Description of an intramonocytic hemoparasite, *Hepatozoon lainsoni* sp. nov. (Apicomplexa: Adeleorina: Hepatozoidae), infecting *Ameiva ameiva* lizard (Reptilia: Squamata: Teiidae) in northern Brazil

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Abstract

The hemogregarine (Apicomplexa: Adeleorina) parasites are considered to be the most common and widespread hemoparasites in reptiles. The genus *Hepatozoon* (Apicomplexa: Adeleorina: Hepatozoidae) can be found parasitizing a broad range of species and, in reptiles, they infect mainly peripheral blood erythrocytes. The present study detected and characterized a hemogregarine isolated from the lizard species, *Ameiva ameiva,* collected from the municipality of Capanema, Pará State, North Brazil. Blood smears and imprints from lungs, brain, heart, kidney, liver, bone marrow and spleen were observed using light microscopy and the parasite was genetically identified by molecular analysis. Morphological, morphometric, and molecular data were obtained. Parasite gamonts were found in 49.5% (55/111) of the blood smears from *Ameiva ameiva*, and were characterized as oval, averaging 12.0 ± 0.8 x 5.9 ± 0.6 µm in size, which displaced the nuclei of parasitized monocytes laterally. Parasite forms resembling immature gamonts were observed in the spleen and bone marrow of the lizards. Furthermore, phylogenetic analyses of 18S rRNA *sequences did* not reveal gene similarity with other *Hepatozoon* spp. sequences from reptiles. Thus, morphological and molecular analyses have identified a new species of *Hepatozoon* parasite, *Hepatozoon lainsoni* sp. nov., which infects monocytes of the *A. ameiva* lizard.

Introduction

The Phylum Apicomplexa contains a large number of species of protozoan parasites, some of which are recognized as important pathogens that cause human diseases (e.g., *Plasmodium* spp. and *Toxoplasma gondii*) or as animal parasites of considerable economic importance (e.g., *Eimeria* spp. and *Neospora* spp.) (Kemp *et al.,* 2013). Apicomplexan parasites have an obligate intracellular existence, and many protozoan parasites also infect the blood cells and organs cells of their vertebrate hosts (Kemp *et al.,* 2013; Al-Quraishy *et al.,* 2021). The group of hemogregarines (Apicomplexa: Adeleorina) is considered a common parasite in reptiles, where *Hepatozoon* is the most commonly reported parasite. Of note, the gamonts of *Hepatozoon* display an important life-cycle characteristic as they infect peripheral blood erythrocytes of lizards (Telford Jr 2009).

Hepatozoon species are heteroxenous parasites, implying a complex variety of life cycles involving vertebrate and invertebrate hosts (ticks, mosquitoes, flies). Merogony occurs in the vertebrate host, producing gamonts (without sexual dimorphism) that are found within blood cells. Vertebrate infection is believed to occur through ingestion of infected invertebrate hosts and, in reptiles, transmission usually occurs through the ingestion of an infected vector or following predation of a vertebrate (paratenic host) (Smith 1996; Paperna and Lainson 2004; Al-Quraishy *et al.,* 2021)

Given that parasites may infect a wide range of host species, many hemogregarines have been described in Brazilian reptiles, including caiman (*Hepatozoon caimani* Carini 1909) and snakes (*Hepatozoon cevapii* O'Dwyer *et al.,* 2013, *Hepatozoon massardii* O'Dwyer *et al.,* 2013, *Hepatozoon cuestensis* O'Dwyer *et al.,* 2013, *Hepatozoon musa* Borges-Nojosa *et al.,* 2017) (Borges-Nojosa *et al.,* 2017; Carini 1909; O'Dwyer *et al.,* 2013). In reptiles of the Amazon, *Hepatozoon* infection has been reported in *Boa constrictor* (*Hepatozoon cf. terzii* Paperna and Lainson 2004), *Caiman caimani* (*Hepatozoon caimani* Carini 1909), and *Ameiva*

ameiva (*Hepatozoon ameivae* Carini and Rudolph 1912) (Paperna and Lainson 2004; Lainson *et al.,* 2003; Picelli *et al.,* 2020).

