ORIGINAL PAPER



Helminth parasites of *Mabuya arajara* Rebouças-Spieker, 1981 (Lacertilia: Mabuyidae) from Chapada do Araripe, northeastern Brazil

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Received: 22 October 2017 / Accepted: 31 January 2018 / Published online: 12 February 2018 © Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

We provide data on the helminth fauna from the digestive tract of the lizard $Mabuya\ arajara\ Rebouças$ -Spieker, 1981 from Chapada do Araripe, northeastern Brazil. Seventy one of the 127 lizards examined (56%) were infected with four nematode species: $Physalopteroides\ venancioi\$ and $Physaloptera\$ sp. (Physalopteridae), $Strongyluris\$ oscari (Heterakidae), and $Parapharyngodon\$ alvarengai (Pharyngodonidae), the latter being the component species (prevalence 53.5%; mean intensity of infection 3.37 ± 2.0 ; discrepancy index D=0.69). The helminth $P.\$ alvarengai infected $M.\$ arajara\ throughout the year and showed increased infection rates in July, at the beginning of the dry season. In addition to the relationship with seasonality, lizards with greater body length and/or body mass were more infected. Relationships between number of parasites and body mass and with the sexes of lizards, on the other hand, were not found. $Mabuya\$ arajara\ represents a new host for these nematodes. This study contributes to the knowledge of the helminth fauna associated with the digestive tract of lizards from South America and the Caatinga domain.

Keywords Caatinga · Nematoda · Parasitism · Parapharyngodon alvarengai · Seasonality

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Introduction

Cosmopolitan and known to encompass interactions between members of all animal classes, parasitism represents the most successful mode of life in terms of animal diversity (Aho 1990; Poulin 1999; Bush et al. 2001). Neglected throughout history, parasite organisms have a great relevance in population dynamics and act directly on several factors of community structure (Poulin and Morand 2000; Bush et al. 2001; Marcogliese 2004). Their actions are reflected in behavioral changes because of metabolic imbalance (Dare and Forbes 2008), or due to their effects on sexual selection and reproductive output in the host populations (Hudson and Greenman 1998; Nordling et al. 1998; Kose and Muller 1999; Marcogliese 2004).

The number of endoparasite species infecting reptiles has long been considered low compared to other vertebrates (Aho 1990; Bush et al. 1990; Rocha and Vrcibradic 2003). The last decade, however, saw the identification of considerable species richness coming from the increased number of studies involving lizards (Ávila and Silva 2010), although in the



Brazilian Caatinga domain, the knowledge of helminth faunas of lizards is still limited (Ávila and Silva 2010; Ávila et al. 2012). Currently, this domain has 78 known species of lizards (Delfin 2012), of which only ~30% have undergone a parasitological survey (e.g., Anjos et al. 2011, 2012; Ávila et al. 2012; Ribeiro et al. 2012a; Brito et al. 2014).

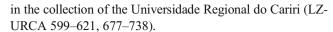
Among the *Mabuya* Fitzinger 1826 (Mabuyidae) occurring in the Caatinga, the only records on parasite infection have been provided by Ribeiro et al. (2012a) for lung pentastomids in *M. arajara* and Brito et al. (2014) for intestinal helminths in *M. heathi*. Although lizards of this genus have a wide Neotropical distribution, in Brazil, most parasitological records are from the midwestern area, characterized by Amazonian, Cerrado, and Pantanal domains and from southeastern regions, characterized by Atlantic Forest domain (e.g., Vrcibradic et al. 2002a, b; Rocha et al. 2003; Rocha and Vrcibradic 2003; Ávila 2009; Ávila et al. 2011; Ávila and Silva 2011).

