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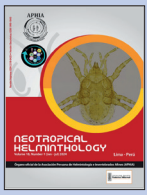

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

# FIRST RECORD OF PARASITISM BY *HEPATOZOON* MILLER, 1908 (APICOMPLEXA: HEPATOZOIDAE) IN ANURANS FROM THE CAATINGA, BRAZIL

# PRIMER REGISTRO DE PARASITISMO POR *HEPATOZOON* MILLER, 1908 (APICOMPLEXA: HEPATOZOIDAE) EN ANUROS DE LA CAATINGA, BRASIL

# PRIMEIRO REGISTRO DE PARASITISMO POR *HEPATOZOON* MILLER, 1908 (APICOMPLEXA: HEPATOZOIDAE) EM ANUROS DE CAATINGA, BRASIL

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## ABSTRACT

This study aimed to register for the first time parasitism by *Hepatozoon* Miller, 1908 in two species of bufonid anurans in areas of the Caatinga in Brazil, and to present morphometric analysis on the parasites and parasitized erythrocytes. During helminthological studies on anurans from the Brazilian Caatinga in 2019, blood smears of three specimens of *Rhinella diptycha* (Cope, 1862) and three of *Rhinella granulosa* (Spix, 1824) in the municipality of Petrolina, state of Pernambuco, Brazil were analyzed. To make blood smears, blood samples were collected intracardiacally or through puncturing the ventral abdominal vein. The smears were analyzed under a light microscope at 1000x magnification. Morphometric dimensions of host cells and parasites were measured. The only parasite forms encountered in the blood of the two anuran species were intraerythrocytic gametocytes of *Hepatozoon* spp., only parasitizing erythrocytes, which had a hypertrophied appearance. Two distinct morphotypes of *Hepatozoon* were found: one in each host species and both of them different from morphotypes previously described and registered in Brazil. Therefore, the present study constitutes the first report of *Hepatozoon* sp. in anurans in the Caatinga biome and also forms the first register of parasites of this genus in *R. granulosa* in this country.


**Keywords:** Amphibians – Bufonidae – Haemogregarines – Hemoparasites.


## RESUMO

O estudo teve o objetivo registrar pela primeira vez o parasitismo por *Hepatozoon* Miller, 1908 em duas espécies de anuros bufonídeos em áreas de Caatinga no Brasil, assim como a morfometria dos parasitos e eritrócitos parasitados. Durante estudos helmintológicos em anuros da Caatinga brasileira em 2019 foram analisados esfregaços sanguíneos de 3 espécimes de *Rhinella diptycha* (Cope, 1862) e três *Rhinella granulosa* (Spix, 1824) no município de Petrolina, estado de Pernambuco, Brasil. Para montagem desses esfregaços sanguíneos, foram utilizadas amostras de sangue coletado via intracardíaca ou por punção da veia abdominal ventral. A análise dos esfregaços foi realizada sob microscópio de luz com a magnificância de 1000x. Células hospedeiras e parasitos tiveram mensuradas dimensões morfométricas. As únicas formas parasitárias encontradas no sangue das duas espécies de anuros foram gametócitos intraeritrocíticos de *Hepatozoon* spp., parasitando somente eritrócitos que se apresentaram hipertrofiados. Foram encontrados dois morfotipos distintos de *Hepatozoon*, um em cada espécie de hospedeiros, todos diferentes dos morfotipos já descritos e registrados no país. Portanto, o presente estudo se constitui no primeiro relato de *Hepatozoon* sp. em anuros no bioma da Caatinga, além de também ser o primeiro registro de parasitos desse gênero em *R. granulosa* no país.


