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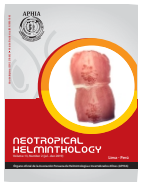

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

# HELMINTHS PARASITES OF THE FROG *PROCERATOPHRYS ARIDUS* CRUZ, NUNES, AND JUNCÁ, 2012 (ANURA: ODONTOPHRYNIDAE) IN A SEMIARID REGION, BRAZIL

# HELMINTOS PARÁSITOS DE LA RANA *PROCERATOPHRYS ARIDUS* CRUZ, NUNES Y JUNCÁ, 2012 (ANURA: ODONTOPHRYNIDAE) EN UNA REGIÓN SEMIÁRIDA, BRASIL

# HELMINTOS PARASITAS DE *PROCERATOPHRYS ARIDUS* CRUZ, NUNES, AND JUNCÁ, 2012 (ANURA: ODONTOPHRYNIDAE) EM UMA REGIÃO DO SEMIÁRIDO, BRASIL

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## ABSTRACT

This work aims to provide information about the diversity of parasites associated with *Proceratophrys aridus* Cruz, Nunes & Juncá, 2012 and to describe and compare the helminth fauna of this host to the other inventoried species of the genus. The specimens were manually collected between 2012 and 2016 in ten localities of Northeast Brazil. A total of 37 *P. aridus* were necropsied and analyzed for endoparasites, of which 22 were parasitized with at least one helminth taxon. To verify the similarity between the parasitic communities, a cluster analysis was performed using the Sorensen index. For this analysis, a matrix with presence and absence data for the helminth species related to the Odontophrynidae taxon was built. A total of 867 helminths were recovered, including nematodes, digenetics, and acanthocephalans, presenting a richness of eight species: *Aplectana membranosa* (Schneider, 1866), *Falcaustra mascula* (Rud. 1819), *Rhabdias breviensis* Nascimento, Gonçalves, Melo, Giese, Furtado, & Santos, 2013, *Oswaldocruzia* sp., Cosmocercidae larvae, unidentified trematodes, and cystacanths. The most abundant species with the highest prevalence and mean intensity of infection was *A. membranosa*. This study provides three new records of helminths parasitizing *P. aridus*, in addition to recording trematode and acanthocephalans infections for this host in northeastern Brazil. *Proceratophrys aridus* is the species of the Odontophrynidae family with the highest helminth richness ever recorded.

**Keywords:** Amphibian – Endoparasite – Host – Northeast – Neotropical realm – Semiarid

## RESUMO

Inventários parasitológicos de anfíbios da região semiárida contribuem para conhecer a diversidade de parasitas associados a espécies da região Nordeste. Este trabalho tem como objetivo fornecer informações sobre a diversidade de parasitas associados a *Proceratophrys aridus* Cruz, Nunes & Juncá, 2012 e descrever e comparar a helmintofauna deste hospedeiro com as demais espécies inventariadas do gênero. Os espécimes foram coletados manualmente entre 2012 e 2016 em dez localidades do Nordeste do Brasil. Um total de 37 *P. aridus* foram necropsiados e analisados para endoparasitas, dos quais 22 foram parasitados com pelo menos um táxon helminto. Para verificar a similaridade entre as comunidades parasitas, foi realizada uma análise de agrupamento utilizando o índice de Sorensen. Para esta análise, foi construída uma matriz com dados de presença e ausência para as espécies de helmintos relacionados ao táxon Odontophrynidae. Um total de 867 helmintos foram recuperados, incluindo nematóides, digenéticos e acantocéfalos, apresentando uma riqueza de oito espécies: *Aplectana membranosa* (Schneider, 1866), *Falcaustra mascula* (Rud. 1819), *Rhabdias breviensis* Nascimento, Gonçalves, Melo, Giese, Furtado, & Santos, 2013, *Oswaldocruzia* sp., Larvas de Cosmocercidae, trematódeos não identificados e cistacantos. As espécies mais abundantes com maior prevalência e intensidade média de infecção foram *A. membranosa*. Este estudo fornece três novos registros de helmintos parasitando *P. aridus*, além de registrar infecções por trematódeos e acantocéfalos neste hospedeiro no nordeste do Brasil. *Proceratophrys aridus* é a espécie da família Odontophrynidae com maior riqueza em helmintos já registrada.

