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Nematodes infecting *Anotosaura vanzolinia* (Squamata: Gymnophthalmidae) from Caatinga, northeastern Brazil

Bruno Halluan S. Oliveira[1], Adonias A. Martins Teixeira[1,*], Romilda Narciza M. Queiroz[1], João A. Araujo-Filho[1], Diêgo A. Teles[1], Samuel V. Brito[2], Daniel O. Mesquita[1]

[1] *Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Departamento de Sistemática e Ecologia – DSE, Centro de Ciências Exatas e da Natureza – CCEN, Universidade Federal da Paraíba – UFPB, Cidade Universitária, Campus I, CEP 58059-900, João Pessoa, PB, Brazil. *Corresponding author.* E-mail: adoniasteixeira01@gmail.com
[2] *Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão, Boa Vista, CEP 65500-000. Chapadinha, MA, Brazil.*

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Abstract. The present study investigated the composition of helminth parasites of the completely unknown lizard *Anotosaura vanzolinia* (Squamata: Gymnophthalmidae) and evaluated the effects of sex, host size, and seasonality on endoparasite abundance in two areas of Caatinga, northeast Brazil. We collected 110 lizards between May 2013 to June 2014 and found 173 nematodes (overall prevalence: 16.3%), with 49 nematodes infecting seven adult males (prevalence: 25%), 84 nematodes infecting six adult females (Prevalence: 23%), and 40 nematodes infecting five juveniles (prevalence: 8.9%), where one nematode was in the lungs and 172 were in the gastrointestinal tracts. We identified all nematodes as *Oswaldocruzia brasiliensis*, representing a new record for the host and for Gymnophthalmidade, showing overall intensity of infection ± SD of 9.6 ± 5.2. Furthermore, abundance of endoparasites was related to the rainy season and sex, but not to host body size (SVL).

Keywords. Parasites, Lizards, Neotropical, *Oswaldocruzia brasiliensis*, Nematodes.

In the last decade, there has been an increasing number of studies about endoparasites affecting reptiles. The last survey in South America recorded about 155 helminth species infecting lizards and amphisbaenians (Ávila and Silva, 2010). Although the number of these studies in Neotropical regions is still increasing, some lizard families are more deeply studied, such as Leiosauridae (Vrcibradic et al., 2008; Barreto-Lima and Anjos, 2014; Dorigo et al., 2014); Phyllodactylidae (Ávila et al., 2010a; Sousa et al., 2010); Teiidae (Brito et al., 2014b), and Tropiduridae (Almeida et al., 2009; Anjos et al., 2012; Pereira et al., 2012). In contrast, studies about Gymnophthalmidae are scarce and mainly regard new host records (Ávila and Silva, 2010). Currently, parasitological studies are only available for ten Gymnophthalmidae species in Neotropical regions: *Cercosaura eigenmanni* and *C. oshaughnessyi* (Bursey and Goldberg, 2004); *Alopoglossus angulatus* (Goldberg et al., 2007b); *Leposoma osvaldoi* and *Neusticurus ecpleopus* (Goldberg et al., 2007a); *Iphisa elegans elegans* (Ávila et al., 2010b); *Bachia scolecoides* and *Cercosaura ocellata ocellata* (Ávila and Silva, 2011); and *Micrablepharus maximiliani* (Brito et al., 2014a).

Gymnophthalmid lizards, popularly known as microteiids, are small (40 to 150 mm snout-vent length) and commonly distributed in Neotropical regions from South Mexico to Argentina, including some islands of Central and South America, totalling 220 species in 48 genera (Presch, 1980). In Brazil, there are about 93 gymnophthalmid species, distributed in 33 genera. One of them is *Anotosaura,* which comprises two species, *A. vanzolinia* and *A. collaris* (Costa and Bérnils, 2015).