**Palavras-chave**: Anfibios – Bufonidae – hemogregarina – hemoparasitos


## RESUMEN

El estudio tuvo como objetivo registrar por primera vez el parasitismo por *Hepatozoon* Miller, 1908 en dos especies de anuros bufonídeos en áreas de Caatinga en Brasil, así como la morfometría de los parásitos y los eritrocitos parasitados. Durante estudios helmintológicos en anuros de la Caatinga brasileña en 2019, se analizaron extendidos sanguíneos de 3 especímenes de *Rhinella diptycha* (Cope, 1862) y *Rhinella granulosa* (Spix, 1824) en el municipio de Petrolina, estado de Pernambuco, Brasil. Para la preparación de los extentidos sanguíneos, se utilizaron muestras de sangre recolectadas mediante punción intracardiaca o de la vena abdominal ventral. El análisis de los extentidos se realizó bajo un microscopio de luz con una magnificación de 1000x. Se midieron las dimensiones morfométricas de las células hospedadoras y los parásitos. Las únicas formas parasitarias encontradas en la sangre de las dos especies de anuros fueron gametocitos intraeritrocíticos de *Hepatozoon* spp., que parasitan solo a eritrocitos que muestran hipertrofia. Se encontraron dos morfotipos distintos de *Hepatozoon*, uno en cada especie de hospedador, todos diferentes de los morfotipos ya descritos y registrados en el país. Por lo tanto, el presente estudio constituye el primer informe de *Hepatozoon* sp. en anuros en el bioma de la Caatinga, además de ser también el primer registro de parásitos de este género en *R. granulosa* en el país.


**Palabras clave**: Anfibios – Bufonidae – hemogregarina – hemoparásitos

## INTRODUCTION

The phylum Apicomplexa comprises lineages of obligate parasites of great diversity, with over 6,000 described species and possibly thousands yet to be discovered. Among these, hemogregarines are the most frequent hemoparasites in vertebrates (Votypka *et al.,* 2017), notably the genus *Hepatozoon* Miller, 1908 (Apicomplexa: Hepatozoidae), which includes species parasitizing animals ranging from anurans to mammals (Smith, 1996). Currently, 48 species of *Hepatozoon* are known from anurans distributed worldwide (Netherlands *et al.*, 2018; Úngari *et al.*, 2021).

Brazil has the highest richness of anurans in the world, totaling approximately 1,180 species, distributed across all biomes of the country (AMPHIBIAWEB, 2024; Frost, 2024). However, only four species of hepatozoids parasitizing anurans are known, mostly occurring in hosts of the family Leptodactylidae (see Costa *et al.*, 1973; Úngari *et al.,* 2021). The hepatozoids parasitizing Brazilian anurans are restricted to six host species, located in areas of the Cerrado and Pantanal biomes in the central-western region (Costa *et al.,* 1973; Leal *et al.,* 2015; Úngari *et al.,* 2021) and in the Atlantic Forest in the southeastern region of the country (Ferreira *et al.,* 2020). However, there are also records of unidentified species of *Hepatozoon* mostly parasitizing leptodactylids, with two records of parasitism in anurans of the family Bufonidae (Ferreira *et al.*, 2020; Úngari *et al.,* 2022).

The scarce records of *Hepatozoon* demonstrate the lack of knowledge about parasitism by *Hepatozoon* in Brazilian anurans, especially in areas within the morphoclimatic domain of the Caatinga. Thus, the aim of this study was to register for the first time parasitism by *Hepatozoon* in two species of bufonid anurans in Caatinga areas in Brazil, along with the morphometry of the parasites and parasitized erythrocytes.

## MATERIAL AND METHODS

In January and February 2019, three individuals of *Rhinella diptycha* (Cope, 1862) and three of *R. granulosa* (Spix, 1824) were collected from an anthropized fragment of shrubby Caatinga in the Senador Nilo Coelho Irrigation Project - Nucleus 01 (9°20'4.68" S; 40°35'11.25" W), in the municipality of Petrolina, state of Pernambuco, Brazil. These anurans were collected through an active search, using a dip net and talc-free nitrile gloves, during both the nocturnal and the diurnal period, near intermittent and perennial water bodies. After collection, these animals were s0ent to the Animal Physiology Laboratory of the Universidade Federal do Vale do São Franciso (UNIVASF), Brazil and at the end of the studies, they were deposited in the Herpetological Collection of the Museum of Caatinga Fauna (Coleção Herpetológica do Museu de Fauna da Caatinga) under the numbers MFCH 5288-5292 and 5295), which is located in the Conservation and Management Center for Caatinga Fauna (Centro de Conservação e Manejo de Fauna da Caatinga, CEMAFAUNA), at UNIVASF.

