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Infection patterns of helminths in *Norops brasiliensis* (Squamata, Dactyloidae) from a humid forest, Northeastern Brazil and their relation with body mass, sex, host size, and season

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Summary

Climatic and ecological factors can influence the parasite load of a host. Variation in rainfall, body size, and sex of the hosts may be related to the abundance of parasites. This study investigated the helminth fauna associated with a population of *Norops brasiliensis*, together with the effect of host biology (sex, body size, and mass) and variation in rainfall regime on the abundance of helminths. Species of three groups of endoparasites were found (Nematoda, Cestoda, and Trematoda), with nematodes as the most representative taxa with eight species, prevalence of 63.2 %, mean intensity of 4.0 ± 0.58 (1 – 25), and mean abundance of 2.66 ± 0.44 (0 – 25). Nine helminth species are new host records for *N. brasiliensis*. The nematode *Rhabdias* sp. had the highest prevalence (53.3 %). There was no significant relationship between abundance of the trematode *Mesocoelium monas* and host sex or season, although the abundance of this parasite increased significantly with host body size and mass, while abundance of nematodes was related to season and host mass. This study increases the knowledge about the diversity of helminth fauna associated with *N. brasiliensis*, revealing infection levels of hosts from northeastern Brazil.
Keywords: Parasite abundance; Nematoda; Cestoda; Trematoda; Lizards; South America

Introduction

Parasites live in constant interaction with their hosts and can influence their way of life and their relationships with the environment, while using them as a source of food and shelter (Aho, 1990; Lozano, 1991; Thomas *et al*., 2010; Hafer & Milinski, 2016). Knowledge of the helminth fauna of a species can provide crucial information for understanding its behavior, diet, and habitat use (Lozano, 1991; Pereira *et al*., 2012, Cabrera-Guzmán & Garrido-Olvera, 2014; Maia-Carneiro *et al*., 2017).

Climatic factors, such as variation in rainfall and temperature, as well as variation in host biology and parasite life cycle, may influence the community structure of helminths (Aho, 1990; Brito *et al.,* 2014; Araújo-Filho *et al.,* 2016; Maia-Carneiro *et al*., 2017). Host size, mass, sex, and reproductive period can also influence parasitism (George-Nascimento *et al*., 2004; Poulin & George-Nascimento, 2007; Pereira *et al*., 2012; Araújo-Filho *et al*., 2016; Oliveira *et al*., 2017).

Lizards act as hosts for many groups of parasites, are good models for ecological studies because they are easy to identify, and are widely distributed around the world (Huey & Pianka, 1981; Ávila & Silva, 2010; Roca & Galdón, 2010). In Brazil, the number of studies on the helminth fauna of lizards is increasing (Ávila & Silva, 2010; Ávila *et al*., 2011; Pereira *et al*., 2012; Macedo *et al*., 2017), although approximately 70 % of the lizard species from the Northeast have still not been investigated for parasites (Ávila *et al.,* 2012; Ribeiro *et al*., 2012; Araújo-Filho *et al*., 2016; Cabral *et al*., 2018).

