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ECOLOGÍA

Nematodes Infecting the Shield Frog *Adelophryne baturitensis* in Rainforest Enclaves of Brazilian Semiarid

Nemátodos infectando a la rana escudo *Adelophryne baturitensis* en enclaves de la selva tropical del semiárido brasileño

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ABSTRACT

Parasitological studies provide important information about host biology. However, most anuran species in mountain environments have their helminth fauna understudied. In the present study, we aimed to inventory the endoparasites associated with the shield frog *Adelophryne baturitensis* from an altitudinal rainforest enclave in the Brazilian semiarid region. We found 57 specimens from five nematode taxa. Perhaps, the high nematode infection on this mountain frog might be related to the host's direct life cycle where they do not need water ponds to lay eggs and reach tadpole development. Trematodes and other endoparasite taxa are commonly reported in water-dependent frogs. Although shield frogs seem to be myrmecophagous, more studies are still needed to investigate how their feeding habits might influence parasitological infection. Our study is an important contribution to understanding the parasite-host ecological relationship in minute frogs.

Keywords: anuran, highland marshes, parasitism, protected areas, threatened species.

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RESUMEN

Los estudios parasitológicos proporcionan información importante sobre la biología del huésped. Sin embargo, la mayoría de las especies de anuros de los entornos montañosos tienen una fauna de helmintos poco estudiada. En el presente estudio, inventariamos los endoparásitos asociados al sapo escudo *Adelophryne baturitensis* de un enclave de selva tropical altitudinal en la región semiárida brasileña. Encontramos 57 especímenes de cinco taxones de nematodos. La elevada infección por nematodos en esta rana de montaña puede estar relacionada con el ciclo de vida directo del hospedador el cual ocurre sin necesidad de charcos de agua para la puesta de huevos y el desarrollo de renacuajos. Los trematodos y otros taxones endoparásitos son habituales en las ranas dependientes del agua. Aunque los sapos escuderos parecen ser mirmecófagos, todavía se necesitan más estudios para investigar cómo sus hábitos alimentarios pueden influir en la infección parasitológica. Nuestro estudio constituye una importante contribución a la comprensión de la relación ecológica parásito-huésped en las ranas pequeñas.

Palabras clave: parasitismo, anuros, pantanos de la sierra, especies en peligro, áreas de protección

INTRODUCTION

Parasitological studies provide important information about the host biology and their relevance to the ecosystems, contributing substantially to a better understanding of species conservation (Marcogliese 2004), in addition, these enable comprehension of natural selection processes, biogeography, species evolution, and parasite-host ecological relationships (Bentz *et al.* 2006, Poulin 2007, Todd 2007). Thus, increasing endoparasite checklists might be crucial to understanding these relationships (Lins *et al.* 2017).

Brazil harbors the most anuran diversity in the world (Segalla *et al.* 2021). These vertebrates are intermediary, paratenic, and definitive hosts of a high endoparasite diversity (Campião *et al.* 2014), being more susceptible to parasitological infection because of their biological aspects, including feeding habits and natural history (Aho 1990, Yoder and Coggins 2007, Campião *et al.* 2012, 2014). Despite the increase in parasitological studies of amphibians in South America, most species are still understudied (e.g., Campião *et al.* 2014, Aguiar *et al.* 2014, Martins-Sobrinho *et al.* 2017).

In the Northeast region, some rainforest enclaves are surrounded by Caatinga vegetation (Andrade-Lima 1982). These mountains harbor one of the most diverse herpetofauna in the Caatinga biome, including some endemism (Roberto and Loebmann 2016). Among these species, *Adelophryne baturitensis* (Hoogmoed, *et al.* 1994), belonging to the Eleutherodactylidae family, is a minute frog distributed in altitudinal rainforest enclaves of the Ceará state (Roberto and Loebmann 2016). It was considered a vulnerable species (Silvano and Borges-Nojosa c2004), but it is still needed the re-assessment of the conservation status of the species (Lourenço-de-Moraes *et al.* 2012).

