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***Raillietiella mottae* (Pentastomida: Raillietiellidae) parasitizing four species of Gekkota lizards (Gekkonidae and Phyllodactylidae) in the Brazilian Caatinga**V. F. LIMA<sup>1\*</sup>, S. V. BRITO<sup>4</sup>, J. A. ARAUJO FILHO<sup>2</sup>, D. A. TELES<sup>2</sup>, S. C. RIBEIRO<sup>3</sup>, A. A. M. TEIXEIRA<sup>2</sup>, A. M. A. PEREIRA<sup>1</sup>, W. O. ALMEIDA<sup>1</sup>

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Accepted January 26, 2018**Summary**

We tested the role of sex, size, and mass of the lizards *Phyllopezus pollicaris*, *Gymnodactylus geckoides*, *Hemidactylus agrius*, *Lygodactylus klugei*, and *Hemidactylus brasiliensis* on the rates of pentastomid infection in the Brazilian Caatinga. We collected 355 individuals of these five species, of which four (prevalence of infection: *P. pollicaris* 15.9 %, *G. geckoides* 1.4 %, *H. agrius* 28.57 %, and *H. brasiliensis* 4.16 %) were infected by *Raillietiella mottae*. Parasite abundance was influenced by host body size and mass only in *P. pollicaris*. Host sex did not influence the abundance of parasites in any species. *Hemidactylus agrius*, *G. geckoides*, and *H. brasiliensis* are three new host records for pentastomids.

**Keywords:** Geckos; Pulmonary parasite; Semi-arid; South America**Introduction**

Pentastomids are endoparasites of the vertebrate respiratory system, whose definitive or intermediate hosts are usually reptiles. There are five pentastomid species of the genus *Raillietiella* that parasitize lizards in the Neotropics: *R. cartagenensis* Ali Riley & Self, 1985, *R. freitasi* (Motta & Gomes, 1968), *R. furcocerca* (Diesing, 1863), *R. frenata* Ali, Riley & Self, 1981, and *R. mottae* Almeida, Freire & Lopes, 2008.

Previous studies found pentastomids infecting insectivorous lizards in Brazil. For example, one study found *Mabuya agilis* (Raddi, 1823) infected by *Raillietiella* sp. (Vrcibradic *et al.*, 2002). Another study in a restinga (Almeida *et al.*, 2009a) found *Cnemidophorus abaatensis* Dias, Rocha & Vrcibradic, 2002 and *C. ocellifer* (Spix, 1824) parasitized by *Raillietiella* aff. *furcocerca* (Almeida *et al.*, 2009a) and *Micrablepharus maximiliani* (Reinhardt

& Luetken, 1862) parasitized by *R. mottae* (Almeida *et al.*, 2009a). Also, Anjos *et al.* (2007) and Almeida *et al.* (2008c) found *R. mottae* and *R. renata*, Sousa *et al.* (2014) recorded only *R. mottae* parasitizing *Hemidactylus mabouia* (Moreau de Jonnés, 1818) in houses, *R. frenata* in the north east coast of Brazil (Bezerra *et al.*, 2016), while *Trachylepis atlantica* (Schmidt, 1945) was infected by *R. freitasi* in the Archipelago of Fernando de Noronha, Pernambuco (Brito *et al.*, 2012). In the Caatinga, *R. mottae* was recorded infecting *Tropidurus hispidus* (Spix, 1825) (Almeida *et al.*, 2008a, 2008b, 2009b; Brito *et al.*, 2014b; Araujo Filho *et al.*, 2016), *T. semitaeniatus* (Spix, 1825) (Almeida *et al.*, 2008b, 2009b, Brito *et al.*, 2014b), *Phyllopezus periosus* Rodrigues, 1986 (Almeida *et al.*, 2008b; Brito *et al.*, 2014b), *P. pollicaris* (Spix, 1825) (Almeida *et al.*, 2008b; Sousa *et al.*, 2010, 2014; Brito *et al.*, 2014b) and *M. arajara* Rebouças-Spieker, 1981 (Ribeiro *et al.*, 2012).