To prepare blood smears, samples of blood were collected via intracardiac puncture or from the ventral abdominal vein in animals that had previously been euthanized with an overdose of 10% benzocaine applied topically. After preparation, the smears were air-dried at room temperature, fixed with absolute methanol for 10 minutes, and stained with 10% Giemsa for 10 minutes. Smear analysis was conducted under a light microscope at a magnification of 1000x, with an attached photographic camera. The prevalence of parasitism was estimated as the percentage of infected individuals for each host species, and the mean intensity of parasitism was calculated based on the number of parasites found per 2,000 erythrocytes (Garrido & Pérez-Mellado, 2013). Morphometric analysis was performed using the ToupView© software, from measurements that included the length, width and area of the parasite, the length, width and area of the parasite nucleus, the length and width of parasitized erythrocytes, and the length and width of their nuclei, with data expressed as the mean and standard deviation (Lima *et al.*, 2021) and range of morphometric variation.

**Ethical approval:** Research permit was provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)/Sistema de Autorização e Informação em Biodiversidade (SISBIO) (Processes n. 29558-1). The collection of specimens of *R. diptycha* and *R. granulosa* was and approved by the ethics committee on the use of animals of the Universidade Federal do Vale do São Francisco (Processes n. 0001/221018).

## RESULTS

The only parasitic forms found in the blood of both anuran species were intraerythrocytic gametocytes of *Hepatozoon* spp., parasitizing only hypertrophied erythrocytes. Three distinct morphotypes of *Hepatozoon* were found, one in each host species (Table 1, Fig. 1). In *R. diptycha*, morphotypes of mature and immature gametocytes were

found, with a prevalence of 100% and a mean intensity of 3.3 ± 3.6, ranging from 2 to 9 parasitized erythrocytes per 2,000 analyzed. In *R. granulosa*, distinct immature gametocytes were found, compared with those of *R. diptycha*, with a prevalence of 66% and mean intensity of 2.0 ± 2.1, ranging from 1 to 4 parasitized erythrocytes per 2,000 analyzed.

The mature gametocytes of *Hepatozoon* sp. from *R. diptycha* were found to have a cylindrical body with curved ends in a C or O shape, with a mean length of 9.3 ± 1.1 µm and a mean width of 4.7 ± 0.9 µm (n = 11) (Fig. 1A-D). In some of these mature gametocytes analysed, it was possible to observe the cell body bent inside the parasitophorous vacuole (Figs. 1A, D). The nucleus had the same cellular body shape, with a size of 6.4 ± 0.8 µm × 2 ± 0.5 µm (Fig 1A). The gametocytes displaced the erythrocyte nucleus to the periphery of the cell (Figs. 1A-D). The parasitized erythrocytes had a size of 19.0 ± 1.6 µm × 13.6 ± 0.6 µm, and their nuclei were 5.4 ± 0.7 µm × 6.3 ± 0.5 µm.

The immature gametocytes had a more robust shape, wider cell body, granular formations in the cytoplasm and very subtle curvatures at the ends (Figs. 1E, F), measuring 8.9 ± 0.8 µm × 4 ± 0.4 µm. The nuclei had a square shape with highly condensed chromatin (Fig. 1E), and measured 3.9 ± 0.4 µm × 3.8 ± 1.1 µm, with less basophilic staining compared with the nucleus of the parasitized cell (Fig. 1F). Additionally, the immature gamont also altered the morphology of the erythrocyte, measuring 16.7 ± 0.1 µm × 13.4 ± 1.7 µm, and pushing the nucleus of 5.1 ± 0.2 µm × 6.1 ± 0.9 µm to the periphery of the cell (Fig. 1E, F).