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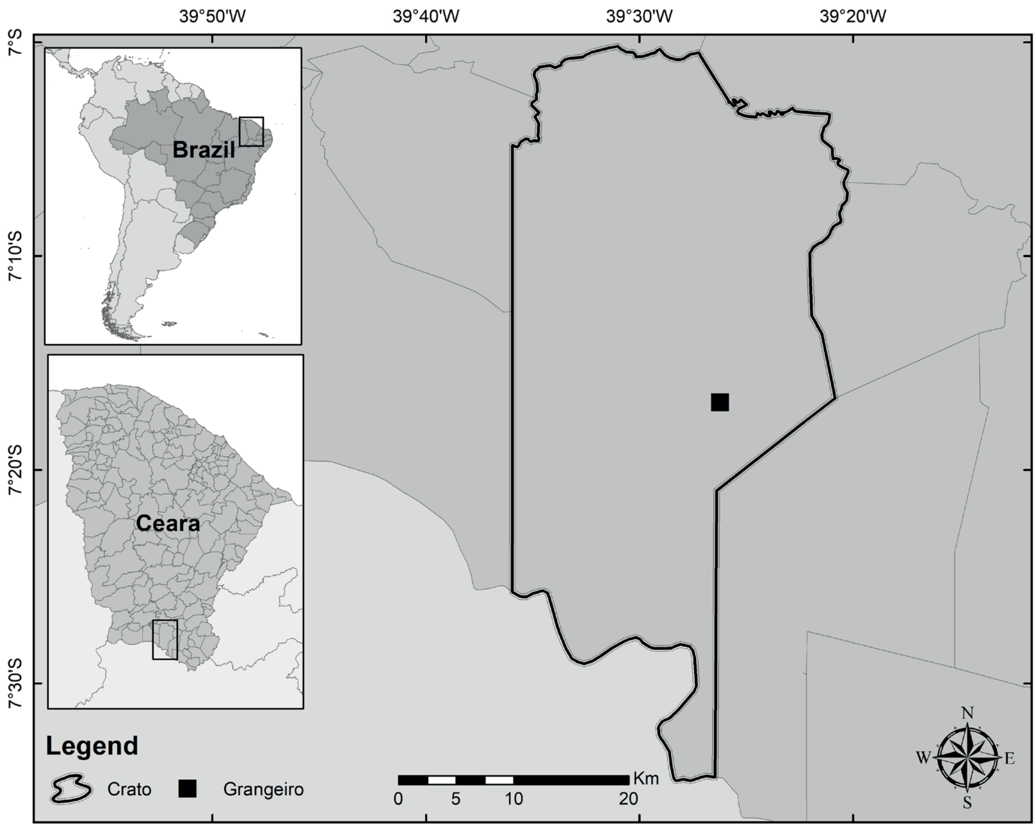

Fig. 1 Location of the studied population of *Norops brasiliensis* in an area of humid forest in Grangeiro, municipality of Crato, state of Ceará, Northeast Brazil.

Norops brasiliensis (Vanzolini & Williams, 1970), is considered endemic to the Cerrado domain, but can also be found in open Amazonian formations (Ávila-Pires 1995; Mesquita *et al.,* 2015). Thus far, for the state of Ceará, *N. brasiliensis* was recorded only in Chapada do Araripe, an area of Cerrado and humid forest (Ribeiro *et al.,* 2012; Roberto & Loebmann, 2016). *Norops brasiliensis* appears to have a generalist diet, feeding mainly on insects, spiders, and gastropods (Mesquita *et al.,* 2015).

Ávila *et al.,* (2011) reported the occurrence of helminths in *N. brasiliensis*, but were limited to a small sample from areas of Cerrado, while Ribeiro *et al.,* (2012) reported parasite infection in the respiratory tract of hosts from areas of humid forest in Northeast Brazil. The present study reports the helminth fauna associated with a population of *N. brasiliensis*, and the effect of host biology (sex, body size, and mass) and variation in rainfall regime on helminth abundance in an area of humid forest in Northeast Brazil.

Material and Methods

This study was carried out on the hillside slope of Chapada do Araripe in an area of humid forest (Tropical Sub-perennial Cloud Forest), at a point located on the Trilha Ecológica do Clube Grangeiro (07°16' S, 39°26' W), the study area is located in Área de Proteção Ambiental do Araripe-APA in the municipality of Crato, state of Ceará, Northeast Brazil (Fig. 1). The area has temperature annual less surrounding areas of Caatinga, ranging from 24 to 26 °C (IPECE 2015).

The rainy season occurs from January to April, while dry season extends from May to December (IPECE 2015). During data collection, the region received a rainfall volume of 100 mm on dry season, and 550 mm on rainy season (Funceme 2016; 2017). Data on rainfall levels were gathered using monthly means from the Fundação Cearense de Meteorologia e Recursos Hídricos – FUNCEME (Foundation of Meteorology and Hydric Resources of Ceará State).

Specimens of *N. brasiliensis* were collected weekly between October 2016 and July 2017. Upon capture specimens were immediately killed with Sodium Thiopental (Thiopentax®), had the snout-vent-length (SVL) taken with digital caliper (precision: 0.01 mm), and were weighed with Pesola® spring scales (precision: 0.1g). The specimens were fixed with 10 % formaldehyde (Franco and Salomão 2002) and vouchers were deposited in the Coleção Herpetológica of the Universidade Regional do Cariri (URCA–H 12667 – 12724, 13004 – 13010, 13012 – 13014; 13016 – 13019; 13093 – 13094; 13129; 13131).

