite abundance is related to host SVL, but not to the sex.

Conflict of Interest

Authors state no conflict of interest.

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