In *R. granulosa*, the immature gametocytes of *Hepatozoon* sp. were found to have a cylindrical shape with subtle curvatures at the ends, taking on a C shape, with dimensions of 9.5 ± 0.7 × 4 ± 0.4 µm (n = 8) (Fig. 1G). Some gametocytes were observed inside parasitophorous vacuoles (Figs. 1G-L). The parasite nucleus (4.0 ± 1.0 × 3.7 ± 0.8 µm) presented basophilic staining but with lower intensity of staining than the erythrocyte nucleus (Fig. 1L). Additionally, the chromatin showed various levels of compaction (Figs. 1G-L). The gametocytes were capable of displacing the erythrocyte nucleus to the periphery of the cell (Fig. 1H-J). The parasitized erythrocytes measured 18.2 ± 1.2 × 12.4 ± 0.8 µm, and their nuclei measured 5.4 ± 1.0 × 5.5 ± 0.9 µm.

## DISCUSSION

In general, studies on the biodiversity of *Hepatozoon* species in Brazilian anurans can be considered scarce. Information regarding this genus parasitizing this group of hosts is concentrated in a few published studies, and most of them reported unidentified species (see Table 1). In Brazil, over the past two decades, with the introduction of molecular tools combined with morphological studies on blood and tissue stages, the panorama of *Hepatozoon* biodiversity in anurans has been changing (see Ferreira *et al.,* 2020; Leal *et al.,* 2015; Úngari *et al.,* 2021, 2022). For many decades, the parasitism of Brazilian anurans was associated solely with *H. leptodactyli* (Lesage, 1908) (Carini, 1908; Pinto, 1925; Cunha & Muniz, 1927; Pessôa, 1970; Costa *et al*., 1973) or with unidentified species of this genus (Table 1). However, recently, three new species of *Hepatozoon* have been discovered in Brazilian anurans, based on morphological studies of blood and tissue stages corroborated by molecular analyses (Úngari *et al*., 2021). This highlights how underestimated the biodiversity of *Hepatozoon* and other hemoparasites in Brazilian anurans is.

The majority of records and descriptions of *Hepatozoon* in Brazil have been made from parasites found exclusively in five species of anurans belonging to the genus *Leptodactylus* Fitzinger, 1826 (Leptodactylidae) in areas of the Cerrado, Pantanal and Atlantic Forest biomes (Carini, 1908; Pinto, 1925; Cunha & Muniz, 1927; Costa *et al.,* 1973; Leal *et al.,* 2015; Ferreira *et al.,* 2020; Úngari *et al.,* 2021, 2022) (Table 1). The only two records of these parasites in Bufonidae in this country are in *R. diptycha* from the Atlantic Forest (Ferreira *et al*., 2020) and in *R. mirandaribeiroi* (Gallardo, 1965) parasitizing by *H. latrensis* in the Pantanal (Úngari *et al.,* 2022) (Table 2).

The mature and immature gametocytes of *Hepatozoon* sp. parasitizing *R. diptycha* were compared in terms of their morphology and morphometry with other species previously described in Brazil. Unlike *Hepatozoon* sp. from *R. diptycha* in the present study, *H. leptodactyli* possesses intraerythrocytic forms apparently without the parasitophorous vacuole, and the cell body does not appear to be bent, but has curvature at both ends (Costa *et al*., 1973). The mature erythrocytes of *H. longinucleus* Úngari, Netherlands, Silva & O'Dwyer, 2021, differ morphometrically from *Hepatozoon* sp. in *R. diptycha* through having thinner elongated cell bodies and nuclei (Table 1), in addition to having a parasitophorous vacuole that takes on the shape of the cell body (Úngari *et al*., 2021). The mature gametocytes of *H. formosus* Úngari, Netherlands, Silva & O'Dwyer, 2021, have