Regarding abundance of pentastomids in Brazil, previous studies,

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found less than 14 % prevalence for pentastomids with at least 15 lizards collected from the same species. For example, *C. abae-tensis* (n = 33, prev. = 6 %, Dias *et al.*, 2005), *C. ocellifer* (n = 40, Prev = 2.5 %), *M. agilis* (n = 28, Prev = 3.6 %, Vrcibradic *et al.*, 2002), *T. hispidus* (n = 18, Prev = 11.1 %, Almeida *et al.*, 2008a; n = 288, Prev. = 1 %, Brito *et al.*, 2014b; n = 411, Prev. = 0.66 %); *T. semitaeniatus* (n = 15, Prev = 13.3 %, Almeida *et al.*, 2008b; n = 120, Prev = 0.51 %, Brito *et al.*, 2014b); *M. maximiliani* (N = 75, Prev = 4 %, Almeida *et al.*, 2009a), and *M. arajara* (n = 125, Prev = 1.6 %, Ribeiro *et al.*, 2012). Only three studies with similar sample size (15 geckos) found a higher prevalence: *H. mabouia* (n = 37, Prev = 45.9 %, Anjos *et al.*, 2007; n = 30, Prev = 20 %, Almeida *et al.*, 2008c) and *P. pollicaris* (n = 22, Prev = 18.18 %, Sousa *et al.*, 2010).

Here we tested the role of host body size, mass and sex on the abundance of pentastomids in the insectivorous geckos *P. pollicaris* and *Gymnodactylus geckoides* (Spix 1825) (Phyllodactylidae), *H. agrius* Vanzolini, 1978, *Lygodactylus klugei* (Smith, Martin & Swain, 1977), and *H. brasiliensis* (Amaral, 1935) (Gekkonidae) in a Caatinga area. These species were chosen because they have been reported to have higher prevalence in the literature.

## Material and Methods

### Lizard sampling and parasite identification

We sampled lizards at the Aiuaba Ecological Station (ESEC Aiuaba), municipality of Aiuaba, state of Ceará, northeastern Brazil (6° 36' 27" S; 40° 08' 00.9" W), which is within the Caatinga. The vegetation is composed of hypoxerophilic plants, shrubs, thorny trees, and open formations (Andrade-Lima, 1981). The climate is warm semi-arid tropical, with an average annual rainfall of 562.4 mm; the average temperature varies from 24 °C to 26 °C, with a short rainy period between February and April (IPECE, 2011).

We collected lizards using visual encounter surveys (McDiarmid, 2012) in September and November 2014 and February and April 2015. We measured Snout-Vent Length (SVL) with a digital caliper (to the nearest 0.01 mm), body weight with a digital scale (to the nearest 0.01 g). Then, we removed the specimens' respiratory tract under the stereomicroscope to inspect and count the parasites. Sex and sexual maturity were determined by analyzing the gonads. Mature males were those individuals with developed testes and epididymas with convolutions, and mature females were those with vitellogenic follicles in ovaries or oviducts. Lizards were killed with a lethal dose of lidocaine and fixed with 10 % formaldehyde and stored in 70 % ethanol.

Parasites were lightened in Hoyer medium (Everhart, 1957), mounted on temporary slides, and preserved in 70 % ethanol. Identification was conducted under microscope coupled with gridded ocular based on the size of mating hooks and spicules of males (Riley *et al.*, 1986; Almeida *et al.*, 2008a). Parasites will be deposited in the Parasitological collection of the Regional University of Cariri – URCA.

