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ORIGINAL ARTICLE / ARTÌCULO ORIGINAL

HELMINTHS INFECTING *RHINELLA DIPTYCHA* COPE, 1862 AND *RHINELLA GRANULOSA* SPIX,1824 FROM NORTHEASTERN BRAZIL

HELMINTOS QUE INFECTAN A *RHINELLA DIPTYCHA* COPE, 1862 Y RHINELLA GRANULOSA SPIX,1824 DEL NORESTE DE BRASIL

HELMINTOS INFECTANDO *RHINELLA DIPTYCHA* COPE, 1862 E *RHINELLA GRANULOSA* SPIX,1824 DO NORDESTE DO BRASIL

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Running Head: Helminths infecting bufonids in Brazil

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ABSTRACT

Brazil is the country with the highest diversity and description rate of amphibian species in the world. The genus *Rhinella* has 100 species of small, medium and large animals, insectivorous, terrestrial or semi-aquatic and nocturnal. *Rhinella diptycha* Cope, 1862 and *Rhinella granulosa* Spix,1824 are widely distributed throughout the Brazilian Northeast. Despite their diversity and wide distribution, infectious diseases caused by helminths have caused a reduction in the populations of these animals. In this study, we determined the parasitic fauna of *R. diptycha* and *R. granulosa* recorded in the states of Piauí and Maranhão, Northeast Brazil. We collected 60 specimens, 30 of *R. diptycha* and 30 of *R. granulosa*, of which 78% of the total (n = 47 individuals) were infected with helminths. The helminths found were *Aplectana membranosa* Schneider, 1866, *Cosmocerca* sp. Diesing, 1861, *Rhabdias* sp Stiles & Hassal, 1905, *Oswaldocruzia* sp. Travassos, 1917, *Physaloptera* sp. Rudolphi, 1819, and *Cylindrotaenia americana* Jewell, 1916. *Cosmocerca* sp. represents the first record of this taxon parasitizing *R. diptycha*. Our findings contribute to expanding knowledge about the diversity of helminths parasitizing bufonids in the Northeast region of Brazil.

More studies are still needed to understand the mechanisms associated with this parasite-host relationship.

Keywords: Anurans – Bufonidae – Caatinga – Diversity – New record – Parasites

RESUMEN

Brasil es el país con mayor diversidad y tasa de descripción de especies de anfibios en el mundo. El género *Rhinella* cuenta con 100 especies de animales pequeños, medianos y grandes, insectívoros, terrestres o semiacuáticos y nocturnos. *Rhinella diptycha* Cope, 1862 y *Rhinella granulosa* Spix,1824 están ampliamente distribuidas por todo el Nordeste brasileño. A pesar de su diversidad y amplia distribución, las enfermedades infecciosas provocadas por helmintos han ocasionado una reducción de las poblaciones de estos animales. En este estudio, determinamos la fauna parasitaria de *R. diptycha* y *R. granulosa* registrada en los estados de Piauí y Maranhão, noreste de Brasil. Se recolectaron 60 ejemplares, 30 de *R. diptycha* y 30 de *R. granulosa*, de los cuales el 78% del total (n = 47 individuos) estaban infectados con helmintos. Los helmintos encontrados fueron *Aplectana membranosa* Schneider, 1866, *Cosmocerca* sp. Diesing,1861, *Rhabdias* sp. Stiles & Hassal,1905, *Oswaldocruzia* sp. Travassos, 1917, *Physaloptera* sp. Rudolphi, 1819, y *Cylindrotaenia americana* Jewell, 1916. *Cosmocerca* sp. representan el primer registro de este taxón parasitando a *R. diptycha*. Nuestros hallazgos contribuyen a ampliar el conocimiento sobre la diversidad de helmintos que parasitan a los bufónidos en la región Nordeste de Brasil. Aún se necesitan más estudios para comprender los mecanismos asociados con esta relación parásito-huésped.

Palabras clave: Anuros – Bufonidae – Caatinga – Diversidad – Nuevo registro – Parásitos

RESUMO

O Brasil é o país com a maior diversidade e taxa de descrição de espécies de anfíbios do mundo. O gênero *Rhinella* possui 100 espécies de animais de pequeno, médio e grande porte, insetívoros, terrestres ou semiaquáticos e noturnos. *Rhinella diptycha* Cope, 1862 e *Rhinella granulosa* Spix, 1824 são amplamente distribuídas em todo o Nordeste brasileiro. Apesar da diversidade e ampla distribuição, as doenças infecciosas causadas por helmintos têm provocado uma redução nas populações destes animais. Neste estudo, nós determinamos a fauna parasitária de *R. diptycha* e *R. granulosa* registrados nos estados do Piauí e Maranhão, Nordeste do Brasil. Coletamos 60 exemplares, sendo 30 de *R. diptycha* e 30 de R*. granulosa*, dos quais 78% do total (n = 47 indivíduos) estavam infectados por helmintos. Os helmintos encontrados foram *Aplectana membranosa* Schneider, 1866, *Cosmocerca* sp. Diesing,1861, *Rhabdias* sp. Stiles & Hassal,1905, *Oswaldocruzia* sp. Travassos, 1917, *Physaloptera* sp. Rudolphi, 1819 e *Cylindrotaenia americana* Jewell, 1916. *Cosmocerca* sp. representa o primeiro registro deste taxon parasitando *R. diptycha*. Nossos achados contribuem para ampliar o conhecimento sobre a diversidade de helmintos parasitando bufonídeos na região Nordeste do Brasil. Mais estudos ainda são necessários para compreender os mecanismos associados nesta relação parasita-hospedeiro.

Palavras-chave: Anuros – Bufonidae – Caatinga – Diversidade – Novo registro – Parasitos

INTRODUCTION

Currently, around 8,722 species of amphibians are recognized in the world, belonging to three orders: Gymnophiona, with 222 species; Caudata, with 822 and Anura, with 7,678 species (Frost, 2024). The most diverse order - Anura, comprises animals that have a life stage with larval morphology (tadpoles) inhabiting terrestrial environments and an adult stage in which they can be found in aquatic, terrestrial and

arboreal environments. They are cosmopolitan, except for extreme latitudes in the North, Antarctica and most of oceanic islands (Stuart *et al*., 2008; Jenkins *et al*., 2013; Frost, 2024).