Although the evaluation of morphological values of the host and parasite life cycle developmental stages are crucial for the correct identification of parasites, molecular data are also essential for differentiating species and genera (O'Donoghue *et al.,* 2017). Therefore, using morphological and molecular analyses, the present study reports on the presence of a new species of *Hepatozoon*, *Hepatozoon lainsoni* sp. nov., with the unique characteristic of parasitizing monocyte/macrophage cells in the *A. ameiva* lizard.

Materials and methods

Lizards

A total of 111 adult *A. ameiva* (Lepidosauria, Teiidae) were hand captured at the municipality of Capanema, Pará State, northern Brazil (S 01º11'37.6" W 047º10'01.4"). Lizards were maintained as described by Silva *et al. (*2004).

Morphological and morphometric analyses

The lizards were restrained manually, and their blood was collected by cardiac puncture into heparinized (100 U/mL) 1mL syringes. The blood smears and the impression smears (imprints) of the lung, brain, heart, kidney, liver, bone marrow, and spleen were air-dried, fixed with absolute methanol, and stained with 10% Giemsa Methylene Blue Eosin Merck® diluted in distilled water (pH 7.0) for 50min, according to Eisen and Schall (2000), to allow analysis of the different parasite forms and stages. The samples were examined, and the infected cells were photographed with a Zeiss Axiophot microscope using the 100ximmersion objective. Prevalence was estimated as the proportion of infected hosts, expressed as a percentage. The intensity of parasitemia in blood monocytes was graded according to Silva *et*

*al. (*2004) as negative, low-level infected, medium-level infected and highly infected.

Measurements of the length, width, and area of the gamonts and host cells (infected and uninfected) were performed. Morphometric data are given in micrometers (μm), and for each metric, the averages, ranges, and standard deviations were also calculated. Measurements of cells were made using a 100x oil immersion objective on a Zeiss Axiphoto microscope, calibrated with a stage micrometer. Unstained samples were analyzed by differential interference contrast microscopy (DIC).

Molecular analysis

DNA was extracted from blood using the Illustra blood genomicPrep Mini Spin Kit (GE Healthcare) and following the manufacturer's instructions. The 18S rDNA gene was amplified with two pair of primers, the HepF300/Hep900 (600 bp) primers (Ujvari *et al.,* 2004) and 4558/2733 (1000 bp) primers (Mathew *et al.,* 2000) to detect the *Hepatozoon* species; reactions were conducted under conditions established by O´Dwyer *et al.* (2013) and Netherlands *et al. (*2014). To check for possible contamination, nuclease-free water was used as a negative control. A blood sample from a *Crotalus durissus terrificus* snake that previously tested positive for *Hepatozoon* spp. was used as the positive control (supplied by the Laboratory of Parasitology of the Institute of Biosciences, UNESP-IB). All reactions were performed in a Mastercycler pro (Eppendorf, Brazil) thermocycler with the following program: 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 60 s, followed by a final 7 min extension at 72 °C.

The amplification products were subjected to electrophoresis at 80V in a 1.5% agarose gel, stained with Gel Red, and observed using an ultraviolet transilluminator. The products of interest were purified by adding 2 μL of ExoSAP IT enzyme (GE Healthcare) to 5 μL of each PCR product, according to the manufacturer's recommendations. Amplicons were then

sequenced using a 3500 Genetic Analyzer capillary sequencer (Applied Biosystems) and the BigDye Terminator Cycle Sequencing Ready Reaction Kit v.3.1 (Applied Biosystems), according to the manufacturer's recommendations. A consensus sequence alignment was created from the forward and reverse electropherograms using the BioEdit software version 7.0.9 (Hall 1999). The sequences obtained (589 nt and 1052 nt) from *A. ameiva* (GenBank number) were compared among them and with those of other *Hepatozoon* isolates available in GenBank.

