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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

Nematodes infecting *Phyllopezus Pollicaris* (Spix, 1825) (reptilia: Phyllodactylidae) in the Palmares national forest, Piauí state, Brazil

Nematódeos infectando *Phyllopezus Pollicaris* (Spix, 1825) (reptilia: Phyllodactylidae) na floresta nacional de Palmares, estado do Piauí, Brasil

Nematodos que infectan a *Phyllopezus Pollicaris* (Spix, 1825) (reptilia: phyllodactylidae) en el bosque nacional de Palmares, estado de Piauí, Brasil

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Running title: Nematodes infecting *Phyllopezus pollicaris*

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ABSTRACT

Parasitological studies are important to understand the host-parasite interaction, providing information on the coevolution, biogeography, morphology, and diet of the species. The present study describes the endoparasite fauna of the lizard *Phyllopezus pollicaris* (Spix, 1825) in the Palmares National Forest, Piauí State, Brazil, a tropical forest covered by transitional vegetation between the Caatinga and Cerrado biomes. Furthermore, we investigated the influence of lizards' size and sex on endoparasite infection patterns. We analyzed 23 individuals of *P. pollicaris*, of which 18 were infected by at least one helminth, belonging to the Nematoda phylum: *Spauligodon oxkutzcabiensis*, *Strongyloides* sp., and *Parapharyngodon* sp. Overall, *S. oxkutzcabiensis* showed the highest prevalence (73.9), mean infection intensity (8.64 ±

12.7), and mean abundance (6.39 ± 11.5). We observed that the lizard size was positively related to the abundance of endoparasites, while the sex of individuals had a poor effect on parasitological descriptors. This study contributes information about the endoparasite fauna associated with *P. pollicaris*, composed exclusively of nematodes. As a pioneering study, it can be considered the starting point for parasitological studies involving lizards in Piauí State, Northeastern Brazil.

Keywords: Geckos – helminths – lizards – parasites

RESUMO

Estudos parasitológicos são importantes para se compreender a relação parasito-hospedeiro, fornecendo informações sobre a coevolução, biogeografia, morfologia e dieta das espécies. O presente estudo descreve a fauna endoparasitária do lagarto *Phyllopezus pollicaris* (Spix, 1825) na Floresta Nacional (Flona) de Palmares, Piauí, Brasil, uma floresta recoberta por vegetações de transição entre o Cerrado e a Caatinga. Além de investigar a influência do sexo e do tamanho dos lagartos nos padrões de infecções. Analisamos 23 exemplares de *P. pollicaris* dos quais 18 estavam infectados por pelo menos um helminto, pertencente a três espécies de nematódeos: *Spauligodon oxkutzcabiensis*, *Strongyloides* sp. e *Parapharyngodon* sp. No geral, a maior prevalência (73,9), intensidade média de infecção (8,64 ± 12,7) e abundância média (6,39 ± 11,5) foi de *S. oxkutzcabiensis*. Nós observamos que o tamanho dos lagartos está positivamente relacionado com a abundância de parasitos, enquanto que o sexo do indivíduo teve pouco efeito nos descritores parasitológicos. Este estudo contribui com informações sobre a fauna de endoparasitas associada a *P. pollicaris*, sendo composta exclusivamente por nematoides. Por ser pioneiro, pode ser considerado o ponto de partida para estudos parasitológicos envolvendo lagartos no estado do Piauí, Nordeste do Brasil.