Brazil is the country with the highest occurrence and description rate of amphibian species worldwide, with 1,188 species, the majority of which belong to the order Anura (Segalla *et al*., 2021). In the country there are 21 families of anurans distributed in more than 100 genera. Among these families, one of the most representative is the Bufonidae family, with 646 described species, distributed in 53 genera, with one of the highlights being the genus *Rhinella* Fitzinger, 1826 (Frost, 2024).

The genus *Rhinella* has 100 species, represented by small, medium and large animals, insectivorous, terrestrial or semiaquatic, and nocturnal activity. Among these species, *Rhinella diptycha* Cope, 1862 and *Rhinella granulosa* Spix, 1824 are widely distributed throughout the Brazilian Northeast, they are large and small animals, respectively, and are nocturnal (Juncá, 2001; Rodrigues, 2003; Frost, 2024). Due to their diversity and wide distribution, occurrence in various types of habitats and sensitive skin, these species often suffer from various diseases and parasitism.

Helminth fauna plays a crucial role in the ecosystem as it affects the ecology, evolution, growth and control of host populations and, consequently, all biodiversity (Marcogliese, 2023). The study of parasitic fauna is extremely significant, contributing to the understanding of biodiversity and the parasite-host relationship (Toledo *et al*., 2017). Furthermore, studies on anurans are essential, as they play an important role as environmental bioindicators (Prestes & Vincenci, 2019).

Studies that describe parasitic species in anurans are essential for research in health and environmental conservation. The helminth group is the most common of all invertebrates that parasitize amphibians. Among them, the most numerous are nematodes that are usually found in the digestive tract, lungs and blood vessels (Vieira

et al., 2021). In the Northeast, several studies have addressed the diversity of helminths parasitizing anurans (e.g., Lins *et al*., 2017; Teles *et al*., 2018; Oliveira *et al*., 2019; Madelaire *et al*., 2020; Vieira *et al*., 2021; Machado *et al*., 2022; Sampaio *et al*., 2022; Oliveira *et al*., 2023; Batista-Oliveira *et al*., 2024).

Several species of helminths have also been described parasitizing bufonids (e.g., Campião *et al*., 2014; Teles *et al*., 2018; Benício *et al*., 2022). However, there is only one article in the literature on helminths parasitizing *R. diptycha* for the state of Piauí (Benício *et al*., 2022) and, to date, no information on helminths parasitizing *R. diptycha* and *R. granulosa* for the state of Maranhão. Thus, the objective of this study was to determine the parasitic fauna of these species found in the states of Piauí and Maranhão, Northeast Brazil.

MATERIAL AND METHODS

Study area and collections

The study was carried out in the municipalities of Picos, in the state of Piauí, and in Timon, in the state of Maranhão, both located in the northeast of Brazil. The location chosen for collections in the municipality of Picos has approximately 1,000 m^2 with an area of tree and shrub vegetation with an ephemeral stream and temporary water puddles. The area is located on the outskirts of the Universidade Federal do Piauí, Picos campus (Fig. 1). The municipality is within the Caatinga ecoregion, a semi-arid climate, with an average annual temperature of 27.2°C and an average annual precipitation of 684 millimeters, with greater precipitation between December and April (Silva *et al*., 2022; Oliveira-Filho *et al*., 2021).

The municipality of Timon, in turn, is located in the eastern region of the state (Silva, 2019). It has a transition area between the Caatinga and Cerrado biomes, a hot climate all year round, with an annual temperature of 27°C and an average annual precipitation of around 790 millimeters (Silva & Coelho, 2018). The location chosen for

collections in the municipality of Timon has approximately 1,000 m^2 composed of a vegetation area with dense forest formations with stretches of riparian vegetation with a stream and peridomiciliary area (Fig. 2).

The anurans were collected between February 2021 and March 2022 during the rainy season, at night between 6pm and 10pm, using the active search method, inspecting all available microhabitats (Crump & Scott Jr., 1994). Specimens of *R. diptycha* were collected at a site located in the rural area of Timon, Maranhão (5°01'39" S, 43°00'10" W), and the specimens of *R. granulosa* were captured surroundings the Universidade Federal do Piauí, Picos campus, Piauí, Northeast Brazil (7°04'54" S, 41°26'05" W).

After collection, the anurans were transported alive to the Laboratório de Zoologia e Biologia Parasitária (ZOOBP) of the Universidade Estadual do Piauí (UESPI), on the Poeta Torquato Neto campus, in the city of Teresina, Piauí, Northeast Brazil for identification in accordance with specialized literature (Roberto *et al*., 2013; Benício & Fonseca, 2014; Benício *et al*., 2014; Benício *et al*., 2021).