Sequences were aligned with the MUSCLE algorithm using Geneious v.7.1.3 (Kearse *et al.,* 2012) for Bayesian inference (BI) and Maximum likelihood (ML) analyses. From this alignment, to compare the 18S rRNA gene in *Hepatozoon* isolates, a pair-wise distance (p-distance) matrix was used (Table 1). For ML phylogeny, the JModelTest v.2.1.10 (Darriba *et al.,* 2012) was used to identify the best evolutionary model. Based on the Akaike Information Criterion (AIC), the TVM+G model was chosen for ML analyses. The phylogeny of the parasite was inferred using PhyML v. 3.0 (Guindon *et al.,* 2010) with 1000 replicate bootstraps (>50%). BI was carried out using MrBayes implemented from the computational resource CIPRES (Miller *et al.,* 2010), and analysis was run with the nucleotide substitution model GTR+I+G. To search with the Markov chain Monte Carlo method, chains were run with 10,000,000 generations, saving one tree Every 1000 generations. On the burn-in, the first 25% of generations were discarded, and the consensus trees were estimated using the remaining trees. Bayesian posterior probabilities (BPP) cutoff was considered > 50%. The trees (BI and ML) were visualized and edited using the FigTree v1.3.1 software program (Rambaut 2012). The sequences from the phylogenetic tree, their hosts and their GenBank number accession are shown in Table 2. The pair-wise distance (p-distance) and gene similarity were executed by the MEGA 7 program (Kumar *et al.,* 2016). The matrix was used to compare the interspecific divergence between the species of *Hepatozoon* sequences isolated

from lizards.

Results

Of the 111 *A. ameiva* lizards screened, 55 (49.5%) showed positivity for hemoparasites infecting monocytes in the peripheral blood (Figure 1 and Figure 2) and organ imprints (Figure 3). The level of infection was usually low (up to two parasites per microscopic field), although 5 (9.1%) of the lizard population presented high-level parasitemia (above three parasites per microscopic field). Developmental stages were observed in the spleen (Figure 3) and bone marrow (not shown), but not detected in other organs. Through morphological, morphometric and molecular evaluation, a previously undescribed species of hemogregarine parasite was identified; this species belongs to the genus *Hepatozoon* Miller 1908 (Adeleorina: Hepatozoidae).

Species description

Phylum Apicomplexa Levine, 1970

Class Conoidasida Levine, 1988

Subclass Coccidia Leuckart, 1879

Order Eucoccidiorida Léger, 1911

Suborder Adeleorina Léger, 1911

Family Hepatozoidae Wenyon, 1926

Genus *Hepatozoon* Miller, 1908

Hepatozoon lainsoni sp. nov. Morais and Silva, 2023

Taxonomic summary

Type-host: Ameiva ameiva (Linnaeus 1758) (Squamata:Teiidae: Teiinae).

Type-locality: In areas of the Capanema municipality, Pará State, northern Brazil (S 01º11'37.6" W 047º10'01.4").

Site of infection: Monocytes and macrophages from peripheral blood, spleen and bone marrow, respectively.

Vector: Remains unknown; we speculate that it is an external blood-sucking vector, such as a tick species.

Etymology: The species is named after the parasitologist, Ralph Lainson, who extensively researched the protozoan parasites of the Brazilian Amazon, and who described this parasite in a preliminary note (Lainson *et al.,* 2003).

Prevalence: Of the 111 lizards analyzed, 55 (49.5%) were positive.

Material deposited: Hapantotypes, one blood smear, one imprint of the spleen and one imprint of bone marrow from the *A. ameiva* lizard were deposited in the collection of the National Institute of Amazonian Research (INPA), Manaus, Brazil [TO BE ADDED].

Gene sequence: The 18S rRNA gene sequences (589bp and 1052bp), obtained from the blood of *A. ameiva,* were deposited in GenBank, under accession numbers [PP003255/PP003256].

Morphological and morphometrical analysis

Gamonts: Gamonts were observed exclusively in monocytes of infected lizards. No division stages were detected in blood films. Gamonts were oval and conspicuous in the monocyte cytoplasm, averaging 10.0-13.5 x 4.5-7.7 (12.0 ± 0.8 x 5.9 ± 0.6) µm (n=50) in size (Table 3), and with an area of 36.9 - 74.1 (57.9± 8.2) µm² (n=50). The parasite cytoplasm stained greyish-blue or pale pink with thin visible reddish staining at the membrane. Some gamonts contained numerous magenta-staining granules. Nuclei were small, averaging 1.9-5.3 x 1.5 - 4.0 (3.6 ± 0.8 x 2.6 ± 0.5) µm (n=50) in size, with an area of 3.2-11. (6.9 ± 1.7) µm² (n=50); they were rounded or irregular shaped, demonstrated dark purple staining, and were located

at one end of the parasite (Figure 1). Differential interference contrast microscopy revealed a thin body, with a prolongation, that was doubled up on itself and with a nucleus situated in the middle of the parasite (Figure 2).