Mabuya arajara Rebouças-Spieker 1981 has a restricted geographical distribution, in enclaves of humid forest (locally known as "brejos de altitude") in Ceará and Piauí States, Brazil (Roberto and Loebmann 2010; Roberto et al. 2012; Ribeiro et al. 2012b). This species is one of the largest genus in Brazil (SVL up to 114 mm) and the females grow larger than males (Ribeiro et al. 2015). Mabuya arajara feeds on a wide diversity of invertebrates, mainly arthropods, but termites tend to dominate the diet (Ribeiro et al. 2015), and has seasonal reproduction occurring in the dry season (Ribeiro et al. 2015). Therefore, this study aimed to provide information on the helminth fauna from the digestive tract of the lizard M. arajara and its correlation with climatic seasonality, time of the year, body size, and sex of the lizards.

Material and methods

This study was conducted in the Crato (07° 15′S, 39° 28′W) and Barbalha Municipalities (07° 21′S, 39° 17′W), in Chapada do Araripe, Ceará State, northeastern Brazil. The localities are within the Área de Proteção Ambiental do Araripe (APA Araripe) (600 to 800-m altitude), with seasonal tropical climate and mean annual temperature of 24 ± 2.1 °C. The rainy season occurs from January to June and the dry season from July to December, and annual average rainfall is 1100 mm³ (MMA 2000; IPECE 2010).

Specimens of *M. arajara* were collected bimonthly from September 2009 to July 2010. Immediately after collection, all animals were weighed with an analytical balance (precision 0.001 mg), killed with a lethal injection of 2% lidocaine, and their snout-vent length (SVL) was measured with a digital caliper (0.1-mm accuracy). The lizards were fixed in 10% formaldehyde solution, preserved in 70% ethanol, and housed



After dissection, the digestive tract (stomach and intestines) was removed for analysis under a stereomicroscope. The nematodes found were cleared using lactophenol, mounted on temporary slides, and analyzed with a light microscope (Carl Zeiss Microimaging GmbH, Gottingen, Germany). Voucher specimens were housed in the collection of the Universidade Regional do Cariri (URCA-P 1203, 1204, 1205, and 1026).

The prevalence and mean intensity of infection were determined according to Bush et al. (1997). The discrepancy index was calculated overall and for each parasite species, as suggested by Poulin (1993):

$$D = 1 - \frac{2\sum_{i=1}^{N} \left(\sum_{j=1}^{i} x_j\right)}{\overline{x}N(N+1)}$$

The value of x is the number of parasites in host j and N is the total number of hosts. The index has a minimum value (0) when the parasites are uniformly distributed among the hosts and maximum value (1) when all parasites are found in a single host. Both evaluations were calculated using Quantitative Parasitology 3.0 software (Rózsa et al. 2000). Student's t test was used to assess the difference in mean total parasite intensity between the dry season and the wet season. The relationship between parasite abundance and SVL, body mass, time of the year, and sex of M. arajara was calculated by the generalized linear model (GLM) of the Poisson model. After GLM, we performed an analysis of variance (ANOVA) comparing the total parasite intensity among the sampled months, followed by the Tukey test. To avoid the influence of ontogenetic factors, only adults were used for statistical analysis. The determination of sexual maturity and snoutvent length measurements were based on Ribeiro et al. (2015). The analysis was performed using the R Core Team (2017) platform and a significance level of p < 0.05.

Results

We analyzed a total of 127 specimens of *Mabuya arajara*, being 65 males, 54 females, and 8 juveniles (Table 1). Two-hundred and eighty helminths were obtained from 71 of the 127 lizards examined, representing an overall prevalence (P) of 56%; mean intensity of infection (I) was 3.5 ± 2.0 and discrepancy index (D) was 0.68. The helminth fauna of M. arajara was composed of four species of nematodes: Parapharyngodon alvarengai Freitas 1957 (Pharyngodonidae), infecting the intestines; Physalopteroides venancioi Lent et al. 1946 (Physalopteridae), infecting the stomach; Strongyluris oscari



Table 1 Overall data on the population of *Mabuya arajara* Rebouças-Spieker, 1981 from Chapada do Araripe, northeastern Brazil