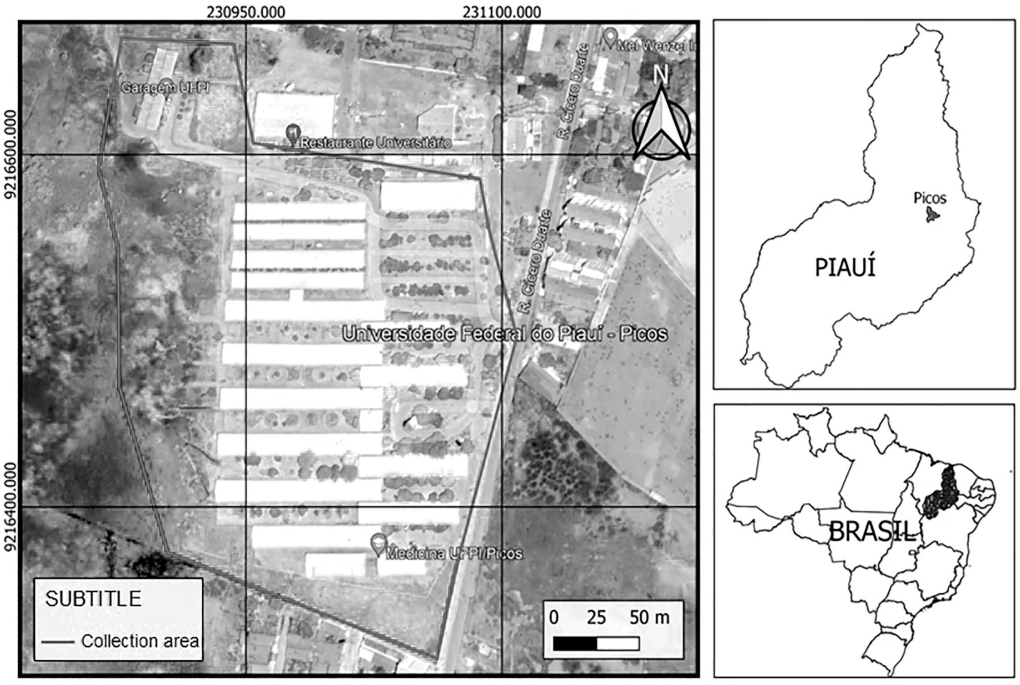


Figure 1. Collection area for *Rhinella granulosa* specimens, Universidade Federal do Piauí, municipality of Picos, state of Piauí, Northeast Brazil.

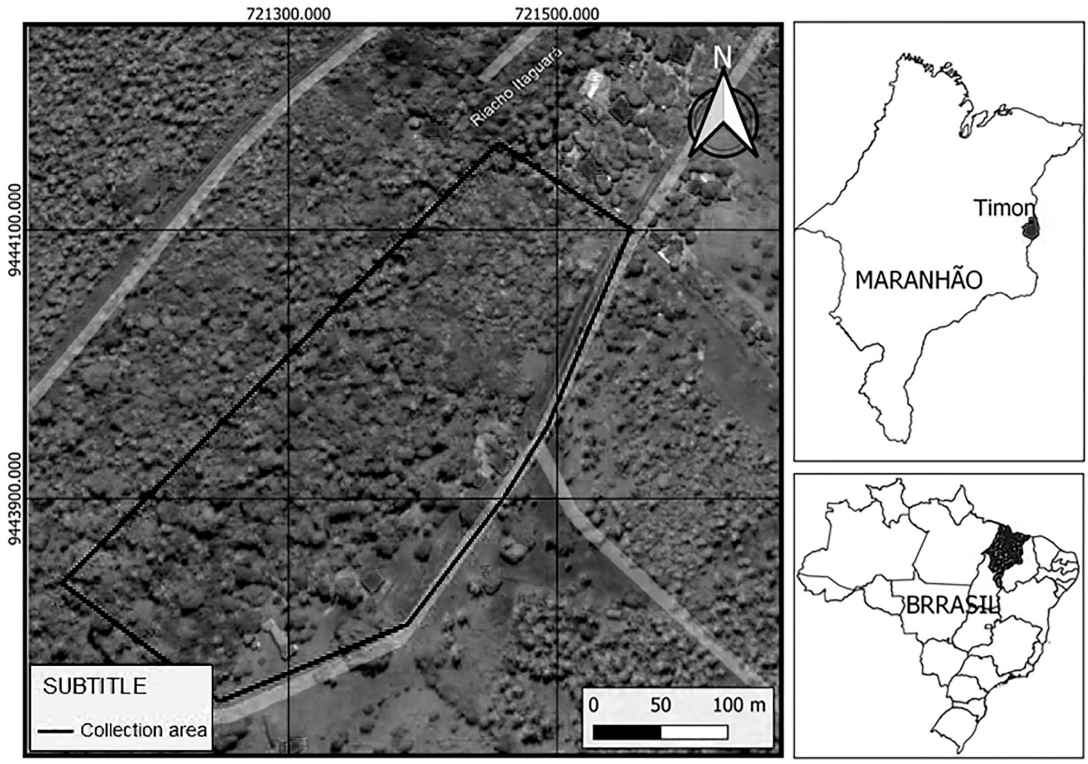


Figure 2. Collection area for *Rhinella diptycha* specimens, in the municipality of Timon, state of Maranhão, Northeast Brazil.

Laboratory procedures

After identifying the anurans, the individuals were measured with the aid of a caliper to obtain the SVL (snout-vent length), weighed on a digital scale, and were subsequently euthanized using the anesthetic compound 5% lidocaine when the anurans were considered small (weighing a maximum of 50 g) and 2% injectable lidocaine when the animal was large (when the animal weighed more than 50 g). The

ointment was applied to the entire ventral region of the individuals. After euthanasia, the organs were separated individually in petri dishes containing 0.9% NaCl saline and taken for analysis under a stereoscopic microscope in search of endoparasites.

The anurans were fixed in 10% formalin solution. After 24h in the fixative, the anurans were transferred to a 70% ethyl alcohol solution. The collected specimens were deposited at the Laboratório de Zoologia e Biologia Parasitária (ZOOBP) of the Universidade Estadual do Piauí (UESPI), Poeta Torquato Neto campus, Teresina, Piauí.

The helminths are deposited in the Helminthological Collection of the Laboratório de Zoologia e Biologia Parasitária (ZOOBP) of the Universidade Estadual do Piauí (UESPI), Poeta Torquato Neto campus, Teresina, Piauí, under registration numbers CHZOOBP54, CHZOOBP55, CHZOOBP56, CHZOOBP57, CHZOOBP58, CHZOOBP59, CHZOOBP60.

All helminths found were fixed in 70% hot ethyl alcohol, according to the protocol by Amato *et al*. (1991). For identification, the cestodes were stained in acetic carmine solution and then clarified with beech creosote, the nematodes were clarified using Amann's Lactophenol solution (Andrade, 2000). Individually, the helminths were observed and measured using Olympus®. CX21 optical microscope, and photographed using the camera of a cell phone with the aid of a microscope adapter.

The taxonomic characters of the parasites were identified using the following keys: Anderson *et al*. (2009), Vicente *et al*. (1990), and articles with specific descriptions of species of the genera found. For statistical analysis, the definitions of frequency (number of hosts infected by a given species of parasite), average intensity of infection (total quantity of a specific parasite on infected hosts) and average abundance of parasites (total quantity of specific parasite on the total number of anurans sampled) were analyzed according to Bush *et al*. (1997).