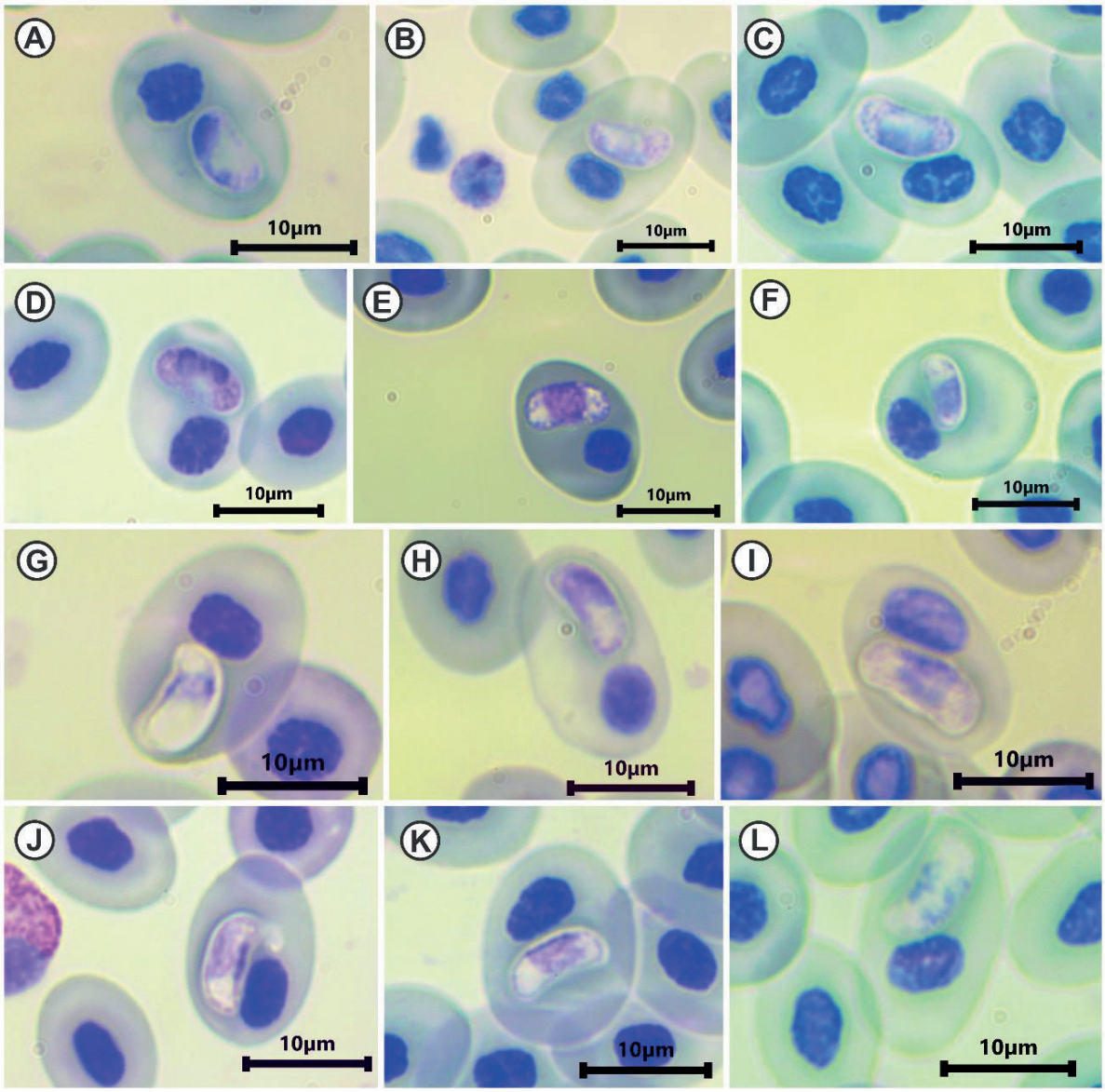


**Figure 1**. Intraerythrocytic gametocytes of *Hepatozoon* sp. in the blood smears. *Rhinella diptycha* (A-F). A-D. Mature gamonts; E-F. Immature gamonts with vacuolization in the parasitic body. *Rhinella granulosa*. G-L. Immature gamonts.