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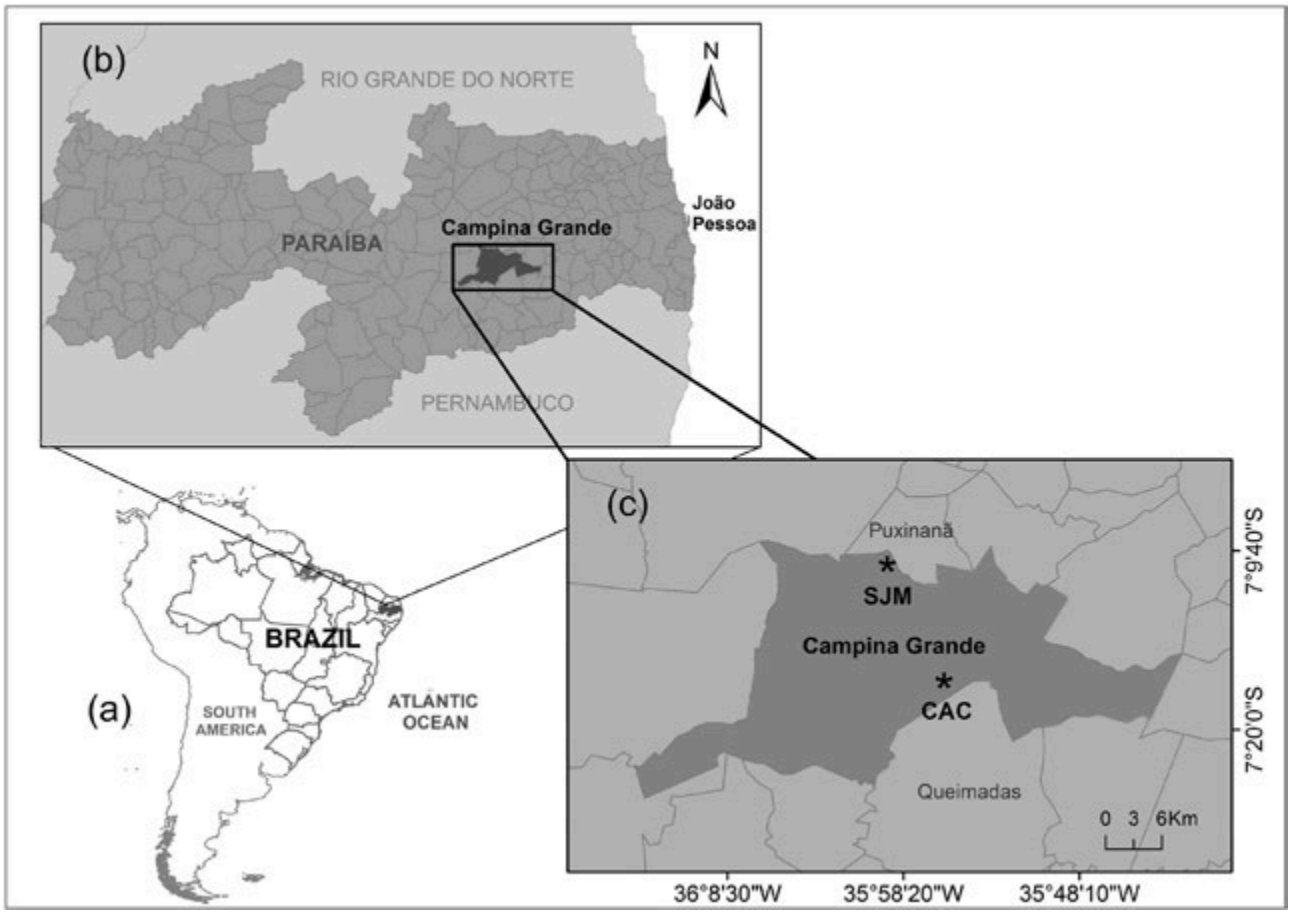

Fig. 1. Collecting sites in Paraíba State (A) in Northeast Brazil, Campina Grande municipality (B). Study areas (C): São José da Mata district (SJM) and Complexo Aluízio Campos Forest Park (CAC).