Ethical procedures

This project was submitted to the Sistema de Autorização e informação em Biodiversidade – SISBIO and to the Ethics Committee on the Use of Animals of the State University of Piauí – CEUA, having been approved through opinion no. 74248-1, 54745 (SISBIO) and 0509/ 2020, 006022/2021-93 (CEUA/UESPI). It was also registered in the National System of Genetic Heritage and Associated Traditional Knowledge – SisGen with number A1A6651.

RESULTS

We collected a total of 60 specimens, of which 30 individuals are *R. diptycha*, seven males and 14 females; and 30 are *R. granulosa*, eight males and 11 females. It was not possible to sex 20 anurans, as they were juveniles. The weight of the animals varied between 38.7 and 758.5 g for *R. diptycha* specimens; and 5.62 to 21.6 g for specimens of *R. granulosa*. The snout-vent length of the animals varied between 6.7 and 20 cm for specimens of *R. diptycha*; and 3.5 to 5.8 cm for specimens of *R. granulosa*. Regarding positivity for parasites, 47 individuals were infected with helminths, around 78.3% of the total sampled, being 40% (n = 24) for *R. granulosa* and 38.3% (n = 23) for *R. diptycha*.

We found seven helminths: *Aplectana membranosa* Schneider, 1866, *Cylindrotaenia americana* Jewell, 1916, *Cosmocerca* sp. Travassos, 1925, Cosmocercidae gen sp. Travassos, 1925, *Oswaldocruzia* sp. Travassos, 1917, *Physaloptera* sp. Rudolphi, 1819, and *Rhabdias* sp. Stiles & Hassall, 1905 (Fig. 3, 4, 5 and 6). Since *A. membranosa*, *Rhabdias* sp. and *Oswaldocruzia* sp. had the highest parasitic rates (Table 1).

The quantity and distribution of parasites varied in the two anuran species. For *R. diptycha* (n = 462): *Oswaldocruzia* sp. (n = 278), *A. membranosa* (n = 109), *Rhabdias* sp. (n = 52), Cosmocercidae gen sp. (n = 17), *Physaloptera* sp. (n = 5) and

Cosmocerca sp. (n = 1); for *R. granulosa* (n = 364): *A. membranosa* (n = 204), Cosmocercidae gen sp. (n = 69), *Oswaldocruzia* sp. (n = 38), *Rhabdias* sp. (n = 35), *Physaloptera* sp. (n = 13) and *C. americana* (n = 5). Overall, the most abundant species were *Oswaldocruzia* sp. and *A. membranosa*, respectively (Figure 7).

Table 1. Hosts examined and their associated parasites. P% = Prevalence; M.A = Mean Abundance; M.I = Mean Intensity; S.I = Site of Infection. S = Stomach; SI = Small Intestine; LI = Large Intestine; LIV = Liver; LUN = Lung.

Hospedeiros	Parasitos	P	M.A	I.M	S.I
Rhinella diptycha	*Oswaldocruzia* sp.	60%	9,2	15,4	S / SI / LI
	Rhabdias sp.	36,6%	1,7	4,7	LUN
	Cosmocercidae gen. sp.	16,6%	0,5	3,4	SI/LI
	Aplectana membranosa	6,6%	3,6	54,5	SI/LI
	Physaloptera sp.	6,6%	0,1	2,5	LIV
	Cosmocerca sp.	3,3%	0,0	1	LI
Rhinella granulosa	*Aplectana membranosa*	33,3%	6,8	20,4	SI / LI
	Rhabdias sp.	23,3%	1,1	5	LUN
	Oswaldocruzia sp.	16,6%	1,2	7,6	SI
	Cosmocercidae gen. sp.	16,6%	2,3	13,8	SI / LI
	Physaloptera sp.	10%	0,4	4,3	S / LIV
	Cylindrotaenia americana	6,6%	0,1	2,5	SI