**Palavras-chaves:** Anfíbios – endoparasitas – hospedeiros – Nordeste – Reino Neotropical –Semiárido

## INTRODUCTION

Parasitological inventories in amphibians from the semiarid region are growing (Campião *et al*., 2014; Teles *et al.,* 2014, 2015; Araujo-Filho *et al.,* 2015; Teles *et al.,* 2017; Martins-Sobrinho *et al.,* 2017; Lins *et al.,* 2017; Alcantara *et al.,* 2018; Oliveira *et al.,* 2019) and have revealed helminth fauna associated with amphibians is rich and diverse. As the sampling effort grows up, with new areas sampled and new host are available the richness tends to increased considerably (Campião *et al.,* 2014; 2015) and collaborate to elucidate the patterns of composition and richness of helminths associated with amphibians species.

Currently, there are around 49 species of Odontophrynidae in Brazil (Segalla *et al.,* 2019) in this is inserted the genus *Proceratophrys* Miranda-Ribeiro, 1920 that is currently composed for 40 species widespread along to south America with records in Argentina, Brazil, and Paraguay (Frost, 2019). In Brazil, four species occur exclusively in Caatinga biome, *Proceratophrys cristiceps* Müller, 1883, *Proceratophrys caramaschii* Cruz, Nunes & Juncá, 2012, *Proceratophrys aridus* Cruz, Nunes & Juncá, 2012, and *Proceratophrys ararype* Mângia, Koroiva, Nunes, Roberto, Ávila, Sant'Anna, Santana & Garda, 2018. These species cited above, only *P. aridus* has been inventoried for its helminth fauna (Teles *et al.,* 2017; Muller *et al.,* 2018).

Teles *et al.* (2017) recorded four taxa for *P. aridus* (*Rhabdias* sp., *Raillietnema spectans* Gomes, 1964, *Physaloptera* sp., and Cosmocercidae larvae), while Muller *et al.* (2018) researching the phylogeny of *Rhabdias* in Brazil, identified through the molecular biology *Rhabdias breviensis* Nascimento, Gonçalves, Melo, Giese, Furtado, & Santos, 2013 associated in a population of *P. aridus* in South of Ceará State. The aim this work is provided information about the diversity of helminths associated the *P. aridus* species widespread along to three Northeastern State. Besides, we describe and to compare the helminth fauna associated the *P. aridus* with other species inventories for the genus in other localities.

## MATERIALS AND METHODS

The specimens were collected between 2012 and 2016 in different municipalities from Ceará State: Aiuaba (6º34'25"S, 40º07'25"W), Barro (7º10'36"S, 38º46'54"W), Farias Brito (6º55'50"S, 39º33'56"W), Mauriti (7º23'21"S, 38º46'28"W), Crato (7°13'39"S, 39°25'05"W), Barbalha (7°19'16"S, 39°18'01"W), Missão Velha (7°14'36"S, 39°09'00"W) and Santana do Cariri (7°11'28"S, 39°44'13"W).

The specimens deposited in Herpetological collection from Cariri Regional University regarding two more states from northeastern also were included: Piauí, Santo Antônio de Lisboa municipality (06º58'53" S, 41º14'03" W) and Pernambuco, Exú municipality (07º30'43" S, 39º43'27" W) (Fig. 1). The sample sites are characterized by presents warm semiarid tropical climate and semiarid smooth tropical climate (IPECE, 2016).

A total of 37 specimens of *P. aridus* were collected manually through of active search, euthanized with thiopental sodium injection and necropsied for helminths. The number of individuals collected in each locality sampled has been provided closely with its parasitological descriptors (Table 1). The specimens had the following organs analyzed under stereomicroscopic: liver, lung, heart, stomach, intestines, celomatic cavity, and kidneys. The following parasitological descriptors: prevalence (P), mean intensity of infection (IMI), and mean abundance (AM) has been calculated following Bush *et al.* (1997) using standard error and range.

The helminths were found alive, washed in saline solution (0.9% NaCl), fixed and preserved in 70% ethanol. The nematodes were cleared in lactophenol or lactic acid while the acanthocephalans were removed their cysts, stained in carmim and cleared in creosote. All the endoparasites were observed under stereomicroscopic of light DMLB (Leica) and DM 5000B with interferential phase contrast and identified at the lowest possible level according to the work of Yamaguti (1961), Sprent (1978), Vicente *et al.* (1991), Anderson (2000) and Gibbons (2010). The helminths were deposited under the number CHIBB 8819 – 8838 in the Helminthological Collection of the Institute of Biosciences (CHIBB), São Paulo State University (UNESP), municipality of Botucatu, SP, Brazil.