	Number	Mean SVL (mm)	Mean body mass (g)	Range (mm)	P (%)
Mabuya arajara	127	82.3 ± 13.7	14.6 ± 6.4	44–114	56
Males	65	80.7 ± 9.6	13.1 ± 4.4	50.8-101	58.5
Females	54	88.7 ± 11.9	18 ± 6.2	54.1-114	61.1
Juveniles	8	51.4 ± 6.1	3.1 ± 1.3	44–60.1	0

SVL snout vent length, P (%) prevalence of infection

Travassos 1823 (Heterakidae) found in the intestine of a single individual; and *Physaloptera* sp. (Physalopteridae) found in the stomach of a single individual (Table 2).

The parasite abundance in *M. arajara* was influenced by seasonality and the highest infection rates were related to the dry season (Student t test = -3.1994, gl = 111.81, p < 0.005).

The relationships between number of parasites \times SVL (GLM; X(1,118) = 9.176, p = 0.005) and number of parasites and SVL \times body mass of the lizards (GLM; X(1,110) = 6.051, p = 0.005) were positively and statistically significant. On the other hand, the relationship between number of parasites and body mass of the lizards was not significant (GLM; X(1,117) = 2.953, p = 0.085), nor was the relationship with sex (GLM; X(1,116) = 0.705, p = 0.401) (Table 3).

We observed a strong relationship between number of parasites and the month of collection for M. arajara (GLM; $X(_{5,111}) = 91.934$, p < 0.001); (ANOVA; $F(_{5,114}) = 6.127$, p < 0.001). While most months showed no differences, the Tukey test identified that July had the highest parasite load (p < 0.001) (Fig. 1). In that month, the recorded species were P. alvarengai, P. venancioi, and Physaloptera sp.

Discussion

The helminth fauna described for *M. arajara* was composed of nematodes known to use many reptile hosts (Vicente et al.

Table 2 Helminthological data of *Mabuya arajara* Rebouças-Spieker, 1981 from Chapada do Araripe, northeastern Brazil

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ntestine
tomach
tomach
ntestine
1

P (%) prevalence, $X \pm SD$ mean intensity of infection, D discrepancy index

Table 3 Results of statistical analysis: generalized linear model (GLM), analysis of variance (ANOVA), and Tukey of infection rates of *Mabuya arajara* from Chapada do Araripe, northeastern Brazil

Correlations	GLM	ANOVA	Tukey
Parasite load~SVL	$X(_{1,118}) = 9.176; p < 0.005$	_	_
Parasite load~SVL × mass	$X(_{1,110}) = 6.051; p < 0.05$	_	_
Parasite load~months	X(5,111) = 91.934; p < 0.001	$F(_{5,114}) = 6.127; p < 0.001$	0.001

pled (Van Sluys et al. 1997; Ribas et al. 1998b; Vrcibradic et al. 2000, 2002a, b; Rocha and Vrcibradic, 2003; McAllister et al. 2010a, b, c; Ávila and Silva 2011). The proportion of specimens infected can define the existence of component parasite species, that is, only those parasites found in ≥ 10% of the host population (Bush et al. 1990). In this study, besides being classified as a component species, since it is was recorded in two thirds of the population of *M. arajara*, the helminth *P. alvarengai* can also be considered a core species, since its infection prevalence was higher than 50% (Aho 1990).