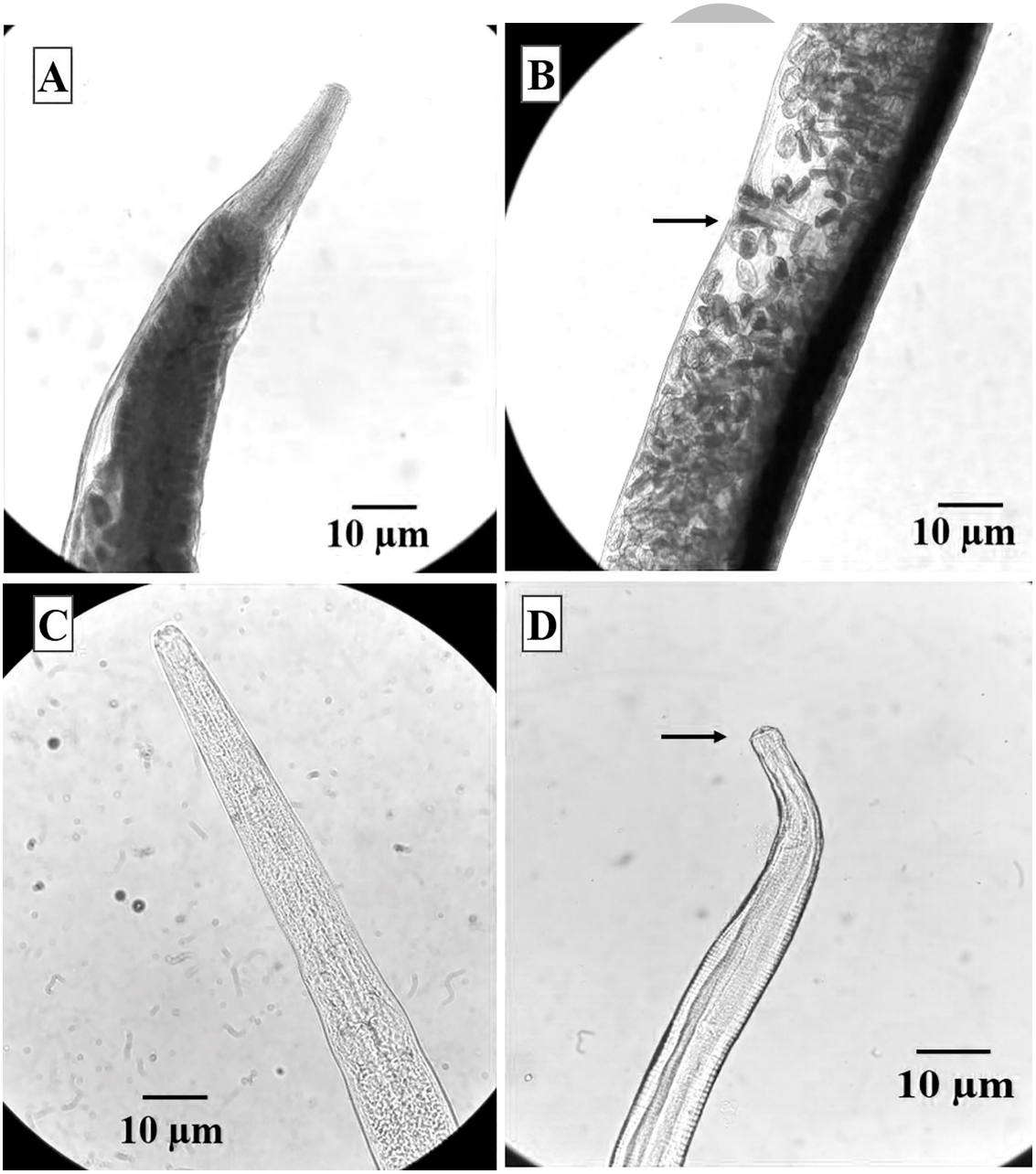


Figure 3. Helminths found in *Rhinella diptycha* and *R. granulosa*, seen by optical

microscopy (10x objective). A) Anterior region of female *Rhabdias* sp. B) Median part of the female *Rhabdias* sp., showing the vulva in detail (arrow). C) Anterior region of Cosmocercidae larvae. D) Anterior region of the larva of *Physaloptera* sp., showing a cephalic collar (arrow).

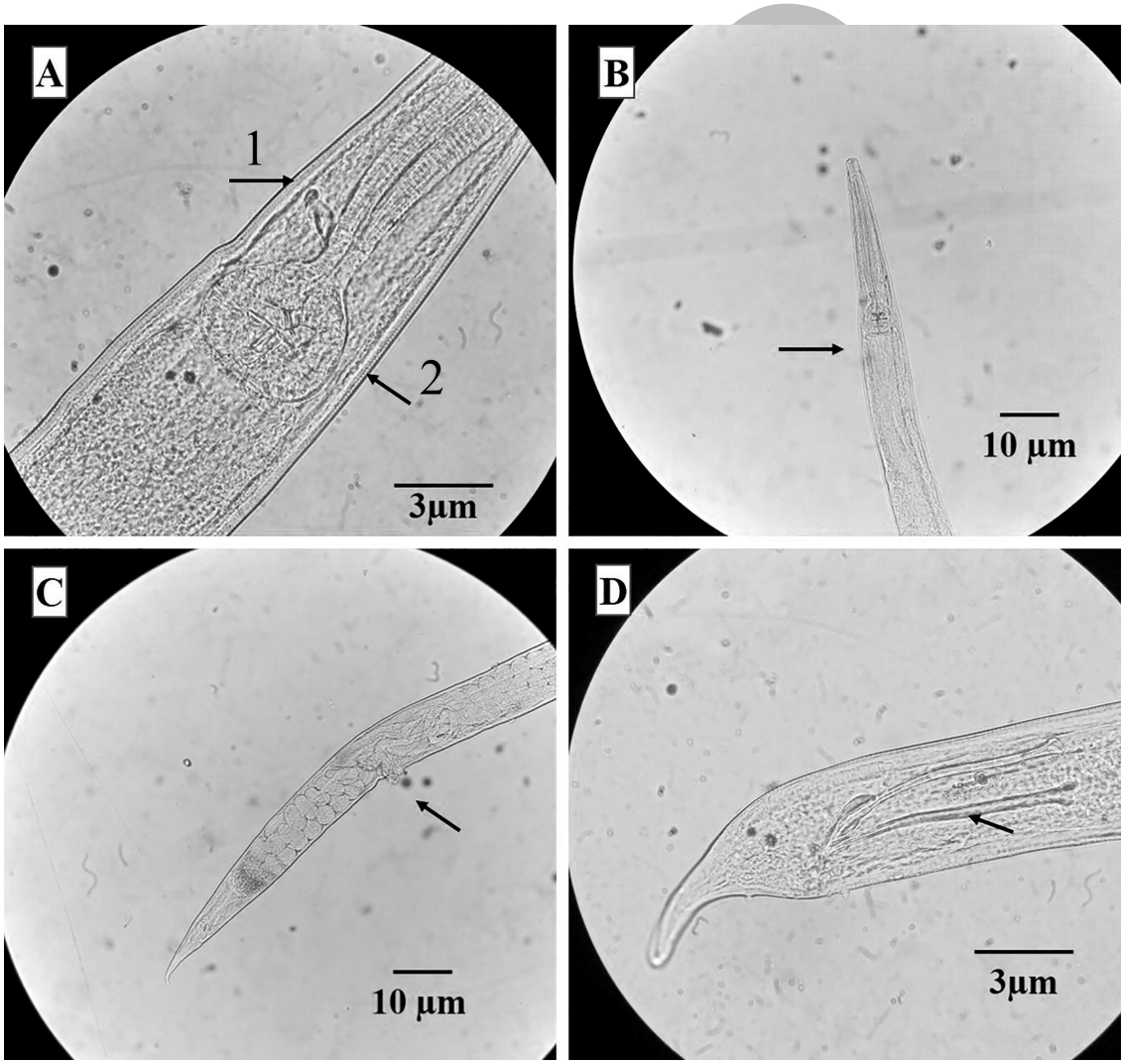


Figure 4. Morphology of the *Aplectana membranosa* parasite found in *Rhinella diptycha* and *R. granulosa*, seen by optical microscopy (A and D – 40x objective; B and C – 10x objective). A) Anterior region of the female, showing the pre-bulbar excretory pore (arrow 1) and bulb posterior to the esophagus (arrow 2) . B) Anterior region

showing intestine immediately in continuation of the medulla oblongata, with the anterior part wider (arrow). C) Vulva just below the middle of the body (arrow). D) Posterior region of the male, showing in detail a pair of approximately equal spicules (arrow).