There is a lack of parasitological studies dealing with Eleutherodactylidae frogs (e.g., Moravec and Kaiser 1994, Espino-del-Castillo *et al.* 2011). Likewise, for *Adelophryne* species (e.g., Oliveira *et al.* 2022a), likely due to being minute frogs that inhabit intact and restricted humid forests at high altitudes. Thus, to fill this gap of knowledge, we aimed to inventory the endoparasites associated with the shield frog *A. baturitensis* from an altitudinal rainforest enclave in a Brazilian semiarid area.

MATERIAL AND METHODS

Study area

This study was carried out in rainforest enclaves of Ceará state, northeastern Brazil (Fig. 1). These mountains are considered real humid forest islands in Brazilian semiarid, surrounded by caatinga vegetation which is shorter and drier vegetation (Ab'Saber 1974, Andrade-Lima 1982), being characterized the former by the presence of arboreal vegetation, high levels of epiphytic, ferns and bryophytes

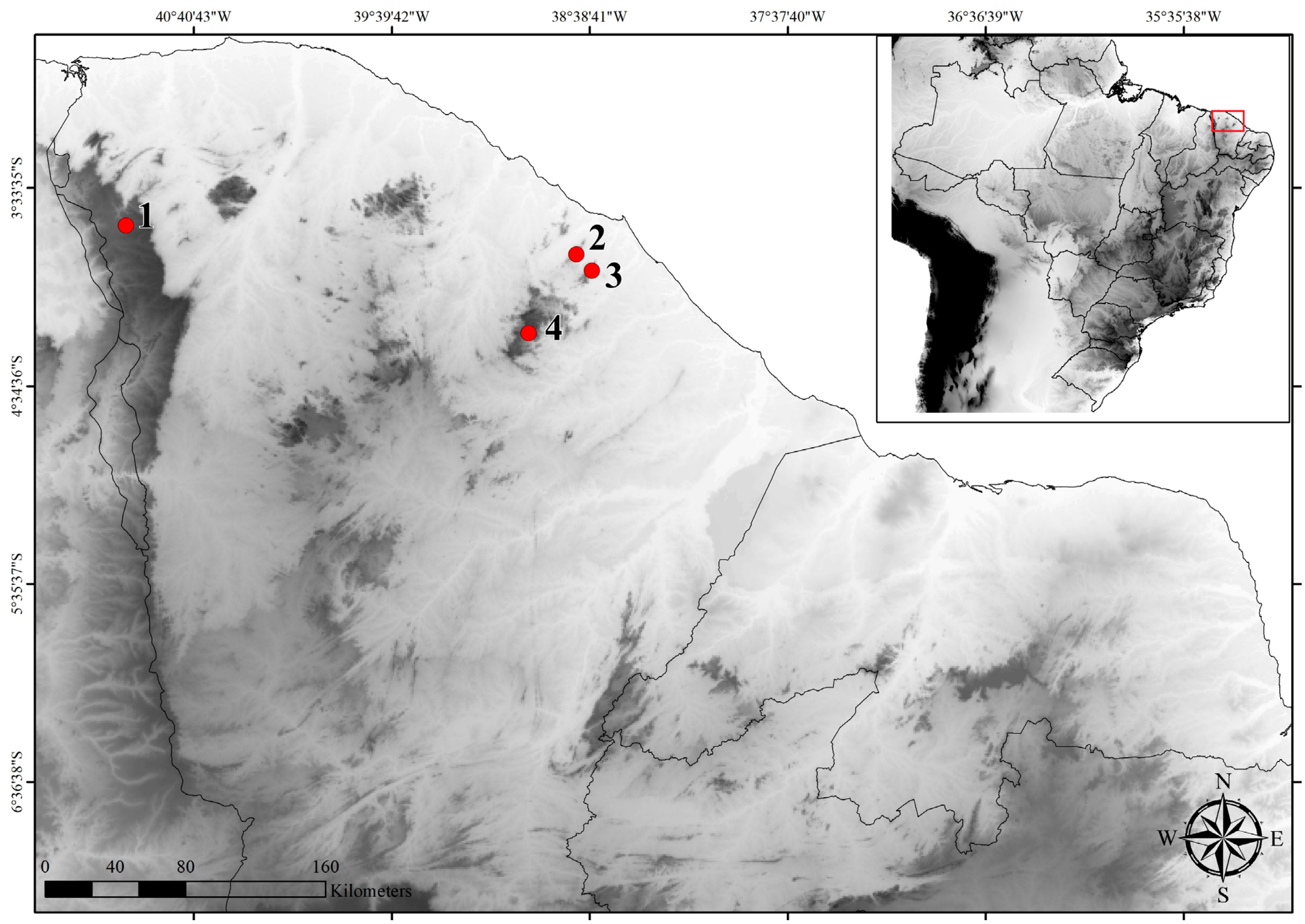

Figure 1. Schematic map of the sampled areas where the ***Adelophryne baturitensis*** specimens were collected in Ceará state, Brazil: Ibiapaba plateau (1), Maranguape (2), Aratanha (3), and Baturité (4) mountains.

at high-altitudes (Moro *et al.* 2015). The anuran samples took place in two of these highlands: Baturité mountain at "Vale das Nuvens", Guaramiranga municipality (4°16'05" South, 38°54'54" West, 805 m a.s.l.), and Maranguape mountain at "Lagoa Verde", Maranguape municipality (3°54'16" South, 38°43'13" West, 700 m altitude). In addition, we used anuran specimens housed in the Herpetological Collection of Universidade Regional do Cariri, Crato, Brazil that were collected in Aratanha mountain, Pacatuba municipality (3°58'59" South, 38°38'05" West, 650 m altitude), and Ibiapaba plateau, Tianguá municipality (3°45'03" South, 41°01'40" West, 780 m altitude).

Sampling

Anurans were sampled during the rainy season (fifteen days per mountain) from 22 March to 01 May 2019, through visual and auditory searches (Heyer *et al.* 1994). The specimens were euthanized following ethical procedures of the Federal Council of Veterinary Medicine - CFMV (2013) after approval by the Ethics Committee of Universidade Regional do Cariri (CEUA/URCA, process #00260/2016.1). Voucher specimens are housed also in the Herpetological Collection of Universidade Regional do Cariri, Crato, Brazil (Appendix 1).