unequal ends of the cell body, with one end thicker than the other, thus taking on a cone shape, which also shows subtle curvature (see Úngari *et al.,* 2021), while the gametocytes of *Hepatozoon* sp. from *R. diptycha* have a curved cell body, larger than the vacuole, and may have a C or O shape (Figs. 1A-D). *Hepatozoon latrensis* Úngari, Netherlands, Silva & O'Dwyer, 2021, possess mature gametocytes without the curvature of the cell body inside the parasitophorous vacuole (see Úngari *et al.,* 2021).

Regarding the immature gametocytes in *Hepatozoon* sp. from *R. diptycha*, these gametocytes are morphologically different from the mature gametocytes (Figs. 1E-F). In the immature stage, the parasite nucleus is centrally positioned, dividing the cytoplasm into two seemingly equal parts, and it possesses vacuolization in its cytoplasm (Fig. 1E). Among Brazilian species, there are morphological descriptions of immature stages of two species: *H. latrensis* and *H. formosus* (Úngari *et al.,* 2021, 2022). The morphotype that parasitizes *R. diptycha* differs from the immature morphotypes of *H. latrensis* and *H. formosus*, as these have immature gametocytes similar to the mature ones but with morphometric differences (Table 1) (Úngari *et al.,* 2021, 2022). In these two species, in the immature stages, the cytoplasmic area of the parasite is small and almost imperceptible, with

**Table 1.** Morphometrics values of intraerythrocytic mature and immature gametocytes of *Hepatozoon* spp. in anurans from Brazil.

| *Hepatozoon* species | Parasite's developmental stage | Morphometrics | | | | | | Host species | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | Length (µm) | Width (µm) | Parasite area (µm²) | Length of nucleus (µm) | Width of nucleus (µm) | Nucleus area (µm²) | | |
| *H. formosus* | Mature | 11.93 ± 0.61 (9.82–13.67) | 4.18 ± 0.24 (3.93–4.67) | 40.20 ± 3.01 (37.76–44) | 5.77 ± 0.23 (5.57–5.97) | 3.91 ± 0.23 (3.33–3.93) | 17.29 ± 1.29 (15.26–18.77) | *Leptodactylus labyrinthicus* | Úngari *et al.* (2021) |
| | Immature | 9.33 ± 0.16 (9.17–9.88) | 3.43 ± 0.11 (3.15–3.83) | 21.27 ± 0.74 (20.33–22.38) | 5.4±1.35 (4.79–6.07) | 3.53 ± 0.56 (3.15–3.83) | 16.6 ± 0.19 (14.15–17.16) | *L. labyrinthicus* | Úngari *et al.* (2021) |
| *H. longinucleus* | Mature | 12.58 ± 0.62 (11.5–13.27) | 4.17 ± 0.41 (2.4–4.36) | 42.24 ± 5.94 (31.37–43.35) | 9.21 ± 0.32 (7.5–10.74) | 2.18 ± 0.54 (1.71–2.94) | 19.16 ± 1.04 (16.78–20.98) | *L. labyrinthicus* | Úngari *et al.* (2021) |
| *H. latrensis* | Mature | 9.88 ± 0.56 (9.06–10.74) | 4.35 ± 0.41 (3.27–5.02) | 37.46 ± 3 (34.91–43.95) | 4.17 ± 0.57 (3.13–5.02) | 3.37 ± 0.6 (2.67–4.02) | 8.87 ± 1.54 (7.02–9.13) | *L. latrans* | Úngari *et al.* (2021) |
| | Mature | 12.28 ± 0.7 | 4 ± 0.1 | 52.37 ± 0.7 | 5.65 ± 0.33 | 4 ± 0.1 | 15.05 ± 0.32 | *Rhinella mirandaribeiroi* | Úngari *et al.* (2022) |
| | Mature | 13.1 ± 0.27 | 4.39 ± 0.34 | 52.42 ± 3.25 | 5.84 ± 0.5 | 3.60 ± 0.35 | 16.30 ± 1.65 | *L. labyrinthicus* | Úngari *et al.* (2022) |
| | Mature | 11.63 ± 0.81 | 4.63 ± 0.49 | 45.97 ± 4.95 | 3.9 ± 0.54 | 3.48 ± 0.93 | 10.19 ± 1.92 | *Leptodactylus* sp. | Úngari *et al.* (2022) |
| | Mature | 13.09 ± 0.75 | 4.12 ± 0.39 | 47.55 ± 4.5 | 5.87 ± 0.84 | 5.92 ± 9.46 | 15.84 ± 3.68 | *L. labyrinthicus* | Úngari *et al.* (2022) |
| | Immature | 9.05 ± 0.8 | 2.86 ± 0.33 | 16.92 ± 2.9 | 4.12 ± 0.37 | 2.83 ± 0.31 | 8.63 ± 0.60 | *L. labyrinthicus* | Úngari *et al.* (2022) |
| *H. leptodactyli* | Unknow | 12–16 | 4–6 | - | - | - | - | *L. latrans* | Carini (1908) |
| | Mature | 5.1–21 | 2.3–8.3 | - | 4.6 | 3 | - | *L. latrans*, *L. pentadactylus* | Costa *et al.* (1973) |