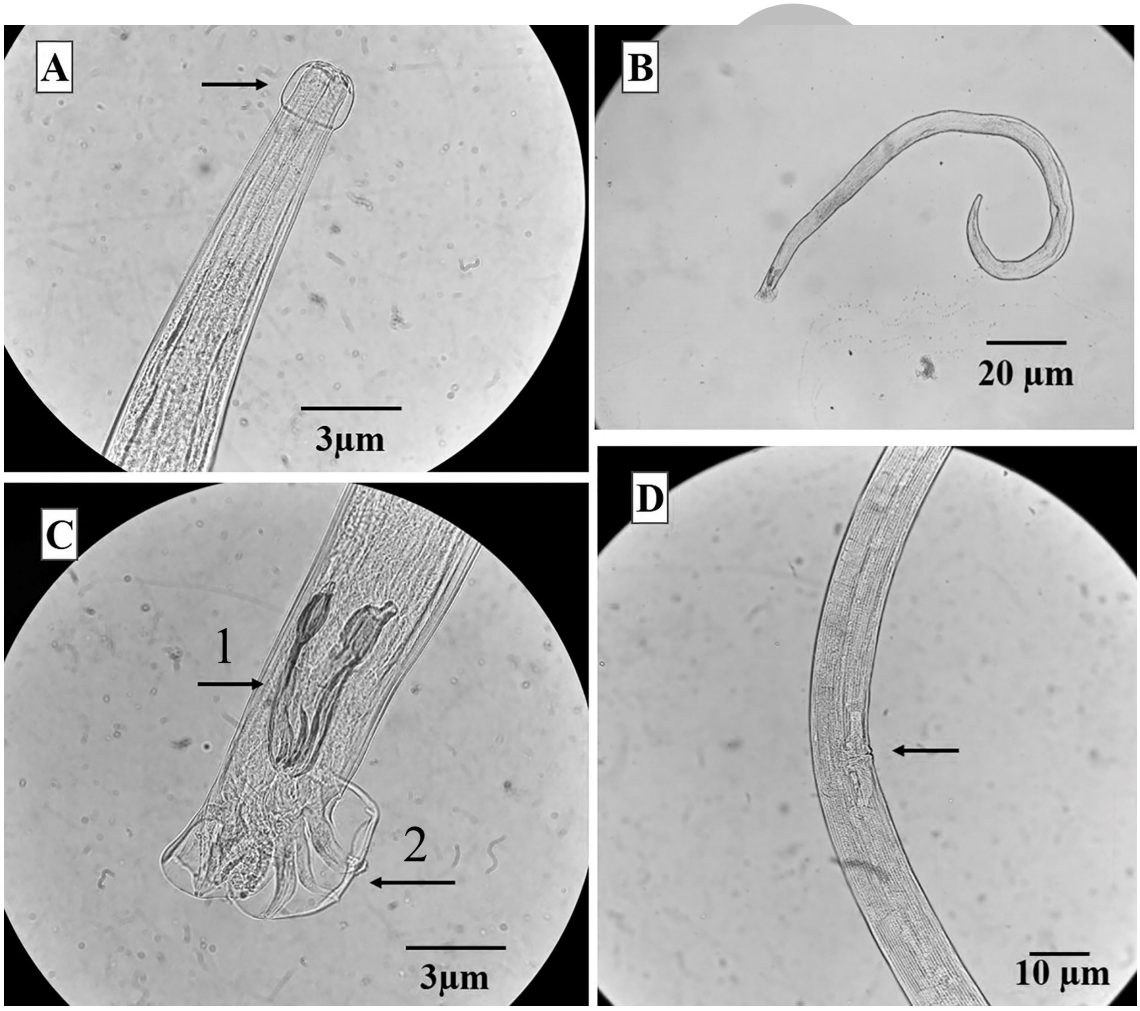


Figure 5. Morphology of the parasite *Oswaldocruzia* sp. found in *Rhinella diptycha* and *R. granulosa*, seen by optical microscopy (A and C – 40x objective; B – 4x objective and D – 10x objective). A) Anterior region of the female, showing cephalic dilation in detail (arrow). B) General view of the male. C) Posterior region of the male, showing a pair of spicules (arrow 1) and the copulatory sac (arrow 2). D) Vulva in the median part

of the female's body (arrow).