Palavras-chave: Gekkos – helmintos – lagartos – parasitos

RESUMEN

Los estudios parasitológicos son importantes para entender la relación parásito-huésped, proporcionando información sobre la coevolución, biogeografía, morfología y dieta de la especie. El presente estudio describe la fauna endoparásita del lagarto *Phyllopezus pollicaris* (Spix, 1825) en el Bosque Nacional de Palmares, Estado de Piauí, Brasil, un bosque cubierto por vegetación de transición entre el Cerrado y la Caatinga. Además de investigar la influencia del sexo y tamaño de las lagartijas en los patrones de infección. Se analizaron 23 ejemplares de *P. pollicaris*, de los cuales 18 estaban infectados por al menos un helminto, pertenecientes a tres especies de nematodos: *Spauligodon oxkutzcabiensis*, *Strongyloides* sp. y *Parapharyngodon* sp. En general, la mayor prevalencia (73,9), intensidad media de la infección (8,64 ± 12,7) y abundancia media (6,39 ± 11,5) fue de *S. oxkutzcabiensis.* Observamos que el tamaño de la lagartija se relaciona positivamente con la abundancia de parásitos, mientras que el sexo del individuo tuvo poco efecto en los descriptores parasitológicos. Este estudio contribuye con información sobre la fauna endoparásita asociada a *P. pollicaris*, que está compuesta exclusivamente por nematodos. Al ser pionero, puede considerarse el punto de partida para los estudios parasitológicos que involucran lagartos en el estado de Piauí, en el noreste de Brasil.

Palabras clave: Gekkos – helmintos – lagartos – parásito

INTRODUCTION

Parasites are organisms that need other living organisms to survive, using the host as a food source and intermediate or permanent home (Windsor, 1995). They are classified as ectoparasites when they live outside or in the host's pits (e.g., ticks) or endoparasites residing inside the host (e.g., nematodes; Rey, 2018). Regarding endoparasites, they are classified as macro or microparasites, for example, arthropods,

106 plathelminths, nematodes, fungi, and bacteria (Pantoja *et al*., 2015). Parasitological
107 studies provide information about the coevolution, biogeography, morphology, and diet
108 of species since parasitic infections significantly affect their hosts (Poulin, 2021).
109 Furthermore, endoparasites play an important role in the ecosystems acting as
110 environmental bioindicators (Poulin, 1999; Zargar & Chishti, 2022).
111 Lizards are interesting ecological models for parasitological studies because of their
112 well-resolved taxonomy and for acting as intermediate and definitive hosts of diverse
113 endoparasite species (Wilson & Carpenter, 1996). This interaction is usually associated
114 with the host forage mode and diet, in which they are infected directly by larvae
115 cutaneous penetration or by ingestion of infected prey (González *et al.*, 2021; Dantas
116 *et al*., 2024).
117 Lizards are one of the most diverse vertebrate groups, with 7,724 species registered in
118 the world (Uetz *et al*., 2024). Morphophysiological adaptations and the water-
119 independence to reproduce contributed to the lizard diversification worldwide (Hickman
120 *et al*., 2013). There are 292 lizard species in Brazil, of which 14 belong to the
121 Phyllodactylidae family (Guedes *et al*., 2023). These lizards are characterized by
122 having smooth skin with the presence of granules, well-developed limbs, non-existent
123 eyelids, and adhesive lamellae that they use to attach themselves to surfaces (Rocha,
124 1994; Gamble *et al*., 2012; Cacciali *et al*., 2018).
125 Among the Phyllodactylidae, *Phyllopezus pollicaris* (Spix, 1825) (Reptilia:
126 Phyllodactylidae) is a mid-size Gecko reaching up to 20 cm in total length,
127 characterized by having a depressed body, flattened head, snout with large granules,
128 well-developed limbs, and distinct coloring between juveniles and adults (Rodrigues,
129 1985; Vanzolini *et al*.,1980). This species is widely distributed in Brazil, being
130 commonly recorded in the Caatinga and Cerrado biomes (Gamble *et al*., 2012; Guedes
131 *et al*., 2023). It is a nocturnal lizard that inhabits rocky outcrops, fallen tree trunks, leaf

litter, and also urban areas. In addition, it is a “sit-and-wait” forager having an insectivore diet (Silva & Araújo, 2008; Gamble *et al*., 2012; Carvalho *et al*., 2014).

Some studies already reported endoparasites infecting *P. pollicaris*, for exemple, nematodes and cestodes were found parasitizing this lizard in the Estação Ecológica de Aiuaba (Lima *et al*., 2017) and in an urban area of Crato municipality (Sousa *et al*., 2014), both in Ceará State, Northeastern Brazil. In these studies, *Spauligodon oxkutzcabiensis* (Chitwood, 1938) had the highest prevalence and mean infection intensity. Specifically in Piauí State, there is one only record of *Raillietiella mottae* (Almeida, Freire & Lopes, 2008) parasitizing this lizard (Sousa *et al.,* 2010). Therefore, due to the lack of parasitological studies dealing with lizard species in Piauí State, the present study aimed to investigate the endoparasites associated with *P. pollicaris* in the Palmares National Forest, Piauí State, Northeastern Brazil, besides testing how the size and sex of the individuals influence the infection patterns.