Host-cell: Host cells were enlarged. Parasitized monocytes measured 11.3-20.9 x 8.9-16.0 (14.9 ± 2.1 x 12.5 ± 7.0) μm (n=50) in size, with an area of 87.2-225.6 (146.2 ± 30.9) μm² (n=50). Nuclei of parasitized monocytes measured 7.3-14.3 x 2.4-8.9 (10.7 ± 1.6 x 5.6 ± 1.4) μm (n=50) in size, with an area of 32.5-88.4 (48.7 ± 10.2) μm² (n=50). Gamonts displace the nuclei laterally, occasionally becoming deformed and flattened by the parasite. Numerous granules in the cytoplasm were occasionally seen. Uninfected monocytes measured 9.6-17.2 x 8.3-15.3 (13.8 ± 1.6 x 12.2 ± 1.5) μm (n=50) in size, with an area of 67.6-186.3(139.3 ± 24.8) μm² (n=50); and nuclei measured 7.8-12.3 x 4.4-10.3 (10.2 ± 1.1 x 7.4 ± 1.4) μm (n=50), with an area measuring 31.6-86.4(61.8 ± 13.2) μm² (n=50).

Tissue developmental stages (mature and immature gamonts): Tissue stages in the spleen and bone marrow were seen. Sometimes macrophages of the spleen presented two parasites in a single cell, with very abundant forms seen in the spleens of some infected lizards (Figure 3). Spleen and bone marrow of some infected lizards showed immature gamonts, characterized by an elliptical shape, which measured 7.4-9.7 x 5.6-6.9 (8.3 ± 0.8 x 6.4 ± 0.4) μm (n=10), and with an area of 29.0-51.0 (43.6 ± 7.4) μm² (n=10). These gamonts had purplish or pinkish cytoplasm with bright granules and fragmented nuclei that occupied the central position or were displaced towards one of the extremities. Another gamont form presented an oval body measuring 8.4-13.5 x 4.4-6.4 (11.3 ± 1.7 x 5.5 ± 0.7) μm (n=10) and with an area that measured 35.2-63.6 (47.7 ± 8.4) μm² (n=10); this form had pink cytoplasm, with rounded or fragmented nuclei that were displaced towards one of the extremities of the parasite, representing mature gamonts. These stages have variable sizes (Figure 3).

Morphological and molecular analyses identifies a new species of *Hepatozoon*

(Apicomplexa: Adeleorina: Hepatozoidae).

Remarks

Prior to this study, *Hepatozoon* spp. were reported infecting erythrocytes of reptiles, and occasionally leukocytes. The hemogregarine described herein appears to infect monocytes of the peripheral blood and division stages were seen only in internal organs. Gamonts of *Hepatozoon lainsoni* sp. nov. presented differences in morphological and morphometrical characteristics at the blood and tissues stages, when compared to other species of *Hepatozoon* described in *A. ameiva* of Minas Gerais and the Amazonas states in Brazil (Carini and Rudolph 1912; Picelli *et al.,* 2020), or any other species currently infecting lizard hosts (Table 4).

Erythrocytic haematozoans of *A. ameiva* were described by Lainson *et al. (*2003) to be slim parasites measuring 13.1 x 3.0 µm and with a nucleus located at the parasite's curved extremity, leading the author to compare these with haematozoans of *H. ameivae* from Minas Gerais, Brazil (Carini and Rudolph 1912). In 2020, Picelli *et al.,* redescribed *H. ameivae* in the peripheral blood of the same host in the Central Amazon by morphological, morphometrical and phylogenetic analyses. Gamonts were found infecting erythrocytes. The parasites were described as measuring 14.2 x 4.5 µm, with curved extremities and with nuclei that were rounded or irregular in shape, and measuring 4.9 x 3.2 µm.