To verify the similarity between parasites communities was building a matrix of data with variables presence / absent to helminths species related to Odontophrynidae family. The degree of similarity between these communities of helminths was calculated employing Sorensen index (So), with a later clustering analyse using Cluster method utilizing the mean of the unweighted pairs (UPGMA). All analyzed were performed using the software PAST 3.0.

Ethical Approval and/or Informed Consent
All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The sampling of the anuran specimens was authorized by the Instituto Chico Mendes de Conservacão da Biodiversidade - ICMBio (permit 29613-1) and to the ethics committee of the Regional University of Cariri (CEUA / URCA, process nº 00260 / 2016.1).

## RESULTS

A total 37 host analyzed, 22 were parasitized at least one helminth taxa (P = 59.5%; IMI = 185.1 ±467.9; AM = 110.1 ±40.5; range 1-190). A total of 867 helminths were collected, included nematodes, digenetics, and acanthocephalans, presenting a richness of eight species: *Aplectana membranosa* (Schneider, 1866), *Falcaustra mascula* (Rudolphi, 1819), *R. breviensis* (Nascimento, Gonçalves, Melo, Giese, Furtado & Santos 2013), *Oswaldocruzia* sp., *Physaloptera* sp., Cosmocercidae larvae, trematodes unidentified and cystacanths (Table 2). The most abundant species with the highest prevalence and mean intensity of infection was *A. membranosa* (n = 625; P = 27%; IMI = 65.5 ±107.1).

The similarity between the helminth communities of the five species of Odontophrynidae inventoried was considered low, with *P. aridus* and *Proceratophrys mantiqueira* Mângia, Santana,


**Figure 1.** Sampled localities of *Proceratophrys aridus* collected in northeast Brazil. Source: QGIS 2.18.

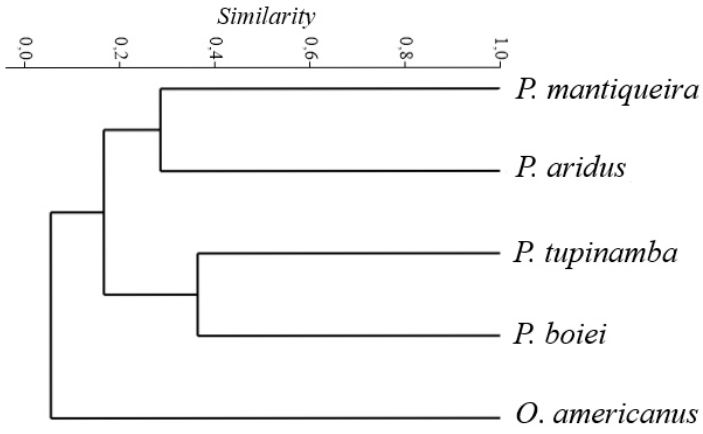


**Figure 2.** Cluster analysis of species of the Odontophrynidae family for helminth fauna composition, cophenetic correlation coefficient (ccc) = 0.92.

Cruz & Feio, 2014 (So = 28.6%) being those with the highest similarity among their parasite populations, followed by *P. aridus* and *Proceratophrys boiei* (Wied-Neuwied, 1825) (So = 15.3%), *P. aridus* and *Proceratophrys tupinamba* Prado & Pombal, 2008 (So = 12.5%) and *P. aridus* and *Odontophrynus americanus* (Duméril & Bibron, 1841) (So = 9.5%). The similarities between the other helminths communities of species *Proceratophrys* genus were *P. tupinamba* e *P. boiei* (So = 36.3%), *P. tupinamba* e *P. mantiqueira* (So = 16.7%) e *P. tupinamba* e *O. americanus* (So = 0), *P. boiei* e *O. americanus* (So = 12.5%), *P. boiei* e *P. mantiqueira* (So = 22.2%) e *O. americanus* e *P. mantiqueira* (So = 0) (Fig. 2).