Lizards were dissected and their gastrointenstinal tract, lungs, and body cavity were examined for endoparasites. Cestodes and Trematodes were dehydrated in an increasing series of alcohol,

Table 1. Prevalence (P), mean abundance (MA), and mean intensity of infection (MII) with standard error (SE) and range (R), and infection site (IS) of the helminth community associated with *Norops brasiliensis* (n = 77) in an area of humid forest in Northeast Brazil. Mean values ± standard error. SI – small intestine, ST – stomach, LI – large intestine, C – coelom, L – lung.

Helminth species	P%	MA ± SE (R)	MII ± SE (R)	IS
Nematoda				
Oswaldocruzia bainae	7.8	0.10 ± <0.1 (0–2)	1.33 ± 0.21 (1–2)	SI
Skrjabinellazia sp.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	SI
Parapharygondon sceleratus	18	0.42 ± 0.1 (0–4)	2.3 ± 0.3 (1–4)	SI, LI
Physaloptera retusa	7.8	0.4 ± 0.2 (0–19)	4.8 ± 2.8 (1–19)	ST
Onchocercidae indet.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	ST
Ophidascaris sp.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	ST
Cosmorcercidae indet.	2.6	0.04 ± <0.1 (0–2)	1.5 ± 0.5 (1–2)	LI, C
Rhabdias sp.	**53**	1.67 ± 0.4 (0–25)	3.17 ± (1–25)	L
Trematoda				
Mesocoelium monas	**33**	1.66 ± 0.4 (0–18)	5.12 ± 1.0 (1–18)	SI
Cestoda				
Oochoristica sp.	1.3	0.013 ± <0.1 (0–1)	1 ± 0 (1–1)	LI

stained with the hydrochloric carmine technique, and cleared in eugenol, while nematodes were cleared in lactophenol. The specimens were mounted on temporary slides and analyzed using a light microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany). The samples were later stored in 70 % ethanol and deposited in the Coleção Parasitológica of the Universidade Regional do Cariri (URCA-P). Parasite prevalence, intensity, and abundance were calculated according to Bush et al. (1997).

Generalized linear models (GLM), adopting the Poisson distribution, were used to evaluate if parasite abundance, considering the species of the groups Nematoda and Trematoda separated, was influenced by the period of collection of the host (dry or rainy season), and/or host sex, snout-vent-length, and mass. The R software was used for all statistical analyses (R Development Core Team 2012).

Ethical Approval and/or Informed Consent

The collecting methods were defined and authorized by the regulatory agency in Brazil Instituto Chico Mendes de Conservação da Biodiversidade, who granted permission for the collection of the animals (ICMbio/SISBio 57530-1, and 8383-1) and ethics committee of Universidade Regional do Cariri (CEUA/URCA, process # 00260/2016.1).

Results

A total of 77 adult lizards, including 26 females (SVL mean: 61.35 ± 3.37, range 55.86 – 67.27 mm) and 51 males (SVL mean: 61.47 ± 4.27, range 52.91 – 70.31mm) were examined. Sixty-four specimens were infected by at least one helminth species, with a total

Table 2. Generalized linear model (GLM) values considering abundance of species of the groups Nematode and Trematoda separated in relation to season, sex, snout-vent-length (SVL) and mass for *Norops brasiliensis* in an area of humid forest in Northeast Brazil. Significant p-values are in bold.

	Nematoda			Trematoda		
GLM	**SD**	**z value**	**P**	**SD**	**z value**	**P**
Intercept	1.253828	-0.749	0.454	1.27401	5.906	**< 0.0001**
SVL	0.025743	-0.251	0.802	0.02482	-7.553	**< 0.0001**
Sex	0.16523	1.915	0.0555	0.19405	-0.551	0.582
Mass	0.081017	4.1	**< 0.0001**	0.08467	9.538	**< 0.0001**
Season	0.142172	2.001	**0.0454**	0.18784	0.484	0.629

of 334 helminths specimens collected. Ten species of helminths were recorded associated with *N. brasiliensis* (Table 1), distributed among three groups: Nematoda, with eight taxa, and Trematoda and Cestoda, with a single species each. All taxa, except *Rhabdias* sp., represent new records for *N. brasiliensis.*

The hosts presented a mean richness of 0.9 ± 0.1 for Nematoda species. Overall prevalence was 63.2 %, overall mean abundance was 2.66 ± 0.44 (0 – 25), and overall mean intensity of infection was 4.0 ± 0.58 (1 – 25) for nematodes. For Cestoda and Trematoda see Table 1.