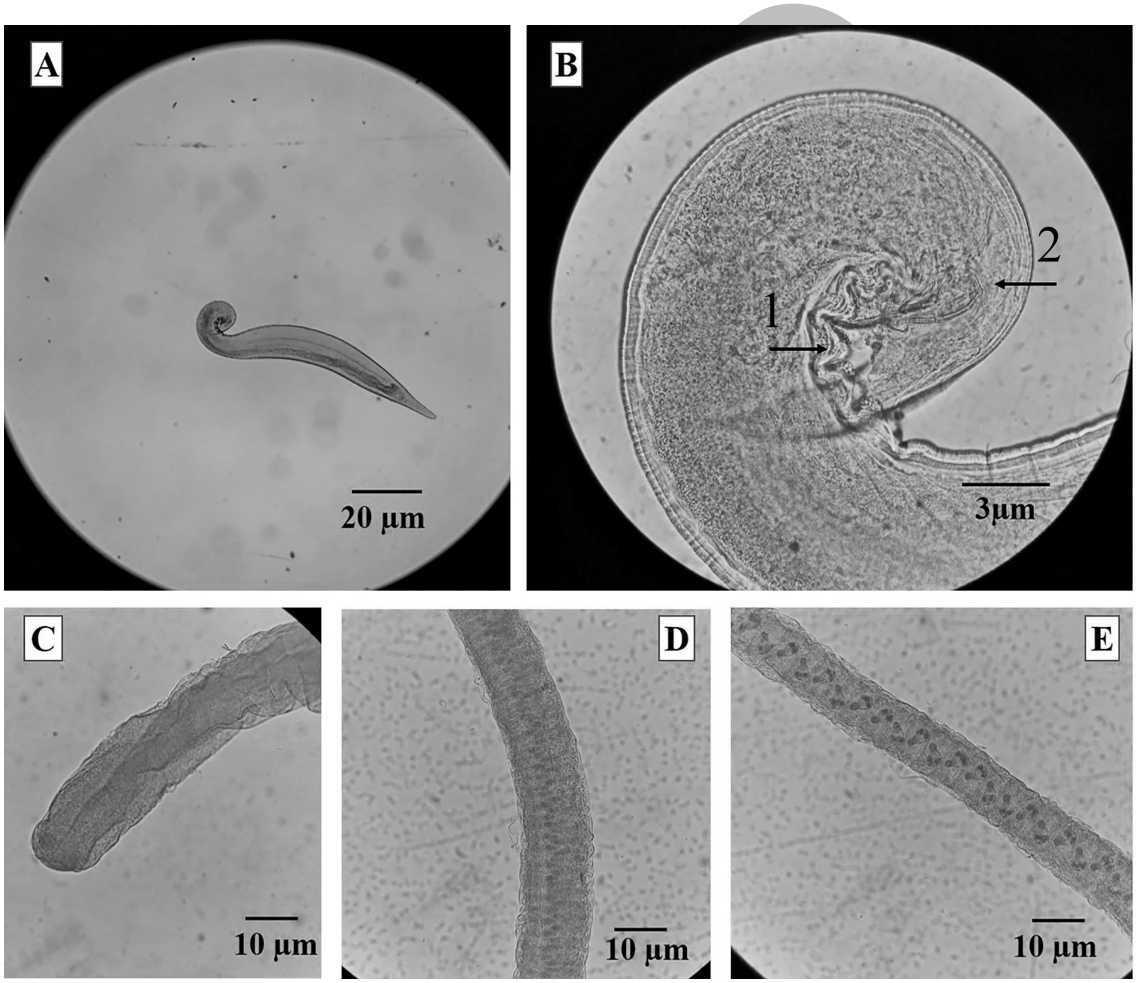
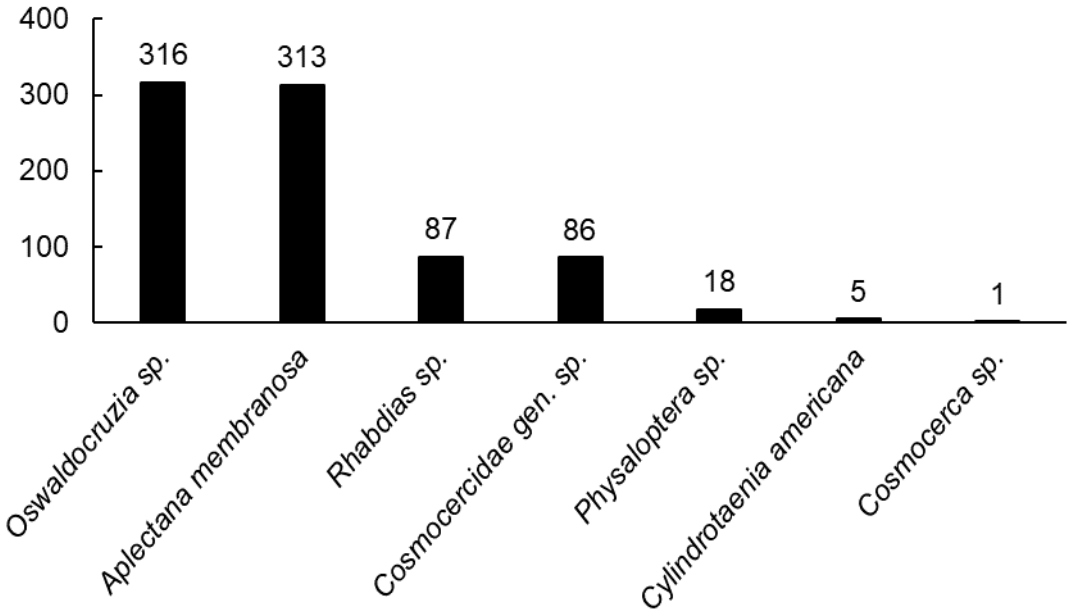


Figure 6. Helminths found in *Rhinella diptycha* and *R. granulosa*, seen by optical microscopy (A – 4x objective; B – 40x objective; C, D and E – 10x objective). A) General view of the male *Cosmocerca* sp. B) Posterior region of male *Cosmocerca* sp. with plectanas, (arrow 1) and rudimentary spicules (arrow 2). C) Anterior region, D) Mature proglottids, E) Pre-pregnancy proglottids (*C. americana*).



DISCUSSION

Figure 7. Total parasite load in *Rhinella diptycha* and *R. granulosa*, from the municipalities of Picos and Timon, states of Piauí and Maranhão, respectively, Northeast Brazil.

Nematodes from the Cosmocercidae, Rhabdiasidae and Strongyloididae families have been recorded parasitizing several species of amphibians in South America, being the most common parasites found in amphibians on this continent (Campião *et al*., 2014). Among the anuran species commonly found in anthropic environments in

Brazil, *Rhinella diptycha* and *R. granulosa* are ecologically considered generalists and opportunists, having a diet composed mainly of arthropods (Pereira-Junior *et al*., 2013; Barbosa *et al*., 2018). Thus, the exclusive infection by nematodes in these species may be related to the life cycle of these hosts and their foraging strategies, that is, the time spent in water or on land. In terrestrial habitats, most nematodes infect anurans by penetrating the skin (e.g., *Rhabdias* spp. and some cosmocercides) or by ingesting eggs (Anderson, 2000; Pinhão *et al*., 2009; Teles *et al*., 2018; Amorim *et al*., 2019).

The Cosmocercidae family is made up of viviparous and oviparous nematodes (Felix-Nascimento *et al*., 2020). They are frequently recorded infecting reptiles and amphibians and have been found parasitizing frogs of the genus *Rhinella* (Campião *et al*., 2014; Teles *et al*., 2018). Despite this, in this study we found only one individual of *Cosmocerca* sp. parasitizing *R. granulosa*. This, however, represents the first record of this parasite for the host *Rhinella granulosa* in the state of Maranhão, northeastern Brazil.