Anotosaura vanzolinia is a small lizard restricted to semi-arid regions in northeastern Brazil, locally known as Caatinga, with fossorial habits, occurring in sites with accumulated leaf litter, its diet is comprised basically of microarthropods associated with sites where these lizards live (Oliveira and Pessanha, 2013). However, there are no reports about parasites of *A. vanzolinia*, possibly because *A. vanzolinia* is a rare lizard in Caatinga. Therefore, this study aims to evaluate the effect of sex, host size, and seasonality on the composition and abundance of helminths associated with the lizard *Anotosaura vanzolinia*, using a considerable sample from Caatinga, northeast of Brazil.

We collected *Anotosaura vanzolinia* specimens during expeditions in the Complexo Aluízio Campos Forest Park (7°16'34"S, 35°53'7"W), a Caatinga area with an altitude of approximately 500 m, with shrubby vegetation represented mainly by Bromeliaceae and Cactaceae, a larger number of rock outcrops, and accumulated leaf litter (Alves et al., 2010; Silva et al., 2010) and in the São José da Mata district (7°11'2.85"S, 35°59'6.17"W), located between the arboreal formations locally known as "Brejo" and Caatinga, with an altitude of approximately 700 m. The site is settled between the "Agreste" and "Sertão", being probably one of the last remains of the transitional arboreal vegetation of Paraiba, with typical plant species from Caatinga and the Atlantic Forest (Barbosa et al., 2007). Both sites are located in Paraiba, northeastern Brazil (Fig. 1). Climate is tropical, with a mean temperature of 22.9ºC; highest precipitation occurs between March and August (99 mm) and lowest between September and February (29 mm) (Climate-Date, 2016).

We collected 110 lizards between May 2013 to June 2014 (Table 1) by hand and using pitfall-traps (six sets in each area), constructed with four buckets (20 L each), totalling 24 buckets per area, arranged in three lines of three meters each, forming angles of 120° from a same central point and connected by a plastic fence fixed with staples on wooden stakes.

Table 1. Number of lizards collected in each sampled area, months of the year and season. CAC = Complexo Aluízio Campos Forest Park; SJM = São José da Mata District.

Months	Locality			
	CAC		SJM	
	Wet season	Dry season	Wet season	Dry season
May	8	–	1	–
Jun	9	–	1	–
Jul	4	–	0	–
Aug	8	–	2	–
Sep	–	7	–	2
Oct	–	9	–	6
Nov	–	4	–	2
Dec	–	7	–	1
Jan	–	16	–	1
Feb	–	7	–	1
Mar	6	–	0	–
Apr	7	–	1	–

We killed the lizards with a lethal injection of 2% lidocaine hydrochloridec and measured snout-vent length (SVL) with digital calipers. Subsequently, we sexed them, preserved them in 10% formalin, and stored them in 70% alcohol. All lizards were kept in the Coleção Herpetológica da Universidade Federal da Paraíba (CHUFPB). In the laboratory, we removed the respiratory and gastrointestinal tracts and analyzed them in a stereomicroscope to search for endoparasites. The endoparasites found were cleared with Hoyer's solution, counted, identified, stored in 70% alcohol, and kept in the Coleção de Invertebrados Paulo Young, in Universidade Federal da Paraíba (UFPB-NEM: 0001; 0002).

The following infection rates were calculated according to Bush et al.(1997), where parasite abundance is defined as the total number of parasites found in a sample (individual host, host population and / or host community); mean intensity of infection is the total number of parasites found in a sample, divided by the number of hosts infected with that parasite; prevalence (P%) is the number of host infected with one or more individuals of a particular parasite species divided by total host number. Throughout the text, means appear ± 1 SD.

To analyze the influence of SVL on endoparasite abundance, we performed simple linear regression (excluding juveniles), using the Statistica Software, version 8.0 (Statsoft, 2007). In addition, we performed a generalized linear model (GLM), adopting Poisson's distribution, using the Software R, package "R commander" (R core team, 2008) to evaluate whether parasite abundance was influenced by host sex, seasonality and interaction between sex and seasonality.