With regard to other species from host lizards, *H. lainsoni* sp. nov. has a similar size range to those of *Hepatozoon caimani* Carini 1909 (12.15 x 4.3 µm) from the caiman *Caiman c. crocodilus* (Lainson *et al.,* 2003b), *Hepatozoon terzii* Sambon and Seligman, 1907 (12.3 x 4.3 µm) from snake *B. constrictor* (Paperna and Lainson 2004), *Hepatozoon quagliatus* Úngari, Netherlands, Silva and O'Dwyer 2021 (13.58 x 6.22 µm) from the sleep snake *Dipsas mikanii* (Úngari *et al.,* 2021) and are smaller than *Hepatozoon musa* Borges-Nojosa, Borges-

Leite, Maia, Zanchi-Silva, da Rocha Braga and Harris 2017 (18.9 x 3.8 μm) from snake *Philodryas nattereri* (Borges-Nojosa *et al.,* 2017).

The new species, *H. lainsoni* sp. nov., is characterized by elongated gamonts, which appear to have an oval shape and are not bent at the end. Gamonts were surrounded by a delicate capsule that increased the size of the host cell. Additionally, *H. lainsoni* sp. nov. have small nuclei that are located at one end of the parasite. *H. lainsoni* sp. nov. share certain characteristics with *H. caimani*, since the gamonts doubled up on themselves, and had a visible capsule where both ends were rounded. However, *H. caimani* exhibit gamont nuclei that are located laterally in the parasite, and extracellular parasites can be seen occasionally in the slim form.

In contrast to *H. lainsoni* sp. nov., gamonts of *H. musa* are longer and thinner, and are curved at both ends with their nuclei in the central position. Mature gamonts of *H. quagliatus* are elongated with a thin parasitophorous vacuole and are slightly curved at one end; the gamont nucleus is larger than that of *H. lainsoni* sp. nov. and slightly displaced to one side of the parasite.

With regard to tissue stages, *H. lainsoni* sp. nov. exhibits immature and mature gamonts in the spleen and bone marrow, with high parasitemia in the spleen macrophages of some lizards. These gamonts presented a rounded body, colorless cytoplasm and were variable in size.

Molecular analysis

The both newly amplified sequences from this study have shown 100% similarity among them. The large sequence (1052 nt) was used to construct BI and ML trees. The trees resulted in identical topologies with supported node value clades (Figure 4). The trees are formed by hemogregarine isolates, hemogregarine (Hemogregarinidae, Karyolysidae and Hepatozoidae)

and haemococcidia isolates as an outgroup. The main clade was subdivided into five subclades. The first subclade (Clade I) comprises *Hepatozoon* isolates from reptiles, anurans and marsupials, including the isolate from this study; furthermore, the isolate from this study grouped together with three isolates, two from marsupial hosts (FJ719813 / FJ719814) and one from lizard (KM234614). The second subclade (Clade II) comprises *Hepatozoon* isolates from mammals; the third subclade (Clade III) describes isolates from *Karyolysus* and *Hepatozoon* isolates from lizards; the fourth subclade (Clade IV) presents *Hemolivia* isolates; the fifth subclade (Clade V) comprises *Dactylosoma* isolates from anurans; and the sixth (Clade VI) comprises *Haemogregarina* isolates from turtles.

Discussion

Species of *Hepatozoon* have a wide geographic distribution, infecting amphibians, reptiles, birds and mammals. To date, species of *Hepatozoon* have been reported mainly infecting erythrocytes of reptiles. However, other species infecting mammals and birds are found mainly infecting leukocytes (Smith 1996; Godfrey *et al.,* 2011; Ebani and Mancianti 2022). This study provides molecular analysis and morphological and morphometric descriptions of the blood forms and tissue stages of *Hepatozoon lainsoni* sp. nov., a species that infects monocytes of the lizard *A. ameiva*.

In the present investigation, blood smears from infected lizards usually showed low parasitemia. A previous study analysing blood from *A. ameiva* reported only 10% of the lizard population with high parasitemia and a higher relative increase in the population of blood monocytes, many of which were infected with *H. lainsoni* sp.nov. (Lainson *et al.,* 2003b; Bonadiman *et al.,* 2010). Similarly, Úngari *et al. (*2018) observed low-level parasitemia in captive snakes from Brazil with positive blood smears. Other studies of *Hepatozoon* sp. in reptiles reported 0.25% parasitemia in the sleep snake *Dipsas mikanii* (from Goiás State,

Brazil) when infected with *H. quagliassus* Úngari and O'Dwyer 2021 (Úngari *et al.,* 2021) and 0.73% parasitemia in *A. ameiva* from Central Amazonia infected with *H. ameivae* Carini and Rudolph 1912 (Picelli *et al.,* 2020).