Rhabdias spp. are frequently seen infecting the lungs of anurans (Teles *et al*., 2018). Infections in anurans can occur via direct penetration of the host's skin, however, the life cycle of these parasites varies between a free-living and a parasitic phase, with only females acting as parasites (Anderson, 2000; Amorim *et al*., 2019). This explains the fact that we found only females parasitizing the two anuran species in this study. Some species of the genus *Rhinella* (e.g., *R. schineideri*, *R. crucifer*, *R. icterica*, *R. marina*, *R. ornata*) have already been reported to be parasitized by *Rhabdias* sp. (Graça *et al*., 2017), including *R. diptycha* (Benício *et al*., 2022).

The genus *Physaloptera* has nematodes with a heteroxenous life cycle, that is, they are parasites that require one or more intermediate hosts. Specimens of the genus *Physaloptera* are frequently found in the stomachs of vertebrates such as reptiles, mammals, birds, fish and amphibians, including frogs of the family Bufonidae, where this parasite is commonly seen in the larval stage (Anderson, 2000; Campião *et*

347 *al*., 2014; Teles *et al*., 2018; Amorim *et al*., 2019). In this study, we found larvae of
348 *Physaloptera* sp. encysted in the liver membrane of both frog species and in the
349 stomach of *R. granulosa*. This infection can be caused by the ingestion of larvae in the
350 intermediate host (insect) (Vieira *et al*., 2021).

351 *Aplectana membranosa* is known to only infect anurans. This parasite occurs in
352 the large intestine and rectum of its hosts (Teles *et al*., 2018). Vieira *et al*. (2021) found
353 a high prevalence of *A. membranosa* parasitizing the small intestine of *Leptodactylus*
354 *macrosternum*. We found specimens of this nematode parasitizing both species of
355 frogs, both in the small and large intestines. This fact can be justified by the high
356 parasitic load found in animals, causing helminths to migrate to other sites of infection.

357 Nematodes of the genus *Oswaldocruzia* are known to infect anurans (Teles *et al*.,
358 2018). During the reproductive period of these anurans there is a greater intensity of
359 infection by *Oswaldocruzia* sp. This parasite has a direct life cycle with active larvae
360 penetrating the host's skin. It is possible that during the rainy months, when the soil is
361 very moist, these larvae have lower desiccation rates, higher survival rates and,
362 consequently, greater ease in infecting their hosts (Madelaire *et al*., 2012).
363 *Oswaldocruzia* sp. has already been recorded parasitizing *R. diptycha* and *R.*
364 *granulosa* (Teles *et al*., 2018; Benício *et al*., 2022).

365 *Cylindrotaenia americana* belongs to the Nematotaennidae family and was found
366 in our study parasitizing *R. granulosa*. This family is made up of parasites that infect
367 the small intestine of amphibians and reptiles and are transmitted by ingestion of
368 pregnant proglottids without the need for an intermediate host. Self-infection is
369 common, and it is possible to find hosts with a large parasite load (Melo *et al*., 2011),
370 although this was not observed in our study. *C. americana* has a direct life cycle and is
371 widely reported to infect anurans from the Bufonidae, Hylidae and Leptodactylidae
372 families (Martins *et al*., 2018), including *R. diptycha* and *R. granulosa* (Madelaire *et al*.,
373 2020; Benício *et al*., 2022).

374 *Cylindrotaenia* sp. has already been recorded parasitizing species of the genus
375 *Rhinella* (e.g., *R. fernandezae*, *R. icterica*, *R. schneideri*) (Justo *et al*., 2017; Martins *et*
376 *al*., 2018), including *R. diptycha* (Benício *et al*., 2022). However, this is the first record
377 of *Cylindrotaenia* sp. parasitizing *Rhinella granulosa* in the state of Maranhão,
378 northeastern Brazil.

379 The richness and species composition of parasites recorded in this study varied
380 little between different populations of the two species (Teles *et al*., 2018; Amorim *et al*.,
381 2019; Madelaire *et al*., 2020; Neta *et al*., 2020; Benício *et al*., 2022). For example, for
382 both *R. diptycha* and *R. granulosa*, species richness ranged from six to seven.
383 Regarding species composition, there was a large number of shared species, with
384 some taxa occurring in almost all populations studied. The most commonly shared
385 parasites were: *Aplectana membranosa*, *Cylindrotaenia americana, Physaloptera* sp.
386 and *Rhabdias* sp. This is only the second article in the literature on helminths
387 parasitizing *R. diptycha* for the state of Piauí (i.e., Benício *et al*., 2022; this study) and
388 the first for both species in the state of Maranhão.

389 In this study, the high prevalence of parasitized hosts (~80%) and the high
390 abundance of parasites recorded (n = 826) in just two species draw attention to the
391 health of these anuran populations, possible associated diseases and inherent
392 declines, as well as the environmental quality of the ecosystems where these species
393 were collected (in general, highly anthropized locations). Several studies have
394 demonstrated how anthropogenic activities, pathogens, agrochemicals and changes in
395 the landscape, for example, can enhance the effect of parasitism (such as coinfections
396 and increased diseases) on amphibian populations around the world (e.g., Carrasco *et*
397 *al*., 2021; Herczeg *et al*., 2021; Jacinto-Maldonado *et al*., 2022; Oliveira *et al*., 2024).
398 Thus, studies like this – which map the diversity and distribution of parasites in
399 amphibians, can help us better understand the complex parasite-host relationship, as
400 well as propose more effective measures for the conservation of amphibian species

and local ecosystems.

Our findings contribute to expanding knowledge about the parasitic fauna of bufonids in the Northeast region of Brazil. We found a high prevalence (78%) and abundance (n = 826) of helminths parasitizing the two frog species (*R. diptycha* and *R. granulosa*) in the states of Piauí and Maranhão, including new records of helminths. However, more studies are needed to understand the mechanisms associated with this parasite-host relationship, since these anurans are considered to be reservoirs of a great diversity of helminths, and their relationships are not yet fully understood.

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