Table 2. Parasitological data from population of *Anotosaura vanzolinia* from Caatinga, Northeast Brazil, infected by *Oswaldocruzia brasiliensis*. P% = prevalence; SD = standard deviation; I = intestine, L = lung.

Host sex and maturity	Host sample size	SVL/SD (mm)	P% (Host infected)	Mean intensity of infection/ SD	Site infection
Males	28	37.9 ± 2	25 (7)	7 ± 3.8	I
Females	26	42.6 ± 2.3	23 (6)	14 ± 8.5	I,L
Juveniles	56	26.7 ± 5.2	8.9 (5)	8 ± 3.4	I
Total	110	-	16.3 (18)	9.6 ± 5.2	-

We examined 110 *Anotosaura vanzolinia* specimens, of which 26 were adult females (SVL: 42.6 ± 2.3), 28 adult males (SVL: 37.9 ± 2), and 56 juveniles (SVL: 26.7 ± 5.2). We found 173 nematodes (overall prevalence: 16.3%), with 49 nematodes infecting seven adult males (prevalence: 25%), 84 nematodes infecting six adult females (prevalence: 23%), and 40 nematodes infecting five juveniles (Prevalence: 8.9%), where one nematode was in the lungs and 172 were in the gastrointestinal tracts. We identified all nematodes as *Oswaldocruzia brasiliensis*, representing a new host record and the first record for Gymnophthalmidade, showing overall intensity of infection of 9.6 ± 5.2 (Table 2).

Simple linear regression revealed a non-significant relationship between SVL and endoparasite abundance ($F_{1,12}$ = 4.24; R^2 = 0.26; P = 0.061). We observed a significant variation between the abundance of endoparasites and sex of the host (GL = 3; Z value = - 2.669; P = 0.0076), where females had more parasites than males (Fig. 2). In addition, abundance of endoparasites was higher in the rainy season (GL = 3; Z value = -4. 318; P <0.0001) (Fig. 2). However, abundance of endoparasites was not influenced by interaction sex-season (GL= 4; Z value = 0.024; P = 0.981) (Fig. 2).

Several factors can influence parasitism by helminths in reptiles (Aho, 1990), especially host size (Poulin and George-Nascimento, 2007), sex (Galdino et al., 2014), season (Brito et al., 2014), and life cycle of the parasite (Araujo-Filho et al., 2016). Usually, larger hosts (mass and body size) have the capacity to provide shelter to a greater number of parasites, consequently, more resources for parasite development and reproduction (George-Nascimento et al., 2004). However, in the present study, we did not observe a significant relation between SVL and parasite abundance in *A. vanzolinia*. For lizards, this hypothesis is supported mainly by Tropiduridade: *Tropi-*