The mean abundance of trematodes was significantly positive related to host SVL (z = -7.553, $P < 0.001$) and host mass (z = 9.538, $P < 0.001$). However, the relationship among mean abundance of trematodes and host sex (z = -0. 551, P = 0.582), and season (z = 0.484, P = 0.629) was not significant. Mean abundance of nematodes was significantly positive related to host mass (z = 4.100, P = < 0.001), and season (z = 2.001, P = 0.045), but not to sex (z = 1.915, P = 0.055) and SVL (z = -0.251, P = 0.802) (see Table 2). Cestoda occurred in a single host, with only one parasite recorded.

Discussion

Species of the phylum Nematoda showed high abundance and prevalence of infection in *N. brasiliensis*, which is a common pattern found in many studies with lizards worldwide, including members of the family Dactyloidae (Goldberg *et al*., 2006; Ávila & Silva, 2010; Cabrera-Guzmán & Garrido-Olvera, 2014; Araújo-Filho *et al*., 2016; Sowemimo & Oluwafemi, 2017; Cabral *et al*., 2018). The high abundance of these endoparasites in reptiles may be related to their life cycle, diet, and lifestyle that favors infections by this group (Cabrera-Guzmán & Garrido-Olvera, 2014; Sowemimo & Oluwafemi, 2017).

All helminth species found, with the exception of *Rhabdias* sp. (a new species in process of formal description), represent new records for *N. brasiliensis*. Ávila *et al.,* (2011) reported parasitic infection by the nematode *Subulura lacertilia* in a population of *N. brasiliensis* from a Brazilian Cerrado area, and Ribeiro *et al.,* (2012) reported infection by *Rhabdias* sp. in the respiratory tract of this host species from areas of humid forest in Northeast Brazil. Ribeiro *et al.,* (2012) found prevalence of 28.6 % for *Rhabdias* sp., in *N. brasiliensis* which is lower than what we observed the present study (53.3 %). The high prevalence reported herein may be due to factors such as microhabitat use and host behavior (Aho, 1990; Goldberg *et al.,* 2006). *Rhabdias* (Stiles & Hassall, 1905) are nematodes with monoxenic life cycles, with infection by active penetration of the larva through the skin of the host (Anderson, 2000), commonly found infecting the lungs of lizards (Goldberg *et al.,* 2006; Vrcibradic *et al.,* 2007; Almeida *et al.,* 2009; Ávila *et al.,* 2011, Ribeiro *et al.,* 2012; Cabrera-Guzmán & Garrido-Olvera, 2014; Dorigo *et al.,* 2014). For the genus *Norops* in Brazil, there are reports of species of *Rhabdias* infecting only the species *N. fuscoauratus* (D'Orbigny 1937) and *N. brasiliensis* (Goldberg *et al.,* 2006; Ribeiro *et al.,* 2012).

In the present study, the monoxenic parasites *Parapharygondon sceleratus* and *Oswaldocruzia bainae,* as well as *Rhabdias* sp., showed high prevalence but low mean intensity of infection. This may be related to the evolutionary strategies of the parasites and to the host's ecology (Aho, 1990; Goldberg *et al.,* 2006). This is the first record of *P. sceleratus* infecting a species of *Norops*, while *O. bainae* has been previously recorded infecting two species of the genus in Brazil, *N. fuscoauratus* and *N. chrysolepis* (Ávila & Silva, 2010).