MATERIAL AND METHODS

Study area

The present study was conducted in the Palmares National Forest (Fig. 1), situated in the municipality of Altos, Piauí State, Northeastern Brazil (05°03'25.90"S, 42°35'34.04"W, elev. 160 m). It is a small federal conservation unit comprising 170 ha, created by decree S/N of 21 February 2005, that aims to maintain and protect wild animals and native forest species in Piauí (ICMBio, 2022). It is a seasonal semideciduous (ecotonal) forest inserted in a transition area between the Cerrado and Caatinga biomes (IBGE, 2019). The climate is tropical with two well-defined seasons (rain and dry seasons), annual rainfall ranging from 1200 to 1400 mm, and annual mean temperature of 28º C (Brandão *et al.*, 2020; ICMBio, 2022; INMET, 2024).

Sampling

We searched for individuals of *P. pollicaris* using visual active searches in microhabitats usually used by them, such as trunks, trees, and sandstone outcrops (see Recoder *et al.*, 2012; Gonçalves *et al.*, 2019). We carried out two samplings of five consecutive days in February and March, totaling 10 collection days. Four researchers conducted the samplings in the morning (9 to 12 h) and night (18 to 23 h), totaling 320 hours of sampling effort. We euthanatized the collected lizards following the guidelines established by the National Council for the Control of Animal Experimentation in accordance with Law 11.794 of 2008, article 14 § 1º and with Normative Resolution 37, February 15, 2018 (Concea, 2018), and the Brazilian Guide for the Production, Maintenance or Use of Animals in Teaching or Scientific Research Activities (Jared *et al.*, 2023).

Parasitological procedures

In the lab, we measured the snout-vent length of each collected individual using a digital caliper (precision of 0.01 mm), and we necropsied the individuals with a ventral incision and removed their organs with the help of a stereomicroscope. Firstly, we analyzed the presence of endoparasites in the oral cavity and, thereafter, the following organs: digestive tract, lungs, heart, liver, and kidneys (Amato *et al*., 1991). The collected endoparasites were fixed in 10% formalin, preserved in 70% alcohol, and housed in the Helminthological Collection of the Universidade Federal do Ceará, Fortaleza, Brazil.

Data analyses

According to Bush *et al*. (1997), we used the following parasitological descriptors: richness (total number of endoparasite species), abundance (total number of endoparasites), mean abundance (total number of a given species of parasite in an individual/number of hosts examined), prevalence (number of infected hosts/total number of hosts analyzed), and mean infection intensity (total number of a species of endoparasites/number of hosts infected by the same parasite). After that, we used the

abundance data to investigate how the lizard sizes influence the endoparasite infection. For this, we used the non-parametric regression of Kernel (Nadaraya, 1964) because our data did not follow the assumptions for the linear regression test. We used the R packages Vegan (Oksanen *et al.*, 2024) and ggplot2 (Wickham, 2016) to perform this analysis.

Ethic aspects: All the procedures used in this work comply with the ethical standards of the relevant national and institutional guidelines on the care and use of laboratory animals. Collection authorization Chico Mendes Institute for Biodiversity Conservation - ICMBio (#86665-2). Voucher specimens were housed in the Biological Collection of Instituto Federal de Educação, Ciência e Tecnologia do Piauí, Campus Pedro II (CBPII).

RESULTS

We collected 23 *P. pollicaris* individuals (12 males and 11 females), of which 18 had at least one helminth parasite (total prevalence = 78.2%). Overall, we found 149 nematodes with a mean abundance of 6.47 endoparasites per individual and 8.27 mean infection intensity. The highest endoparasite abundance was found in the larger intestine (139 nematodes), whereas 10 were recorded in the stomach and five in the small intestine (Table 1).