Parasitological procedures

We analyzed the presence of ectoparasites under the epidermis and oral cavity and endoparasites in the following organs: the digestive tract, lungs, heart, liver, and kidneys, according to Amato *et al.* (1991). We collected and prepared the endoparasites for taxonomic identification according to Amato and Amato (2010). Nematodes were clarified with lactophenol (Anderson 2000), and temporary slides were examined in the light microscope with a computerized image analysis system (Carl Zeiss Microimaging GmbH, Gottingen, Germany). The identified nematodes are deposited in the Parasitological Collection of the Universidade Regional do Cariri, Crato, Ceará state, Brazil.

Figure 2. The male of ***Adelophryne baturitensis*** registered in Maranguape Mountain, Ceará State, northeastern Brazil. Photographer: Robson W. Ávila

The parasitological descriptors prevalence, abundance, mean abundance, and mean infection intensity were measured according to Bush *et al.* (1997), using the software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000).

RESULTS

We analyzed 29 specimens of *Adelophryne baturitensis* (Fig. 2), eight (five females and three males, both adults) were infected with at least one endoparasite species (overall prevalence = 27.6 %). We found 57 nematodes with a mean overall abundance of 1.97 ± 1.01 and a mean overall infection intensity of 7.13 ± 2.25. The highest endoparasite abundance infecting *A. baturitensis* was found in the lungs (n = 35) and small intestine (n = 11). By contrast, the liver was the less infected (n = 1). Regarding sexual differences, we observed the number of nematodes (NH = 28), mean abundance (MA = 1.65), and mean intensity (MI = 4.67 ± 3.16) was lower in females than in males (NH = 29; MA = 2.64; MI = 14.5 ± 5.41). Nevertheless, the overall prevalence was higher for females (P = 35.3 %) than for males (P = 18.1 %).