We provide the first infection record of *P. alvarengai* on *M. arajara*, and the second occurrence of this nematode

1993; Anderson 2000; Ávila and Silva 2010). Our data

showed a low helminth diversity and intensity of infection,

but high prevalence, a common characteristic of South

American Mabuya (Vrcibradic et al. 1999; Rocha and

Vrcibradic 2003; Rocha et al. 2003). Parasitological studies

with representatives of the genus are restricted to 41% of the

species from South America (Fig. 2), but it is important to

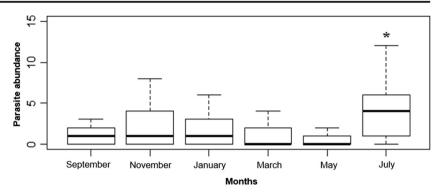
mention that some of these, such as M. agilis,

M. macrorhyncha, and M. nigropunctata, were widely sam-

We provide the first infection record of P. alvarengai on M. arajara, and the second occurrence of this nematode among South American Mabuya; the other record is for M. heathi (infection rates P=25%; I=2.25) (Brito et al. 2014). Evolutionarily, Parapharyngodon spp. infected lizards and transferred to amphibians (Sousa et al. 2015; Montes-Oca et al. 2016). Among lizards, previous host records include the



Fig. 1 Parasite abundance in *Mabuya arajara* from Chapada do Araripe, northeastem Brazil, in different periods of the year



families Dactyloidae (Cabrera-Guzmán and Garrido-Olvera 2014), Gekkonidae (Anjos et al. 2011; Bezerra 2014; Brito et al. 2014), Iguanidae (Mayén-Peña and Salgado-Maldonado 1998), Scincidae (Freitas 1957; Brito et al. 2014), Teiidae (Padilha and Duarte 1979; Macedo et al. 2017), and Tropiduridae (Brito et al. 2014; Galdino et al. 2014; Bezerra et al. 2015; Araújo-Filho et al. 2016; Václav et al. 2017). Among amphibians, hosts include members of the Bufonidae (Luque et al. 2005) and Hylidae families (Montes-Oca et al. 2016).

Climatic seasonality, a fundamental factor responsible for the dry season having the highest parasite abundance in *M. arajara*, has a considerable influence on the ecology of species, such as habitat use and feeding (Grifftiths and Christian 1996; Vrcibradic and Rocha 1995, 2005; Brito

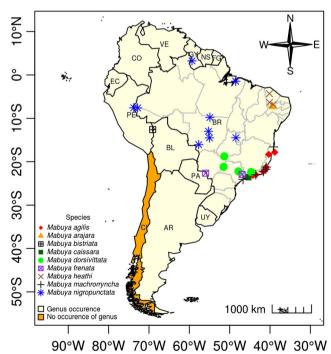


Fig. 2 Map of areas of South America represented by studies with lizards of the *Mabuya* genus parasitized by intestinal helminths. Country names are abbreviated: *AR* Argentina, *BL* Bolívia, *BR* Brazil, *CH* Chile, *CO* Colômbia, *EC* Ecuador, *FG* French Guiana, *GY* Guiana, *PA* Paraguay, *PE* Peru, *NS* Suriname, *UY* Uruguay, *VE* Venezuela

2003). These aspects, although determinants in the structuring and composition of the parasite assemblages Caatinga lizards (Brito et al. 2014), are apparently not observed in M. arajara, since the mode of life of this species varies little throughout the year (Ribeiro et al. 2015), and P. alvarengai has a monoxenous life cycle (Anderson 2000). A probable explanation for the increased infection rates in this period, therefore, is the consumption of contaminated particles of the substrate. On the other hand, during the rainy season, parasite eggs may be carried away by rain, providing less direct contact between hosts and parasites. This phenomenon is known to influence infection rates in *Tropidurus torquatus* (Pereira et al. 2012). The increased infection rates may also be related to physiological mechanisms in these animals, such as energy imbalance and hormonal changes (Aho 1990; Marcogliese 2004; Roberts et al. 2004; Martin et al. 2008). Although the low intensity of infection is a common characteristic among organisms infected by P. alvarengai (Pereira et al. 2012), there is evidence that susceptibility to infection by this parasite has an impact on the reproduction of host species (Bezerra 2014; Galdino et al. 2014).