Phyllopezus pollicaris was exclusively parasitized by nematodes belonging to three species: *S. oxkutzcabiensis*, *Strongyloides* sp., and *Parapharyngodon* sp. (Fig. 2). Among these, *S. oxkutzcabiensis* had highest prevalence, abundance, and mean infection intensity. The other two nematode species parasitized a single male individual. Additionally, we observed that males had the highest abundance (NH = 89), mean abundance (MA = 7.42), prevalence (P = 83.3), and mean infection intensity (IM = 8.90) than females (NH = 60, MA =5.45, P = 72.3, IM = 7.50).

It is interesting to note that the abundance of endoparasites was positively related to the lizard size (F = 4.481, P = 0.025), thus, larger individuals tended to be more parasitized (Fig. 3).

DISCUSSION

We found a high abundance of helminths parasitizing *P. pollicaris* belonging to three species, composed of nematodes exclusively. These helminths are usually found parasitizing reptiles (Anderson, 2000; Lacerda *et al*., 2023), and exclusively nematodes infection was already reported for other lizards such as *Tropidurus hispidus* (Spix, 1825) and *T. semitaeniatus* (Spix, 1825), and even Geckos such as *Hemidactylus mabouia* (Moreau de Jonnés, 1818) (Ávila *et al.*, 2012; Anjos *et al.,* 2013).

Among the *Phyllopezus* species, our results support the findings this lizard tend to be predominantly parasitized by nematodes, mainly for *S. oxkutzcabiensis* in the Caatinga biome (Ávila *et al.*, 2012; Sousa *et al*., 2014; Lima *et al.*, 2017). The high infections taxa of *S. oxkutzcabiensis* may be associated with its monoxenic life cycle, in which parasitic infection can occur through the ingestion of eggs (Anderson, 2000). This helminth had the highest abundance and prevalence in the present study, and it is commonly reported parasitizing other geckos in different Brazilian biomes, as Cerrado, Caatinga, and Mata Atlantica (Ávila & Silva, 2010; Ávila *et al*., 2012; Ávila *et al*., 2010; Araujo *et al*., 2020). It is noteworthy to note that the host's biological characteristics also influence the parasitic infection patterns (Aho, 1990).

We found only one *Strongyloides* sp. specimen parasitizing the larger intestine of a single male individual of *P. pollicaris*. The genus *Strongyloides* can be usually found in the gastrointestinal tract of reptiles, birds, amphibians, and mammals (Little, 1966; Roca & Hornero, 1992). These parasites have a complex life cycle with two phases: in the free-living phase, male and female individuais are found that develop through indirect development and exchange gametes (sexual or heterogonic reproduction), and

in the parasitic phase, parthenogenetic females are found only that produce eggs without the need for a male (asexual or homogonic reproduction) (Viney & Lok, 2007). This is the first report of this genus parasitizing *P. pollicaris*, despite this nematode being frequently reported to infect reptiles (Ávila & Silva, 2010; Mati *et al*., 2013; Mesquita *et al*., 2020). Because we only found one female with a broken posterior region, we were unable to count the number of turns the posterior ovary makes around the intestine, a characteristic crucial to species identification (Little, 1966). As a result, we were unable to identify this helminth to the species level.

Parapharyngodon is a nematode genus widely distributed and commonly recorded infecting insectivore reptile species (Adamson, 1981), including lizards such as *P. pollicaris, Ameiva ameiva* (Linnaeus, 1758), and *T. semitaeniatus* (Lacerda *et al*., 2023). Once this helminth has a monoxenic lifecycle (Anderson, 2000), host infection probably occurs during foraging activity, where this lizard accidentally ingested the eggs of infectious parasites that were attached to its prey or in the environment. However, further investigations are necessary to prove the validity of this proposal. *Parapharyngodon* sp. was not identified to species level because only a single female was recorded, and the identification of *Parapharyngodon* species is carried out based mainly on the reproductive characteristics of males, such as morphology and size of the spicule, number and arrangement of caudal papillae, presence or absence of genital cone, and shape of the anterior lip of the cloaca (Bursey & Goldberg, 2005).