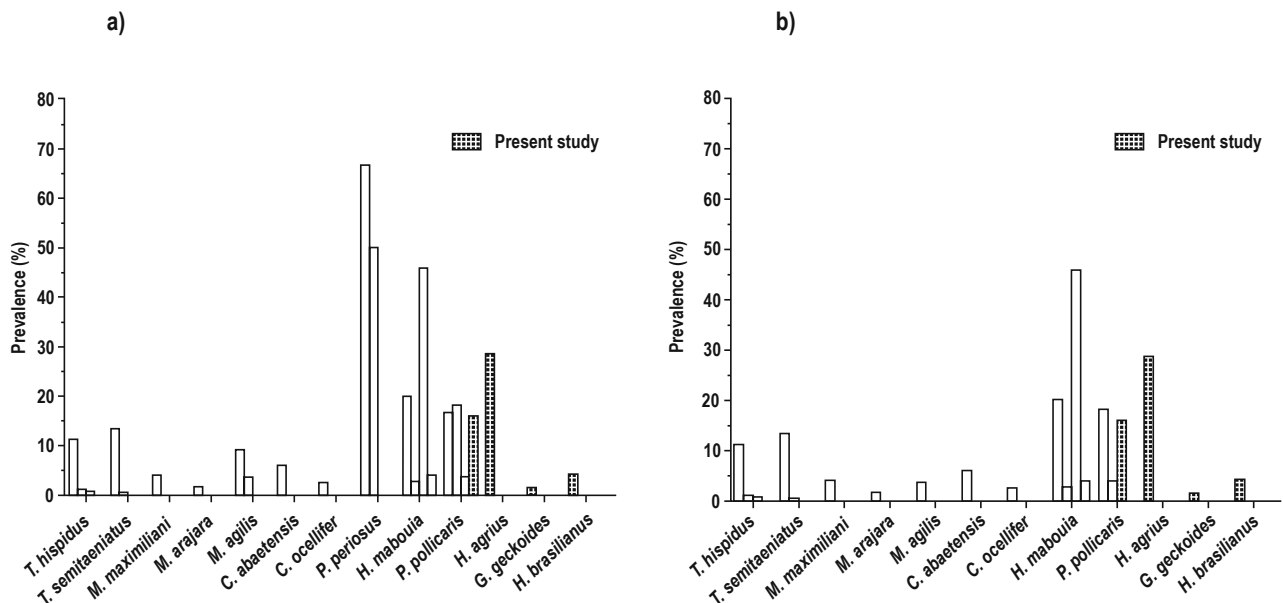


Fig. 1. Pentastomids as parasites of insectivorous lizards in Brazil. Data from the literature and the present study. **a)** All infection records for pentastomids in Brazil including this study, **b)** includes only studies that sampled more than 15 individuals of the same lizard species. References with number of hosts in parenthesis: *Tropidurus hispidus* (n = 18, Almeida *et al.*, 2008a; n = 288, Brito *et al.*, 2014b; n = 411, Araujo-Filho *et al.*, 2016), *T. semitaeniatus* (n = 15, Almeida *et al.*, 2008b; n = 120, Brito *et al.*, 2014b), *Micrablepharus maximiliani* (n = 75, Almeida *et al.*, 2009a), *Mabuya arajara* (n = 125, Ribeiro *et al.*, 2012), *M. agilis* (n = 11, n = 28, Vrcibradic *et al.*, 2002), *Cnemidophorus abae-tensis* (n = 33, Dias *et al.*, 2005), *C. ocellifer* (n = 40, Dias *et al.*, 2005), *Phylllopezus periosus* (n = 6, Almeida *et al.*, 2008b; n = 6, Brito *et al.*, 2014b), *Hemidactylus mabouia* (n = 37, Anjos *et al.*, 2007; n = 30, Almeida *et al.*, 2008c; n = 76, Sousa *et al.*, 2014; n = 277, Bezerra *et al.*, 2016), *P. pollicaris* (n = 6, Almeida *et al.*, 2008b; n = 22, Sousa *et al.*, 2010; n = 94, Brito *et al.*, 2014b; n = 132, this study) *H. agrius* (n = 63, this study), *Gymnodactylus geckoides* (n = 71, this study) and *H. brasiliensis* (n = 24, this study).