**Table 1.** Parasitological descriptors of helminths associated *P. aridus* in ten municipalities from Northeastern region, Brazil. Prevalence (%), mean abundance (AM), mean intensity of infection (IMI), standard error (SD), and range.

| Locality | Host | % | AM±SD | IMI±SD | Range |
|---|---|---|---|---|---|
| Aiuaba, CE | 17 | 35 | 36.2±16.7 | 102.7±34.4 | 0-190 |
| Barro, CE | 5 | 80 | 15.6±0.4 | 19.5±70.7 | 0-75 |
| Farias Brito, CE | 3 | 33.3 | 1±1 | 0.33 | 0-1 |
| Santana do Cariri, CE | 3 | 66.7 | 1.5±1 | 1 | 0-2 |
| Nova Olinda, CE | 3 | 100 | 1±1 | 1 | 0-1 |
| Crato, CE | 2 | 100 | 1 | 1 | 0-1 |
| Barbalha, CE | 1 | 100 | 3 | 3 | 0-3 |
| Mauriti, CE | 1 | 100 | 24 | 24 | 1-24 |
| Exú, PE | 1 | 100 | 16 | 16 | 1-16 |
| St. Ant. de Lisboa, PI | 1 | 100 | 1 | 1 | 0-1 |

**Table 2.** Helminth component community of *P. aridus* (n=37) from ten municipalities from northeast region, Brazil. Prevalence (%), mean abundance (AM) and mean intensity of infection (IMI) following by standard error (SD), site of infection (SI), and range. (*) new records.

| Helminths | % | AM±SD | IMI±SD | SI | Range | Locality |
|---|---|---|---|---|---|---|
| **Nematoda** | | | | | | |
| *Aplectana membranosa** | 27 | 16.9±4.32 | 65.5±107.1 | Li, Si | 1-181 | Ba, Ma, No, Bo, Ab |
| *Falcaustra mascula** | 2.7 | 0.05±0.16 | 2±2 | Li | 0-2 | Sc |
| *Rhabdias breviensis* | 13.5 | 0.14±0.24 | 1±0.9 | L | 1-5 | Cr, No, Bo |
| *Oswaldocruzia* sp. | 2.7 | 0.03±0.08 | 1±1 | Si | 0-1 | No |
| *Physaloptera* sp. | 5.4 | 0.51±1.95 | 9.5±15.82 | S | 2-19 | Bo, Ab |
| Cosmocercidae larvae | 10.8 | 5.22±0.16 | 48.25±150.1 | S, Li, Si | 1-170 | Ex, Sc, Ab, Fb |
| **Digenea** | | | | | | |
| Trematoda unidentified* | 2.7 | 0.54±1.62 | 20±20 | Kd | 0_20 | Ab |
| **Acanthocephala** | | | | | | |
| Cystacanth | 5.4 | 0.05 | 1±0.97 | Cc | 1¯2 | Sa, Bo |

(Site of infection: Cc = Coelomic cavity; Lung= L; Kidney = Kd; S = Stomach; Li = Large intestine; Si = Small intestine. Locality: Ba – Barbalha, Ma – Mauriti, No - Nova Olinda, Bo - Barro, Ab - Aiuaba, Sc - Santana do Cariri, Cr - Crato, Fb - Farias Brito, Ex – Exú, and Sa - Santo Antônio de Lisboa).

**Table 3.** List of helminths related to the species of the family Odontophrynidae.

| Host | Parasites | References |
|---|---|---|
| *O. americanus* | *Myxidium immersum*, Lutz 1889 | Cordero (1928) |
| | *Oligacanthorhynchus* sp. | Smales (2007) |
| | *Rhabdias elegans* Gutierrez, 1945<br>*Cosmocerca parva* Travassos, 1925<br>*Cosmocerca podicipinus*, Baker and Vaucher, 1984 | González & Hamann (2009) |
| | *C. uruguayensis* Lent & Freitas 1948<br>*Travtrema* aff. *stenocotyle* Cohn, 1902<br>Echinostomatidae gen. sp.<br>*Aplectana membranosa* (Schneider, 1866) Miranda, 1924<br>*A. meridionalis* Lent & Freitas 1948<br>*Styphlodora* sp.<br>*Opisthogonimus* sp. | Hamann & González (2009); Campião *et al.* (2014) |
| *O.* cf. *barrioi* | *Falcaustra sanjuanensis* González, Sanabria & Quiroga, 2013 | González *et al.* (2013) |
| *P. tupinamba* | *Aplectana delirae* Fabio, 1971<br>*Cosmocerca brasiliense* Travassos, 1925<br>*Cosmocerca cruzi* Rodrigues & Fabio, 1970<br>*Physaloptera sp.*<br>*Rhabdias androgyna* Kloss 1971<br>*Schulzia travassosi* Durette-Desset, Baker and Vaucher, 1985<br>Unidentified Cestoda | Boquimpani-Freitas *et al*. (2001) |
| *P. boiei* | *Aplectana delirae* Fabio, 1971<br>*Cosmocerca parva* Travassos, 1925<br>*Oxyascaris oxyascaris* Travassos, 1920<br>*Physaloptera* sp. | Klaion *et al*. (2011) |
| *P. mantiqueira* | Cosmocercidae larvae<br>*Physaloptera* sp.<br>*Oxyascaris* sp.<br>*Oswaldocruzia lopesi* Freitas & Lent, 1938<br>*Rhabdias* sp. | Almeida-Santos *et al*. (2017) |
| *P. aridus* | *Rhabdias breviensis*<br>*Railietnema spectans*<br>*Physaloptera* sp.<br>Cosmocercidae larvae<br>*Aplectana membranosa*<br>*Falcaustra mascula*<br>*Oswaldocruzia* sp.<br>Unidentified Trematoda<br>Cystacanth | Teles *et al*. (2017)<br>Muller *et al*. (2018)<br>Present study |