The overall prevalence in this study was 49.5%, which is close to that reported in the preliminary note about this parasite infecting *A. ameiva* (41.7%) (Lainson *et al.,* 2003a). The prevalence of *Hepatozoon* sp. infection observed in our study is in agreement with another study of *A. ameiva* infected with *Hepatozoon ameivae* Carini and Rudolph 1912 from Brazil, which found 55.5% of prevalence in 72 lizards in the Amazonas state (Picelli *et al.,* 2020). Furthermore, a higher prevalence of the occurrence of *Hepatozoon* sp. infecting free-ranging caimans (*Caiman crocodilus yacare)* was reported in a study in the State of Mato Grosso, Brazil (70.8%) (Bouer *et al.*, 2017) and in snakes (*Python regius*) in Ghana (78.2%) (Sloboda *et al.,* 2007), both using blood smear examination. Lower prevalences of 2% to 17.6% were observed in lacertid hosts from the Maghreb region of North Africa (Maia *et al.,* 2011) and in Asian snakes (22.2%) (Haklová *et al.,* 2014). Although different methodologies were used in these studies, Maia *et al. (*2011) suggested that infection with *Hepatozoon* is age-related, considering that the prevalence among snakes seems to be higher than among lizards, and that snakes live much longer than lizards, so that age is related to a higher chance of acquiring the infection, which might persist for a long time (Javanbakht *et al.,* 2015; Jakes *et al.,* 2003).

To date, species of *Hepatozoon* have been reported as infecting the erythrocytes and leukocytes of reptiles, mammals and birds hosts (O'Donoghue *et al.,* 2017; Godfrey *et al.,* 2011; Ebani and Mancianti 2022). In this study, we further characterized a species of *Hepatozoon* infecting monocytes and macrophages. Morphological and morphometric characteristics and phylogenetic analysis distinguished this species from other *Hepatozoon* species of lizards, such as *H. ameivae* Carini and Rudolph 1912, *H. tupinambis* Laveran and Salibeni 1909, *H. missoni* Carini 1909, *H. cnemidophorus* Carini 1941 and *H. sinimbui* Carini

1942 (Carini 1909; Carini 1941; Carini 1942; Laveran 1909). Through phylogenetic analysis, it was possible to observe that the *Hepatozoon* species from this study was grouped closer to *Hepatozoon* from marsupial hosts than lizard hosts. This fact corroborates with the morphological data observed. *Hepatozoon lainsoni* was found infecting defense cells, a characteristic normally found in mammals; while in reptiles it is common to be found in erythrocytes. Therefore, the morphological and molecular data suggest that *Hepatozoon lainsoni* may have an ancestor closer to mammals than to reptiles.

The morphometric and morphological analysis showed that the average size and tissue stages of *H. lainsoni* sp. nov. closely resembled those previously described by Lainson *et al.* (2003a). Gamonts of *Hepatozoon* from this current study revealed differences compared to the other species of *Hepatozoon* described in reptiles. In the literature, the *Hepatozoon* species described in *A. ameiva*, *H. ameivae* Carini and Rudolph 1912 is recognized to have a slim and elongated form, with both rounded and slightly arched extremities, with nuclei located at its curved extremity, and to infect erythrocytes. In contrast, *H. lainsoni* gamonts had an oval shape with rounded extremities and a small nucleus and infected monocytes. Indeed, interference contrast microscopy revealed the gamont to have a slim body. These data were supported by ultrastructural analysis of the blood forms, which showed an elongated form, with some embracing the monocyte cytoplasm and, sometimes in intimate contact with the host cell nucleus, suggesting a type of nutrient capture (Silva *et al.,* 2004). Again, these results corroborate the description of a new species of *Hepatozoon*.