We found five taxa associated with *A. baturitensis*, all nematodes: *Physaloptera* sp., *Raillietnema* sp., *Rhabdias breviensis* (Nascimento*et al.*2013), Cosmocercidae larvae, and an unidentified nematode larva (Fig. 3). *Physaloptera* sp. and *R. breviensis* were the most prevalent nematode, whereas Cosmocercidae larvae were less abundant (Table 1). Among males we found three parasite taxa: Cosmocercidae larvae (n = 1), *Physaloptera* sp. (n = 1), and *R. breviensis* (n = 27), while females were parasitized by four parasite taxa: *Physaloptera* sp. (n = 4), *R. breviensis* (n = 9), unidentified nematode larva (n = 13), and *Raillietnema* sp. (n = 2).

DISCUSSION

The parasite load associated with *Adelophryne baturitensis* comprises only nematodes, which usually infect the hosts through the ingestion of prey infected (heterogenic nematodes) or cutaneous penetration (monogenic nematodes, Aho 1990, Anderson 2000). Other studies found a high predominance of nematodes associated with terrestrial frogs (e.g., Teles *et al.* 2017, Alcantara *et al.* 2018, Amorim *et al.* 2019). In addition, the congeneric species *A. maranguapensis* (Hoogmoed *et al.* 1994) was also infected with nematodes (Oliveira *et al.* 2022a). Perhaps, the high nematode infection in these mountain frogs might be related to the host life cycle (water-independent) because other parasite taxa as trematodes are commonly found in water-dependent frogs (Hamann *et al.* 2006, Toledo *et al.* 2017). Similar assumptions were supported for *A. maranguapensis* (Oliveira *et al.* 2022a). Although shield frogs seem to be myrmecophagous (MacCulloch *et al.* 2008, Lourenço-de-Moraes *et al.* 2012), detailed studies are still needed to investigate the influence of feeding and foraging habits might have on parasitological infections.

The low prevalence rate of parasites in *A. baturitensis* is like that found in *A. maranguapensis*, which might be associated with these species' high resilience and good immunological health (Oliveira *et al.* 2022a). Although amphibians usually are highly parasitized (Campião *et al.* 2014), some species might have low prevalence rates (e.g., Calil *et al.* 2017, Silva-Neta *et al.* 2020, Mascarenhas *et al.* 2021, Oliveira *et al.* 2022b). In addition, larger frogs usually tend to have higher helminth fauna richness (Campião *et al.* 2015), thus, it might be expected low prevalence rates in minute and terrestrial frogs. In this case, the foraging habit (water-independent frog) of *A. baturitensis* might be also taken into consideration because frogs with aquatic reproductive modes are more susceptible to being parasitized (Poulin and Morand 2000), which may explain the low prevalence rate of parasites in this species.

Physaloptera spp. use invertebrates as intermediate hosts (González and Hamann 2006), and the amphibian infection might have occurred due to the ingestion of infected prey (Anderson 2000, Campião *et al.* 2015). *Physaloptera* spp. are usually found in the stomachs of terrestrial vertebrates (Anderson 2000, Ávila, and Silva 2010, Campião *et al.* 2014). By contrast, only larvae have been found in anurans (e.g., Aguiar *et al.* 2014, Campião *et al.* 2014, Alcantara *et al.* 2018). Likewise, *Raillietnema* spp. are nematodes that might infect hosts through ingestion of infected prey or cutaneous penetration (Anderson 2000). This last one was found just in females.

The rhabditid nematodes present a monogenic life cycle adhering to tissue mucous, being considered specific to lungs in amphibians and reptiles (Anderson 2000, Ávila and Silva 2010, Campião *et al.* 2014). By contrast, we found *Rhabdias breviensis* parasitizing the liver besides the lungs of *A. baturitensis*. This finding supports the assumption of rhabditid infections in other organs besides the lungs (Imai *et al.* 2009, Pizzatto *et al.* 2016, Kelehear *et al.* 2011).

In addition, we found one Cosmocercidae larvae infecting *A. baturitensis*, which was not identified due to its immature stage. The presence of larvae might be associated with the parasite monogenic cycle (Anderson 2000) and likely is a recent infection. Cosmocercidae species are common to different vertebrates, including amphibians (Ávila and Silva 2010, Campião *et al.* 2014).