The heteroxenic nematode *Physaloptera retusa* (Rudolphi 1819) was found in this study with high prevalence and mean intensity of infection, which may be related to host diet (Aho, 1990; Anderson, 2000). *Norops brasiliensis* feeds mainly on termites, crickets, gastropods, insect larvae, and spiders (Mesquita *et al.,* 2015), being exposed to infection by the helminths that may be using these invertebrates as intermediate hosts (Aho, 1990; Anderson, 2000; Cabrera-Guzmán & Garrido-Olvera, 2014).

Other nematodes, (Onchocercidae Leiper 1911 (Filarioidea); Cosmocercidae Travassos 1925; *Ophidascaris* sp.; *Skrjabinelazia* sp.) and the cestode *Oochoristica* sp. were also recorded herein with low prevalence and mean infection intensity, which could be explained by the life cycle of the parasites or as casual infections (Aho, 1990; Anderson, 2000). Nonetheless, they are common parasites of the intestines of amphibians and reptiles, including lizards (Ávila & Silva 2013; Campião *et al.,* 2014; Amorim *et al.,* 2017).

The trematode *Mesocoelium monas* (Rudolphi 1819) was also recorded in this study, being the helminth with the second highest prevalence (Table 1). This parasite species generally uses gastropods as intermediate hosts (Escudero & Murillo, 2007). *Mesocoelium monas* is a widely distributed parasite, and has been previously documented infecting lizards of the genus *Norops* (Ávila & Silva, 2010). The high prevalence recorded herein may be related to the diet and habitat use of the hosts (Escudero & Murillo, 2007; Mesquita *et al.,* 2015).

The abundance of species of nematodes in *N. brasiliensis* was significantly different regarding rainfall regime. However, the abundance of *M. monas* did not differ significantly. In some species, parasite infection may be related to seasonal variation, due to the life cycle of the parasites and possible changes in habits of the hosts throughout the year (Aho, 1990; Brito *et al.,* 2014; Araujo-Filho *et al.,* 2016). *Norops brasiliensis* may be exposed to *M. monas* throughout the year, because they prefer humid microhabitats and are active in the coldest hours of the day, a lifestyle common to the intermediate hosts of these parasites, which may explain their abundance being not related to season (Escudero & Murillo 2007; Vitt *et al.,* 2008; Mesquita *et al.,* 2015).

There were no significant differences in the average abundance of parasites regarding host sex. Previous studies also reported no occurrence of intersexual variation related to the abundance of parasites (Amo *et al.,* 2005; Sowemimo & Oluwafemi, 2017;

Cabral *et al.,* 2018; Ribeiro *et al.,* 2018). This may be related to the reproductive period, which may indicate that investment in reproduction is greater than in defense against parasites for both sexes (Zuk & McKean, 1996; Amo *et al.,* 2005; Roberts *et al.,* 2004; Martin *et al.,* 2008; Cabral *et al.,* 2018), as well as behavior and lifestyle of the host species that influence the exposure to parasites (Sowemimo & Oluwafemi, 2017; Ribeiro *et al.,* 2018).

The abundance of trematodes was significantly related to host body size and mass, while for nematodes the abundance was related only to mass. Generally, larger hosts (body size and/or mass) have greater capacity to provide shelter and resources for the development of a greater number of parasites (Aho, 1990; George-Nascimento *et al.,* 2004; Poulin & George-Nascimento, 2007). Another important factor is the life span of the hosts because, compared to juveniles, older individuals have a greater period of time for exposure to infection by parasites, as well as larger body size and mass, which may result in higher rates of parasitism (Aho, 1990; Poulin, 1997).

The results of the present study showed that the seasonality does not influence the abundance of trematodes, but is significant for infection with nematodes. The sex of the hosts did not influence the parasitic infection of *N. brasiliensis* for any of the parasites groups. However, body size and mass of the host were significantly related to helminth abundance, suggesting that larger individuals with longer exposure time are more susceptible to helminth infections. This study also highlights the apparent variation in relation to infection by species of Nematoda and Trematoda groups, which may be related to the life cycle of endoparasites and/or a host response to exposure to these groups of helminths, although these are issues that still need to be addressed.

This study contributes to the knowledge of the helminthological fauna of *N. brasiliensis* by revealing infection levels, besides reporting nine new parasite records for this host species, increasing the knowledge of helminths of South American lizards.

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Conflict of Interest

Authors state no conflict of interest.

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