### Statistical analyses

We calculated the prevalence (the number of hosts infected by one or more parasites divided by total hosts number), abundance (the number of parasites of a given host whether or not it is infected), range (the number of parasites in a given host species, with a smallest number is given the number one, and other numbers represent the largest number of parasites found in an individual of the host species) and mean intensity of infection (the total number of parasites species found in a sample divided by the number of hosts infected with that parasite) for each lizard species following Bush *et al.* (1997). First, we tested whether there is a relationship between pentastomids abundance (response variable, log-transformed and using a Poisson response distribution) and SVL, sex, species and interaction between sex and species of lizards. Second, we tested whether there is a relationship between infection (presence/absence and using a binomial response distribution) and the explanatory variables, mass, sex, species and interaction between sex and species of lizards, the selected statistical test Generalized Linear Model (GLM) is efficient to avoid the confounding effect of host size (Poulin, 1997). We only used host species with more than ten individuals infected (at least five males and five females) in the analyses. Only *P. pollicaris* and *H. agrius* met this criterium, because *G. geckoides* and *H. brasiliensis* presented only one lizard infected by pentastomids. All analyzes were performed using the Software R, package "R commander" (R core team, 2008) (Wilson & Grenfell, 1997).

### Ethical Approval and/or Informed Consent

The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

### Results

We collected 355 individuals from five lizard species. Of these,

three species belonged to the family Gekkonidae *H. agrius*: 28 males (SVL = 47.07 ± 3.83 mm) and 35 females (SVL = 47.82 ± 3.78 mm); *L. klugei*: 25 males (SVL = 27.52 ± 2.58 mm) and 40 females (SVL = 28.02 ± 2.41 mm); and *H. brasiliensis*: 11 males (SVL = 45 ± 5.93 mm) and 13 females (SVL = 43.61 ± 5.70 mm). And another two species belonged to the family Phyllodactylidae *P. pollicaris*: 57 males (SVL = 64.22 ± 8.40 mm) and 75 females (SVL = 62.58 ± 11.93 mm); *G. geckoides*: 30 males (SVL = 37.76 ± 3.70 mm) and 41 females (SVL = 37.53 ± 4.12 mm) (Fig. 1). The only parasite species found in four of the five lizard species was *Raillietiella mottae* (Table 1).

Body size and mass were significant for the abundance of pentastomids only for *P. pollicaris*, only when related by host sex. However, the SVL and mass only become significant in the part of the response variable (infected or uninfected host), regardless of the intensity of the infection (number of parasites per infected lizard). Thus, the SVL and mass determine whether or not the lizard will be infected, but not how many parasites it will harbor. Both sex and species were significant in both parts of the model, that is, these factors influence the infection and the amount of parasites (Table 2). We recorded three new hosts for *R. mottae*: *H. agrius*, *H. brasiliensis*, and *G. geckoides*. There was no damage to the lung tissue of infected lizards.

### Discussion

All lizard species found are of nocturnal activity and have a sit-and-wait foraging mode (Vanzolini, 1968). However, they differ in the strata used to forage. For example, *Hemidactylus agrius* and *P. pollicaris* are common in rocky outcrops, while *Hemidactylus brasiliensis* and *L. klugei* are tree dwellers, and *G. geckoides* forages in the leaf litter (Vitt *et al.*, 1995; Colli *et al.* 2003; Rocha *et al.*, 2005; Mesquita *et al.*, 2006; Sousa *et al.*, 2010; Recorder *et al.*, 2012; Albuquerque *et al.*, 2013; Passos *et al.*, 2014). The similar nocturnal activity of the species may render similar insect prey and allow the infection by *R. mottae*, which seems to be a generalist parasite,

Table 1. Total infection rates for each lizard species discriminated by sex in the Ecological Station of Aiuaba, Ceará, Brazil.

Species	<i>Raillietiella mottae</i>					
	Prevalence (%)		Intensity (range)			
	Total		Male		Female	
Gekkonidae						
<i>Hemidactylus agrius</i>	28.57 %	1.66 (1 – 5)	39.28 %	1.27 (1 – 2)	20 %	2.28 (1 – 5)
<i>Hemidactylus brasiliensis</i>	4.16 %	1 (1)	0	0	7.69 %	1 (1)
Phyllodactylidae						
<i>Phyllopezus pollicaris</i>	15.9 %	1.9 (1 – 5)	87.7 %	2 (1 – 5)	21.33 %	1.87 (1 – 3)
<i>Gymnodactylus geckoides</i>	1.4 %	5 (5)	0 %	0	2.43 %	5 (5)

Table 2. Generalized Linear Model (GLM) in Poisson distribution on the variation of the pentastomids abundance related to the host specie (*Phyllopezus pollicaris*), sex, mass, Snout-Vent Length (SVL) and interaction SVL, mass with sex × host species. Values of P presented in italics are statistically significant.