The month of July, i.e., the beginning of the dry season, was highlighted by the highest parasite infection rates in M. arajara. This is a key period in the reproduction of this species (Ribeiro et al. 2015; Cabral 2017). Among males, seminiferous tubules go through a regenerative phase (Cabral A.N., unpublished data), peak hormonal production occurs, spermatogenesis begins, and the development of sexual structures is initiated (Fox 1977; Krohmer et al. 2004). It is known that testosterone is mostly used for reproductive functions, inducing decreased immunity in the organism, which increases susceptibility to opportunistic species (Nordling et al. 1998; Roberts et al. 2004; Martin et al. 2008). Among the females of M. arajara, there was a decrease in fat bodies with concomitant accelerated embryo development during that period (Ribeiro et al. 2015). Although this relationship is unclear, the investment of resources to the production of offspring leads to an energy imbalance in females, inducing a decrease in immunity similar to that observed in males (Nordling et al. 1998; Roberts et al. 2004). This harmful parasite-reproduction relationship has also been observed in



Table 4 List of geographical coordinates for parasitological studies of South American Mabuya lizards

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Species	Coordinates	Locality	Nematode richness	Total helminth richness	Host sample size	References
Mabuya agilis	19° 18′S, 40° 19′W	Linhares/ES	2	4	19	Van Sluys et al. (1997)
	22° 17′S, 41° 41′W	Jurubatiba/RJ	4	4	8	Vrcibradic et al. (2000)
	21° 44′S, 41° 02′W	Grussaí/RJ	5	6	28	Vrcibradic et al. (2002a)
	21° 15′S, 40° 58′W	Praia das Neves/ES	4	∞	11	
	23° 02′S, 43° 31′W	Grumari/RJ	4	7	42	Vrcibradic et al. (2002b)
	17° 49′S, 38° 49′W	Abrolhos/BA	2	2	11	Rocha and Vrcibradic (2003)
	23° 8′S, 44° 14′W	Ilha Grande/RJ	2	2	19	
M. arajara	07° 15′S, 39° 28′W	Chapada do Araripe/CE	4	5	127	Ribeiro et al. (2012) This study
	07° 21′S, 39° 17′W					
M. bistriata	01° 27′S, 48° 30′W	Belém/PA		1	7	Bain (1974)
	12° 35′S, 69° 05′W	Reserva Cuzco/Peru	4	4	11	Bursey et al. (2005)
M. caissara	23° 36′S, 45° 21′W	Caraguatuba/SP	2	2	30	Rocha and Vrcibradic (2003)
M. dorsivittata	22° 15′S, 47° 49′W	Estação Ecológica Itarapina/SP	2	2	12	Vicente et al. (2002)
	22° 23'S, 44° 40'W	Parque Nacional Itatiaia/RJ	2	2	16	Rocha et al. (2003)
	18° 46′S, 51° 20′W	Parque Nacional das Emas/GO		1	4	Ávila et al. (2011)
	21° 12′S, 51° 30′W	RPPN Rio Aguapeí/SP		3	5	Silva (2014b)
M. frenata	22° 56′S, 46° 55′W	Valinhos/SP	4	5	232	Vrcibradic et al. (1999) and
						Vicente et al. (2000)
	22° 40′S, 55° 57′W	Amambay/Paraguay	0	0	1	McAllister et al. (2010a)
M. guaporicola	14° 30′S, 55° 00′W	Chapada dos Guimarães/MT	_	3	11	Ávila (2009)
M. heathi	$06^{\circ} 41'S, 40^{\circ} 17'W$	Estação Ecológica Aiuaba/CE	2	3	9	Brito et al. (2014)
	$04^{\circ}\ 19'S$, $40^{\circ}\ 09'W$	Santa Quitéria/CE	2	3	2	
	06° 47′S, 17° 45′W	Várzea Alegre/CE	2	3	~	
M. macrorhyncha	22° 17′S, 41° 41′W	Jurubatiba/RJ	4	4	40	Vrcibradic et al. (2000)
	16° 35′S, 39° 06′W	Trancoso/BA	3	7	~	Vrcibradic et al. (2001)
	21° 44′S, 41° 02′W	Grussaí/RJ	4	7	14	Vrcibradic et al. (2002a)
	$21^{\circ} 15'S$, $40^{\circ} 58'W$	Praia das Neves/ES	4	∞	11	
	23° 02′S, 43° 31′W	Grumari/RJ	1	1	6	Rocha and Vrcibradic (2003)
	22° 29′S, 46° 41′W	Ilha da Queimada Grande/SP	3	4	19	Rocha and Vrcibradic (2003)
						Vrcibradic and Rocha (2005)
M. nigropunctata	01° 27′S, 48° 30′W	Belém/PA	1	1	7	Bain and Sulahian (1974)
	07° 35′S, 73° 45′W	Ucayali/Peru	8	3		McAllister et al. (2010b)
	03° 25′S, 59° 34′W	Guyana		1	4	McAllister et al. (2010c)
	16° 04′S, 57° 40′W	Cáceres/MT	0	0	1	Ávila and Silva (2011)
	09° 47′S, 54° 54′W	Guaratã do Norte/MT	3	4	4	
	12° 59'S, 55° 15'W	Nova Ubiratã/MT	1	1	2	
	14° 28′S, 48° 27′W	Niquelândia/GO	0	1	3	Ávila et al. (2011)
	07° 37′S, 72° 48′W	Floresta Rio Moa/AC	1	1	1	Albuquerque et al. (2012)