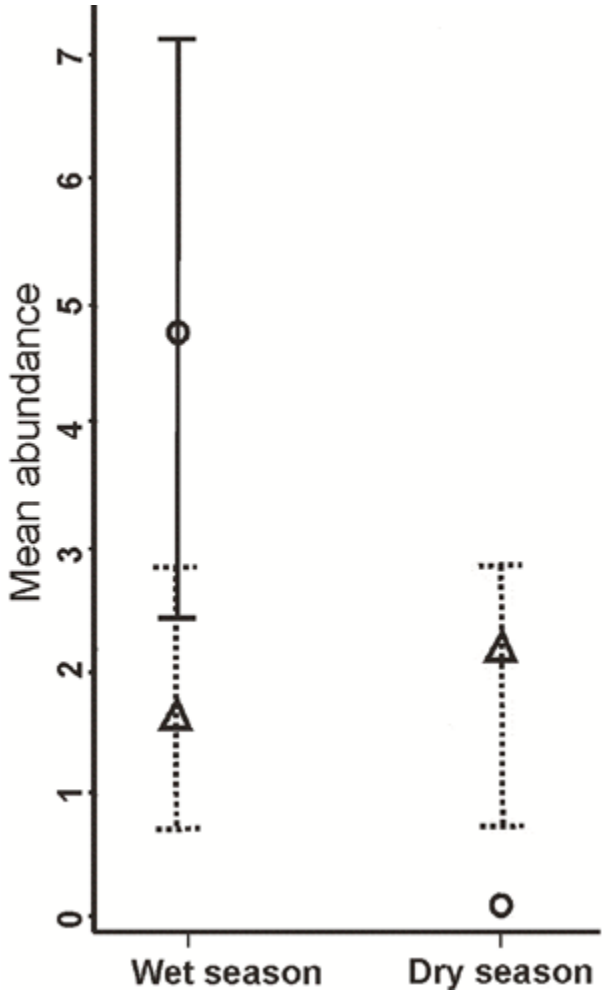

Fig. 2. Mean abundance of nematodes between seasons for species of lizard *A. vanzolinia*, males (triangle) and female (circle).

durus torquatus (range of intensity: 22.1 ± 20.2) (Ribas et al., 1998); *T. hispidus* (range of intensity: 8.5 ± 1) (Anjos et al., 2012); *T. torquatus* (range of intensity: 8.2 ± 6.9) (Pereira et al., 2012); and Teiidae species: *Ameiva ameiva* (range of intensity: 7.2 ± 7.3) (Ribas et al., 1998); *A. festiva* (intensity of infestation: 21, range: 1-115) (Ramírez-Morales et al., 2012). Nevertheless, besides body size and host mass, other factors can explain the variation in parasite abundance, such as ecology (Aho, 1990), physiology (Poulin and Mouillot, 2004; Poulin, 2007), behaviour, and phylogeny (Poulin and Mouillot, 2005; Patterson et al., 2008; Brito et al., 2014a).

Furthermore, the small size of *A. vanzolinia* (SVL = 42 mm) can constrain the diversification of its parasitic fauna, hindering niche differentiation and microhabitat segregation by endoparasite competitors (Kuris et al., 1980; Ávila et al., 2010a) thus explaining the presence of only a single nematode species infecting the host studied. This observation was also recorded in other small lizard species, such as *Liolaemus lutzae* (SVL = 61 mm), *Aspronema dorsivittatum* (SVL = 64 mm), and *Phyllopezus lutzae* (SVL = 42 mm), which exhibit up to two endoparasites species (Rocha, 1995; Rocha et al., 2003; Ávila et al., 2010a).

We found significant differences in the abundance of nematodes in relation to host sex, where females were more parasitized than males. According to Poulin (1996), physiological, morphological, and behavioral differences between males and females may explain the differences of infection rates between sexes. However, due to the absence of research that verified possible differences in the ecology between the sexes for the lizard *A. vanzolinia*, we can not explain what factors may have influenced the increase of the parasite abundance in females in the present study.

The nematode *Oswaldocruzia brasiliensis* has a monoxenous life cycle (Lent and Freitas, 1935), and congeneric species infect mainly frogs (Well Slimane and Durette-Desset, 1996), lizards (Bursey et al., 2006), and snakes (Durette-Desset et al., 2006). According to Anderson (2000), monoxenous nematodes can easily be influenced by variations in temperature and humidity. Our results support this hypothesis, considering that *A. vanzolinia* presents a greater endoparasite abundance in the rainy season, mainly because of its fossorial habits, increasing the possibility of infection by *O. brasiliensis* through accidental ingestion of eggs (in the faeces of other hosts, substrate in general) or by active penetration of infective larvae (via the skin and / or mucosa).This can also explain the higher parasite abundance in females, because they lay eggs during the rainy season and in humid microhabitats to avoid egg desiccation (Oliveira and Pessanha, 2013).

The present study revealed that the parasitic fauna of *A. vanzolinia* from Caatinga consists of a single nematode, *Oswaldocruzia brasiliensis*, representing the first record of infection to the host and to Gymnophthalmidae. Furthermore, endoparasite abundance is related to the rainy season and sex, but is not correlated with host body size (SVL).

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