(Continued Table 1)

(Continued Table 1)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Hepatozoon* sp. | Mature | 9.3 ± 1.1 (8.2–10.4) | 4.7 ± 0.9 (2.1–5.6) | 37.4 ± 8.9 (16.8–48.3) | 6.4 ± 0.8 (5.3–7.3) | 2.0 ± 0.5 (0.9–2.5) | 11.1 ± 1.6 (8.1–13.4) | *R. diptycha* | **Current study** |
| | Immature | 8.9 ± 0.8 (8.3–9.4) | 4 ± 0.4 (3.7–4.3) | 32.3 ± 8.8 (26.1–38.5) | 3.9 ± 0.4 (3.6–4.2) | 3.8 ± 1.1 (3–4.6) | 12.8 ± 4.4 (9.7–15.9) | *R. diptycha* | **Current study** |
| | Immature | 9.5 ± 0.7 (8.7–10.8) | 4 ± 0.4 (3.2–4.5) | 39 ± 5.4 (30.4–45.4) | 4 ± 1 (2–5.3) | 3.7 ± 0.8 (2.2–4.6) | 14.1 ± 3.8 (6.8–19.4) | *R. granulosa* | **Current study** |
| | Unknow | 9.7 ± 2.7 (4.9–13.9) | 4.5 ± 0.7 (3.2–5.7) | 35.05 ± 10 (20.7–50.0) | 4.7 ± 1.4 (2.1–8.6) | 4.2 ± 0.75 (2.7–5.4) | 16.5 ± 5.3 (6.7–27.8) | *L. macrosternum* | Leal *et al.* (2015) |
| | Unknow | 13.95 ± 3.3 (7.1–17.2) | 4.6 ± 0.55 (4–5.8) | 43.8 ± 6.2 (30.15–49.2) | 4.7 ± 1.1 (2.3–6) | 4.2 ± 0.5 (3.65–5.3) | 15.3 ± 3.9 (9.5–21.05) | *L. podicipinus* | Leal *et al.* (2015) |

the difference that in *H. formosus*, the nuclei of immature gametocytes are larger in relation to the total area of the parasite (Table 1) (Úngari *et al.,* 2021, 2022).