	Estimate	Std. Error	z value	P
Abundance ~ SVL	-0.062	0.035	-1.778	0.075
Abundance ~ Sex	-1.136	0.496	-2.292	<i>0.022</i>
Abundance ~ SVL * Sex	1.512	0.684	2.212	<i>0.027</i>
Abundance ~ Mass	0.344	0.072	4.790	0.167
Abundance ~ Sex	0.117	0.366	0.320	0.749
Abundance ~ Mass * Sex M	-1.080	0.517	-2.088	<i>0.037</i>

infecting several lizard species (Dias *et al.*, 2005; Vrcibradic *et al.*, 2002; Anjos *et al.*, 2007; Almeida *et al.*, 2008a, 2008b, 2008c, 2009a; Sousa *et al.*, 2010, 2014; Ribeiro *et al.*, 2012; Brito *et al.*, 2014b; Araujo Filho *et al.*, 2016).

However, there is no records of insects as intermediate hosts for *R. mottae*, even though Lavoipierre & Lavoipierre (1966) found *Periplaneta americana* as intermediate host of *R. hemidactyli* in Singapore. Almeida *et al.* (2008b) suggested that ants and termites could be intermediate hosts, because these insects are commonly found in lizard diets (Vitt *et al.*, 1995; Colli *et al.*, 2003; Rocha *et al.*, 2005; Mesquita *et al.*, 2006; Sousa *et al.*, 2010; Recoder *et al.*, 2012; Albuquerque *et al.* 2013; Passos *et al.*, 2014) which could possibly contribute to infections.

The infected species (*H. agrius*, *H. brasiliensis*, and *G. geckoides*) represent three new host records for *R. mottae*. We did not find lung lesions, probably because the maximum load of parasites in a single lizard was five. Pulmonary lesions in geckos are more frequent when they are infected by more than nine pentastomids (Riley *et al.*, 1991).

The prevalence of *R. mottae* in Brazilian lizards varies according to the species, but it seems that only geckonids have a prevalence above 14 % (Anjos *et al.*, 2007; Almeida *et al.*, 2008c; Sousa *et al.*, 2010; this study). Pentastomids may have a long co-evolutionary history with geckonids. This would also explain the small prevalence of *R. mottae* in other lizard lineages, even with large sample sizes (Dias *et al.*, 2005; Vrcibradic *et al.*, 2002; Almeida *et al.*, 2008b; 2009a, Ribeiro *et al.*, 2012, Brito *et al.*, 2014b, Araujo Filho *et al.*, 2016). These lizards would be occasional hosts due to opportunistic infections. Future studies are needed to confirm these hypotheses.

Host size and mass influenced the abundance of pentastomids only in *P. pollicaris*, mainly in males (only when related to host sex), but not in the amount of pentastomids per lizards (range). This relationship is identified here for the first time and is probably due to larger individuals having larger home ranges, having a more diverse diet of invertebrates, using a wider range of microhabitat, and being active longer. Thus this ratio of higher SVL and mass, represent higher niche and consequently greater abundance of

pentastomids can be intensified in males of the host species in question. In addition the range might be influenced other factors not tested in the present study, possibly humidity, seasonality, temperature, among others. However, the same does not apply to larger lizards such as *Salvator merianae* and *Ameivula ocellifera* (Brito *et al.*, 2014a; Ramalho *et al.*, 2009; Teixeira *et al.*, 2017).

The present study adds three new host records for *R. mottae*. Also, we found a positive relation between host size and mass in infection by pentastomids in lizards of the families Phyllodactylidae and Gekkonidae in the Caatinga. However, further studies are needed to better understand the ecological and co-evolutionary relationships between lizards and pentastomids in this biome.

#### Conflict of Interest

Authors state no conflict of interest.

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