Name of states of localities are abbreviated: AC Acre, BA Bahia, CE Ceará, ES Espírito Santo, GO Goiás, MT Mato Grosso, PA Pará, RJ Rio de Janeiro, SP São Paulo



1190 Parasitol Res (2018) 117:1185–1193

amphibians (Dare and Forbes 2008), birds (Nordling et al. 1998; Kose and Mueller 1999), and mammals (Barger 1993; Zuk and Mckean 1996).

The low prevalence and intensity of infection may represent an evolutionary strategy of the parasite infecting a large number of hosts or may be due to casual infections (Poulin 2007). Thus, in both cases, the parasites are not considered component species (Bush et al. 1990). In this study, these parasite species were *Physalopteroides venancioi*, *Physaloptera* sp., and *Strongyluris oscari*. Similar records were provided by Ribeiro et al. (2012a) for the pentastomid *Raillietiella mottae*, a lung parasite recorded in *M. arajara*.

Physalopteroides venancioi is the only species of the genus reported for South America (Vrcibradic et al. 2000), and its low prevalence in *M. arajara* is common in lizards of the genus *Mabuya* (Van Sluys et al. 1997; Vrcibradic et al. 2000; Rocha and Vrcibradic 2003). According to Golberg et al. (1993), these characteristics occur due to the existence of physiological mechanisms and make the organism unfavorable to the development of *Physalopteroides*. The life cycle of *P. venancioi* is not completely known, but it is known that this nematode uses insects during the intermediate phase (Anderson 2000). In addition to lizards (e.g., Ávila and Silva 2010; Ávila et al. 2012), *P. venancioi* uses amphibians (Lent et al. 1946), and snakes as the final host (Al-Moussawi 2016).

We could not achieve a specific identification for only one individual of the family Physalopteridae due to immaturity. Widmer (1970), during analyses on the snake *Crotalus viridis*, found the presence of larvae of this family in the third stage of development, identified as *Physaloptera* sp. The author suggested the possibility of using this vertebrate as a paratenic host, which can be extended to *M. arajara*. Recently, Goldberg et al. (2014) indicated that *Physaloptera* larvae need insects as intermediate hosts and are commonly observed in the digestive tract of vertebrates after feeding. *Physaloptera* spp. affect a broad spectrum of hosts (Brandão et al. 2009; Santoro et al. 2010; Sianto et al. 2014), especially amphibians and reptiles in South America (Ávila and Silva 2010; Campião et al. 2014).