In *Hepatozoon* sp. parasites of *R. granulosa* from the present study, only immature gametocytes were found. These were characterized by filling the entire parasitophorous vacuole, with the nucleus following the shape of the parasite, but without marking the cell body (Fig. 1G-L). As mentioned earlier, in Brazil, only *H. latrensis* and *H. formosus* have been shown to have immature gametocytes (see Úngari et al., 2021, 2022). In some immature gametocytes in *R. granulosa*, the nucleus appeared segmented (Fig. 1L), and was also characterized by having a C shape (Fig. 1G-L). This was more evident than in the immature stages of *H. latrensis* and *H. formosus* (see Úngari *et al.*, 2021, 2022). Furthermore, regarding the nuclei, unlike the immature gametocytes of *R. granulosa* (Figs. 1G-L), nuclear segmentation was not observed in the immature gametocytes of *R. diptycha* from the present study. Moreover, the vacuolization observed in the parasitic body in *R. diptycha* (Fig. 1E) was not seen in *R. granulosa*. Additionally, these two species had morphometric differences, compared with *Hepatozoon* sp. from this host species (Table 1).

The morphotypes of *Hepatozoon* found in the anurans of this study each had a prevalence of at least 30%. However, due to the low host sample size (three individuals of each species), we were unable to comparatively analyse the ecological data of *Hepatozoon* sp. parasitism from this study in relation to data on other species of this genus parasitizing anurans in Brazil. Nevertheless, the fact that previously unknown morphotypes of *Hepatozoon* were found in anurans in Caatinga areas leads us to believe that there is still potential for studying entirely unknown biodiversity of *Hepatozoon* species in anurans from this region. Therefore, this study constitutes the first report of *Hepatozoon* sp. in anurans in the Caatinga biome, as well as providing the first record of parasites of this genus in *R. granulosa* in Brazil.

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**Table 2.** *Hepatozoon* spp. in anurans from Brazil, locations and Biomes.

| ***Hepatozoon* species** | **Host species** | **Localities** | **Biomes** | **References** |
|---|---|---|---|---|
| ***H. formosus*** | *Leptodactylus labyrinthicus* | Nova Xavantina, Mato Grosso | Pantanal | Úngari *et al.* (2021) |
| ***H. latrensis*** | *L. latrans* | Nova Xavantina, Mato Grosso | Pantanal | Úngari *et al.* (2021) |
| | *L. labyrinthicus* | Araguaiana and Cocalinho, Mato Grosso | Cerrado | Úngari *et al.* (2022) |
| | *Rhinella mirandaribeiroi* | Cocalinho, Mato Grosso | Cerrado | Úngari *et al.* (2022) |
| | *Leptodactylus* sp. | Cocalinho, Mato Grosso | Cerrado | Úngari *et al.* (2022) |
| ***H. longinucleus*** | *L. labyrinthicus* | Nova Xavantina, Mato Grosso | Pantanal | Úngari *et al.* (2021) |
| ***H. leptodactyli*** | *L. latrans* | São Paulo, São Paulo | Atlantic forest | Carini (1908) |
| | | Rio de Janeiro | Atlantic forest | Cunha & Muniz (1927) |
| | | Rio de Janeiro | Atlantic forest | Pinto (1925) |
| | | Rio Bonito, São João de Meriti, Niterói, Cantagalo and Caxias, Rio de Janeiro; | Atlantic forest | Costa *et al.* (1973) |
| | | Guanabara and Florianópolis, Santa Catarina | | |
| | *L. pentadactylus* | Goiania, Goiás | Cerrado | Pessoa (1970) |
| | | Goiania, Goiás | Cerrado | Costa *et al.* (1973) |
| | | Santos, São Paulo; | Atlantic forest | Costa *et al.* (1973) |
| | | Salvador, Bahia | | |
| ***Hepatozoon* sp.** | *R. diptycha* | Petrolina, Pernambuco | Caatinga | **Current study** |
| | | Jaboticabal, São Paulo | Atlantic forest | Ferreira *et al.* (2020) |
| | *R. granulosa* | Petrolina, Pernambuco | Caatinga | **Current study** |
| | *L. latrans* | Barrinha, São Paulo | Atlantic forest | Ferreira *et al.* (2020) |
| | *L. macrosternum* | Corumbá, Mato Grosso do Sul | Pantanal | Leal *et al.* (2015) |
| | *L. podicipinus* | Corumbá, Mato Grosso do Sul | Pantanal | Leal *et al.* (2015) |

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