## DISCUSSION

*Proceratophrys aridus* is the species of the genus that presented the highest richness of parasites among the others investigated (Cordero, 1928; Boquimpani-Freitas *et al.,* 2001; Smales, 2007; González & Hamann, 2009; Hamann & González, 2009; Klaion, 2011; Almeida-Santos *et al.,* 2017; Teles *et al.,* 2017) (Table 3). This can be explained in the function of the sampled effort, the richness of parasite species is directly influenced by this variable (Campião *et al.,* 2015). Thus, in this study, the sampled effort was important for growing up the richness of helminths associated *P. aridus* among congeners.

Brazil is one of the countries of South America that more research helminths of amphibians (Martins-Sobrinho *et al.,* 2017). The present study provides three new records of helminths parasitizing *P. aridus* in the northeast region from Brazil. Regarding other works as Almeida-Santos *et al.* (2017) and Teles *et al.* (2017) we recording besides of infection by nematodes, the presence of digenetic trematodes and cystacanths associated in this host, thus totalizing fifteen species of Nematoda, one record of Cestoda, Trematoda, and cystacanth for the genus *Proceratophrys*.

The *Aplectana* genus currently is composed of 28 species distributed in the Neotropical realm (Gomez *et al.,* 2017). In this study, *A. membranosa* was species that present the highest prevalence and intensity of infection. Considered monoxenic cycle life this parasite, the infection occurs through the penetration of the host skin (Anderson, 2000), thus the terrestrial habit and forage behavior of the host can have the exposure it highest risk of infection for this parasite.

In Brazil, occurs nine species of the genus *Aplectana* that infecting amphibians: *A. crossodactyli* (Vicente & Santos, 1970), *A. crucifer* Travassos, 1925, *A. delirae* Fábio, 1971, *A. lopesi* Silva, 1954, *A. membranosa* (Schneider, 1866), *A. meridionalis* Lent & Freitas, 1948, *A. micropenis* Travassos, 1925, *A. pintoi* Travassos, 1925 and *A. vellardi* Travassos, 1926. In Brazilian Northeast, only *A. membranosa* has records infecting hosts *Leptodactylus labyrinthicus* (Spix, 1824), *L. syphax* Bokermann, 1969 and *Dermatonotus muelleri* (Boettger, 1885) (Vicente *et al.,* 1991; Lins *et al.,* 2017; Alcantara *et al.,* 2018). This work presents the first record of infection by *A. membranosa* parasitizing a species of *Proceratophrys* genus.

The genus *Falcaustra* Lane, 1915 has 13 species distributed throughout the Neotropical realm (Bursey *et al.,* 2018). In Brazil, there are records of the occurrence of three species parasitizing amphibians, lizards and snakes: *Falcaustra belemensis* Baker and Bain, 1981 (Goldberg *et al.,* 2007), *F. tikasinghi* (Schoenecker, Schmidt & Everard, 1977) Baker & Bain, 1981 (Baker & Bain 1981; González *et al.,* 2013) and *F. mascula* (Rudolphi, 1819; Gomes & Vicente, 1966; Vicente *et al.,* 1991; Goldberg *et al.,* 2007; Teles *et al.,* 2014). Little is known about the life cycle of *F. mascula*, however, some evidence indicates that it has a heteroxenic cycle (Anderson, 2000; González *et al.,* 2013; Teles *et al.,* 2014). *Procetatophrys aridus* represents the second species of the Odontophrynidae family to be recorded infected by the genus *Falcaustra* (González *et al.,* 2013).