Only one individual of *M. arajara* was infected by *S. oscari*. Since it occurred in the same infection site of *P. alvarengai*, we believe that this may be related to an interspecific competition between the nematodes, where the low prevalence of one derives from the competitive superiority of the other. It is known that *S. oscari* needs insects as intermediate hosts and acquisition in lizards occurs after the ingestion of eggs in the infectious stage (Anderson 2000; Barreto-Lima and Anjos 2014). Among lizards of the *Mabuya* genus, this helminth has been previously described only for *M. agilis* (Ribas et al. 1998b) and *M. guaporicola* (Ávila 2009). With wide geographical distribution and registered in all Brazilian domains (Ribas et al. 1998a, b; Bursey et al. 2005; Ávila and Silva 2011, 2013; Ávila and Silva 2013; Barreto-Lima and Anjos 2014; Bezerra et al. 2015), high infection rates are

found in lizards of humid regions (Vrcibradic et al. 2008; Barreto-Lima et al. 2012; Araújo-Filho et al. 2016) (Table 4).

The positive correlation between parasite load/SVL and parasite load /SVL/body mass observed in *M. arajara* was similar to what has been observed in its congeners *M. agilis*, *M. macrorhyncha* (Vrcibradic et al. 2000), and *M. frenata* (Vrcibradic et al. 1999). It is known that older individuals present greater SVL/body mass when compared to younger animals, thus increasing the chances of acquiring parasites due to greater time for contact with the environment throughout life (Ribas et al. 1998a; Pereira et al. 2012). In addition, a large body volume provides the parasite an abundance of resources (space to feed) available for colonization (Aho 1990; Van Sluys et al. 1994; Poulin 1997), even more so if we consider that *M. arajara* has a large body size among lizards of the genus *Mabuya* (Ribeiro et al. 2015).

In terms of overall infection and as the component species, P. alvarengai showed a tendency toward an intermediate distribution between aggregation and uniformity. Although parasite aggregation is the distribution between individuals (Poulin 1993; Bush et al. 2001; Begon et al. 2006; Simões et al. 2010), it is known that as long as the proportion of infected hosts increases, the parasite tends to exploit a growing number of individuals in the population. Thus, it is expected that the parasites are distributed uniformly, resulting in a high number of infected hosts (Poulin 1993). Similar trends were observed in Enyalius perditus (Barreto-Lima et al. 2012), Tropidurus semitaeniatus (Bezerra et al. 2015), and T. torquatus (Pereira et al. 2012). However, low prevalence of secondary helminths coincides with the maximum aggregation of these parasites, reflected in the underutilization of their respective hosts, as described by Poulin (1993).

This study contributes to the knowledge of the helminth fauna associated with the digestive tract of South American lizards inhabiting the Caatinga domain. *Mabuya arajara* is a new recorded host for all four nematodes recorded herein. Our data also show that the helminth *P. alvarengai* infects *M. arajara* throughout the year. However, we believe that more research should be undertaken in order to fill knowledge gaps in this parasite-host relationship.

Acknowledgements We thank the owners of private areas in which fieldwork was conducted: Raimundo Marques (Nascente, Delvechia Farm) and Ivan de Araújo B. Filho (São Joaquim Farm, Itapuí-S.A). We also thank Guilherme Sousa, Ivonildo Dias, Rafael Brandão and Jonathan Ramos for the field assistance. We are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico — CNPq (PQ-302429/2015-8) for the research grant awarded to W.O. Almeida. We thank to referees who carefully reviewed the manuscript and offered great contributions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.



Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical standards The collecting methods were defined and authorized by the regulatory agency in Brazil (ICMBio/SISBio 20388-1 and 23544-1). All processes followed the ethical guidelines provided by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL), the Society for the Study of Amphibians and Reptiles, and Conselho Brasileiro de Biologia (CBO).

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1192 Parasitol Res (2018) 117:1185–1193

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