We observed that larger lizards tended to be more infected (highest endoparasite abundance). Based on the species-area hypothesis (see Tjørve *et al.*, 2008), it is expected that larger hosts will have more space and high niche diversity to house endoparasites (Anjos *et al.*, 2012; Kamiya *et al.*, 2013; Campião *et al*., 2015). This pattern was already reported in lizards, including *P. pollicaris* in an urban area in

Northeastern Brazil (Sousa *et al*., 2014). However, further studies are still needed to understand the main drivers of endoparasite infections in lizard species.

Overall, this study contributes to the knowledge of the parasitic fauna of the lizard *P. pollicaris*, being the first study carried out in the Palmares National Forest, Piauí State, Northeastern Brazil. Aspects related to seasonality, foraging activity and feeding habits of hosts influence endoparasite infections, however, little is known about how parasitological descriptors are affected by the environmental characteristics of the Palmares National Forest. Therefore, we reinforce the need of more parasitological studies in this conservation unit aiming to understand the host-endoparasite in lizard species of Piauí State.

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FIGURE LEGENDS

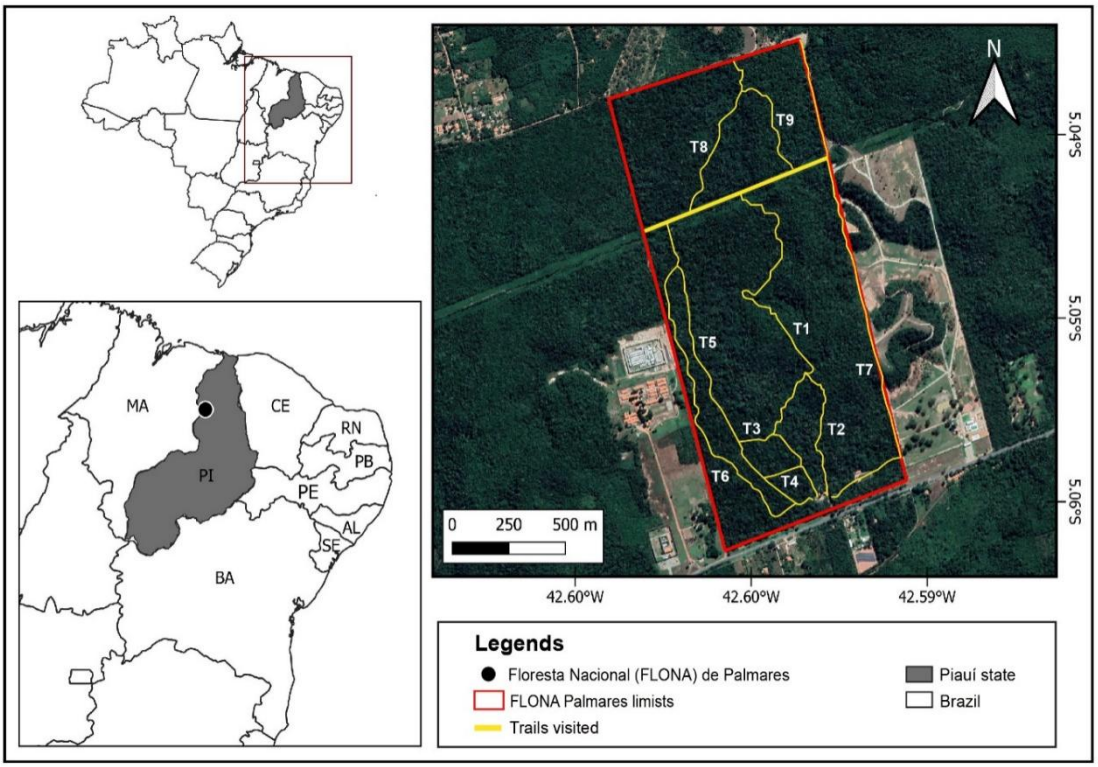

Figure 1. Location map of the Palmares National Forest, Piauí State, Northeastern Brazil, highlighting the main visiting trails.