To *R. breviensis*, Muller *et al.* (2018) reports the multiple lineages presence of this species in Brazil. One of this lineages has been recorded in the Northeast region (Piauí and South Ceará state) associated *Leptodactulus fuscus* (Schneider, 1799), *Rhinella jimi* (Stevaux, 2002) and *P. aridus*.

This reports confirm the presence of this species outside type locality (Pará, Amazônic Region) expanding their spacial distribution and their hosts, which until then were only Leptodactylidae, evidencing its generalist character. Our reports overlap other records as Muller *et al.* (2018) confirm the presence of this lineage of *R. breviensis* in the Northeast region. The records of Teles *et al.* (2017) and Muller *et al.* (2018) were both from Aiuaba locality, Ceará state, thus the records of *Rhabdias* sp. in effect is *R. breviensis*.

The genus *Oswaldocruzia* Travassos, 1917 has 88 species around the world (Svitin & Kuzmin, 2012), 43 reported in Neotropical realm, 14 in a South America, only eight recorded in amphibians and reptiles in Brazil designated to following Bufonidae, Ceratophynidae, Craugastoridae, Dendrobatidae, Hylidae, Leptodactylidae e Microhylidae taxa (Guerreiro, 2013; Campião *et*

*al.,* 2014; Willkens *et al.,* 2016). Almeida-Santos *et al.* (2017) reported for the first time *O. lopesi* Freitas and Lent, 1938 in *P. mantiquiera* in rain forest in Brazilian southeastern. We present the second records for the family and the first to *P. aridus*, expanding this relation from the Brazilian northeast.

*Proceratophrys* genus composes a taxon of diurnal toads, rugged skin that lives in litter leaf. Considering the ecological aspects similarity such as foraging mode and diet of the four species investigated for this genus, we observed that these aspects were not determinant for the helminth assemblage composition. (Boquimpani-Freitas *et al.,* 2001; Almeida-Santos *et al.,* 2017). By the other hand, the geographical distance between the species can be a factor considered (Poulin, 2018). The low similarity between the communities of helminths of *P. aridus* and their congeners can be explained by the distribution of these species (Poulin, 2003; Poulin *et al.,* 2011).

*Procetatophrys aridus* has a restricted occurrence in the Brazilian Northeast (Cruz *et al.,* 2012), while *P. tupinamba*, *P. mantiqueira* and *P. boiei* occur mainly in humid forests of southeastern Brazil (Boquimpani-Freitas *et al*., 2001; Klaion *et al.,* 2011; Almeida-Santos *et al.,* 2017), a factor that justifies higher values of similarity between the helminth communities among these sympatric hosts. Besides, the phylogenetic proximity may also justify the similarities between the species *P. tupinamba* and *P. mantiqueira* (*Proceratophrys appendiculata* group) and *P. boiei* (*Proceratophrys boiei* group) (Prado & Pombal Jr., 2008; Almeida-Santos *et al.,* 2017; Mângia *et al.,* 2018).

All helminths associated with *Procetatophrys* genus can be considered generalists because they occur in several other amphibian taxa (González & Hamann, 2008; Campião *et al.,* 2014; Muller *et al.,* 2018). However, it should be considered that species that compose the community of *P. boiei* parasites such as *Cosmocerca parva* Travassos, 1925 and *Oxyascaris oxyascaris* Travassos, 1920 also have records for the northeastern region (Teles *et al.,* 2015; Silva *et al.,* 2018). The non-occurrence of these species in this study does not imply that they do not parasite *P. aridus* but may be related mainly to the effort employed, corroborating the implications of the sample effort relationship with the richness of parasite species (Campião *et al.,* 2015). Besides, the proximity between the communities of *P. aridus* and *P. mantiqueira* is due more to the number of helminth taxa not determined than the species shared by these hosts.

Parasitic communities provide great models for exploring various subjects related to interspecific associations, community structure, and species richness or diversity determinants (Poulin, 2018). The higher species and localities are inventoried concerning their helminth fauna, the greater the data set that will able to available to trace the distribution, occurrence, and coevolution patterns of endoparasite and their host relationships.

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