Because of the increase in environmental changes such as pollution, habitat disturbance, and diseases, including fungi, viruses, and parasites, amphibian populations are declining (Alford and Richards 1999, Pessier 2008, Collins 2010, Koprivnikar *et al.* 2012). Since parasite-host interactions are bioindicators of environment quality (Galli *et al.* 2001), understanding the parasite-host

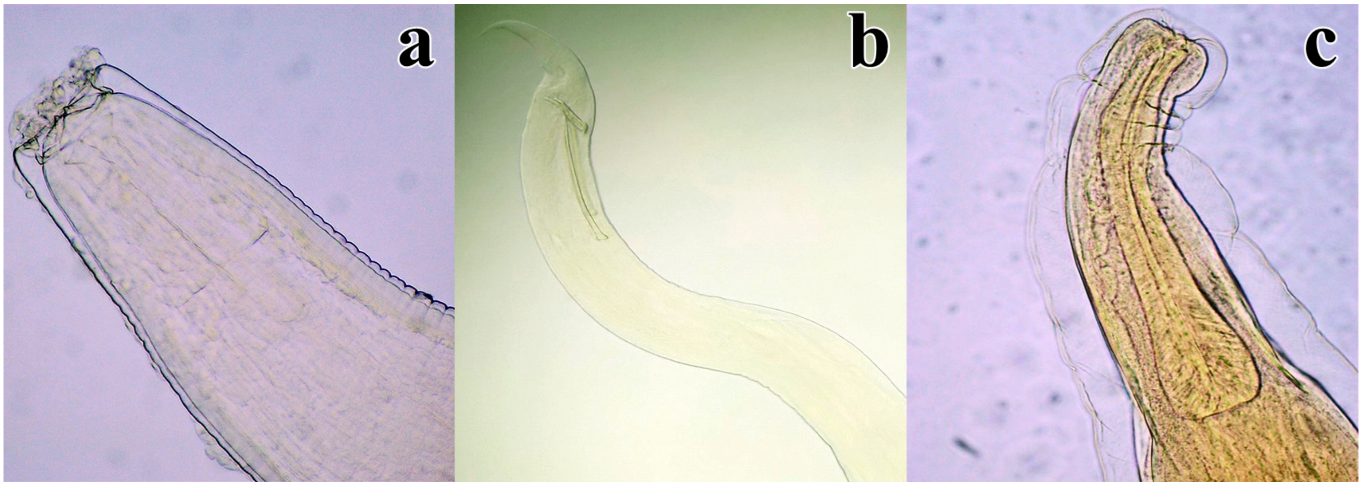

Figure 3. Nematoda taxa associated with ***Adelophryne baturitensis*** in Maranguape Mountain, Ceará State, northeastern Brazil. **a**- ***Physaloptera*** sp., **b**- ***Raillietnema*** sp., **c**- ***Rhabdias breviensis***. Images not to the same scale.

relationship might contribute to monitoring ongoing and future conservational plans. Overall, our study is an important contribution to understanding the parasite-host ecological relationship in minute frogs, besides identifying nematodes as the main source of infection in this species.

PARTICIPATION OF AUTHORS

The manuscript was conceptualized and designed by DBO, KCA, and RWA; DBO, KCA, and CRO performed a literature review and wrote the first draft; RWA and DCL revised the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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APPENDIX

Appendix 1. Voucher anuran specimens used in the present study. Maranguape: URCA-H 15275; URCA-H 15689; URCA-H 15690; URCA-H 15691; URCA-H 15692; URCA-H 15693; URCA-H 15694; URCA-H 15695; URCA-H 15696; URCA-H 15697; URCA-H 15698; URCA-H 15699; URCA-H 15700; URCA-H 15701; Aratanha: URCA-H 13355; URCA-H 13356; URCA-H 13362; URCA-H 13445; Baturité: URCA-H 16275; Ibiapaba plateau: URCA-H 9261; URCA-H 9262; URCA-H 9263; URCA-H 9264; URCA-H 9265; URCA-H 9266; URCA-H 9267; URCA-H 9268; URCA-H 9269; URCA-H 9270; URCA-H 9271