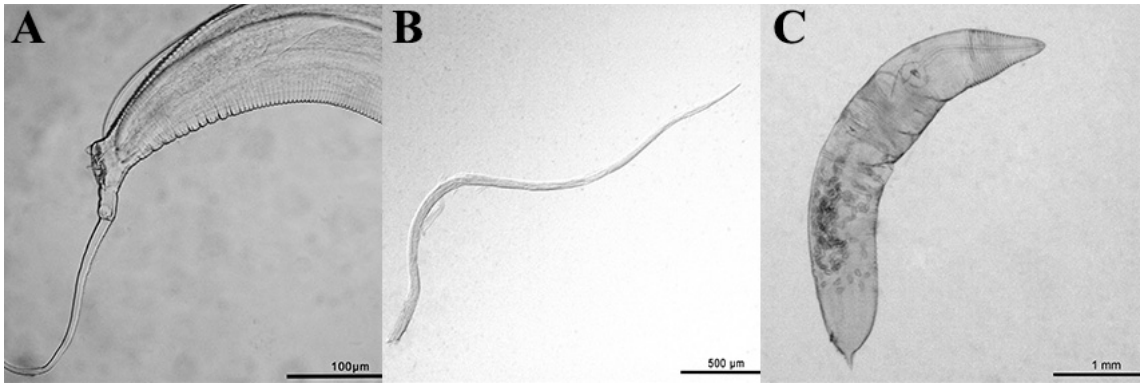

Figure 2. Nematode species parasitizing *Phyllopezus pollicaris* in the Palmares National Forest: **A)** *Spauligodon oxkutzcabiensis*, **B)** *Strongyloides* sp., and **C)** *Parapharyngodon* sp.

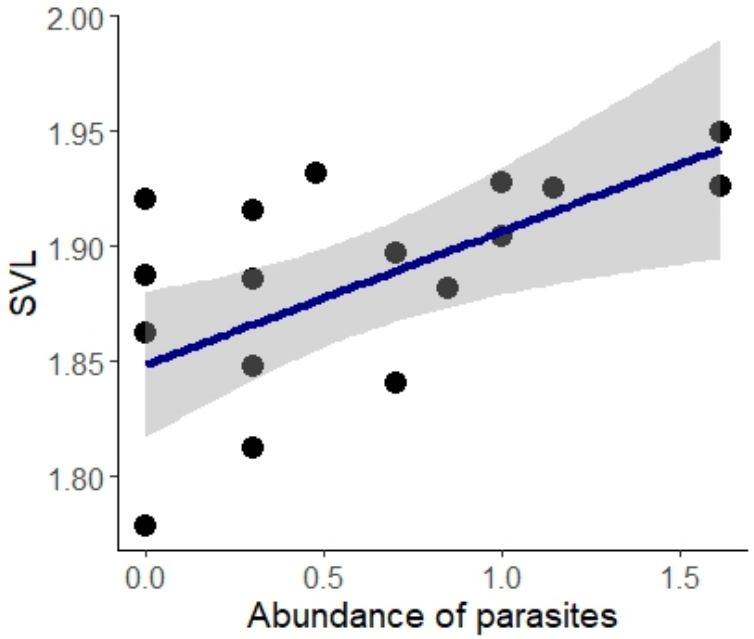

Figure 3. Relationship between lizard size (SVL) and abundance of parasites associated with *Phyllopezus pollicaris* in the Palmares National Forest, Piauí, Northeastern Brazil. We used logarithmized values for a better visualization.

TABLE LEGENDS

Table 1. Endoparasites associated with *Phyllopezus pollicaris* in the Palmares National Forest, Piauí State, Northeastern Brazil, with the respective number of helminths recorded (NH), prevalence (P), mean infection intensity (IM), mean abundance (MA), minimum and maximum number of parasites per individual (Min-Max) and site of infection (IG).

Endoparasitos	**NH**	**P%**	**IM±SD**	**MA±SD**	**Min-Max**	**SI**
Spauligodon oxkutzcabiensis	147	73.9	8.64 ± 12.7	6.39 ± 11.5	1-41	ST, LI, SI
Strongyloides sp.	1	4.34	1	0.04 ± 0.20	1-1	SI
Parapharyngodon sp.	1	4.34	1	0.04 ± 0.20	1-1	LI
TOTAL	149	78.2	8.27 ± 12.4	6.47 ± 11.5	1-41	

Legends: standard deviation (SD), stomach (ST), large intestine (LI) and small intestine (SI).