One noticeable characteristic of the *Hepatozoon* species was the alterations in the monocyte host cell. In the present study, enlargement, and deformation of the nucleus, which was flattened by the parasite, were observed. Similar morphological alterations in the host monocyte after parasite infection were previously observed by Lainson *et al.* (2003a) and Silva *et al.* (2004) using optical and transmission electron microscopy, respectively.

Furthermore, accumulation and association of mitochondria and Golgi complex vesicles at the intracellular parasite were also observed by Silva *et al. (*2004). Shape alteration, lateral displacement of the nucleus and enlargement of infected blood cells have already been reported in snakes *Hydrodynastes gigas* and *Crotalus terrificus* infected by *Hepatozoon* sp. in Brazil (Moço *et al.,* 2002). In the current study, tissue stages were observed in the spleen and bone marrow, with the presence of immature and mature gamonts in some lizards; merogony and cystic forms were not seen. Indeed, we did not examine the skeletal muscle and intestines of infected lizards, where the occurrence of merogony and tissue cysts containing cystozoites may be possible, as seen in the host cells of mammal hosts infected with *H. felis*, *H. americanum* and *H. silvestres* (Hodžić *et al.,* 2017).

Dividing meronts were observed in the lungs and/or liver tissues of reptiles infected with *H. terzii* (Paperna and Lainson 2004), *H. kisrae* (Paperna *et al.,* 2002)*,* and *H. quagliattus* (Úngari *et al.,* 2021). Conversely, Lainson *et al. (*2003b) described *H. caimani* Carini 1909 in *Caiman c. crocodilus* and detected meronts only in the lamina propria of experimentally infected caimans, suggesting that merogony is limited to the lamina propria of the small intestine and justifying the failure of other authors to detect meronts in the viscera of naturally-infected caimans. It is probable that a similar pattern of merogonic stages may occur in *H. lainsoni* and further investigative endeavors are needed to clarify this.

Therefore, these discoveries underscore the need for expanded research on hemoparasite infections in lizards and other reptiles*,* especially in the Brazilian Amazon, which represents a geographic region with a great diversity of hosts and the possibility of finding genetically distinct hemogregarines.

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Table 1. The shaded matrix (upper) shows the similarity percentage (%) of the nucleotide sequences and the unshaded matrix (lower) shows the

pair-wise distance (p-distance) among *Hepatozoon* spp. isolates from lizards (1052 nt – sequence length).

ISOLATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. This study		96.74	96.72	97.19	97.66	96.72	96.02	96.25	97.89	97.89	91.47	92.51	96.60	96.49	97.89	96.25	95.55	96.02	96.96	95.32
2. MN833640	0.033																			
3. KM234612	0.031																			
4. KM234613	0.026																			
5. KM234616	0.021																			
6. KM234618	0.031																			
7. KM234650	0.038																			
8. KU680423	0.029																			
9. KU680459	0.019																			
10. KU680460	0.019																			
11. KU680462	0.075																			
12. KU680463	0.067																			
13. KU680466	0.031																			
14. KU680465	0.031																			

15.	HQ734806	0.019
16.	HQ734792	0.029
17.	HQ734793	0.036
18.	HQ734798	0.031
19.	AY252108	0.028
20.	JX531941	0.039

Table 2. Hosts, localities and GenBank accession numbers for the *18S rDNA* sequences of *Hepatozoon* spp., *Haemogregarina* spp. and *Hemolivia* spp. used in the phylogenetic analyses (except the sequence from this study and outgroup).

Parasite	Host	Locality	GenBank
Haemogregarina pellegrini	*Platysternon megacephalum*	China	KM887509
Haemogregarina sacaliae	*Sacalia quadriocellata*	Vietnam	KM887507
Haemogregaruina stepanowi	*Emys orbicularis*	Bulgaria	KF257928
Haemogregarina balli	*Chelydra serpentina serpentina*	Canada	HQ224959
Haemogregarina podocnemis	*Podocnemis unifilis*	Brazil	MF476204
Hemolivia parvula	*Kinixys zombensis*	South Africa	KR069083
Hemolivia mauritanica	*Testudo marginata*	Greece	KF992710
Hemolivia sp.	*Rhinoclemmys pulcherrima manni*	Nicaragua	KF992714
Hepatozoon ewingi	*Haemaphysalis bancrofti*	Australia	MG593275
Hepatozoon sp.	*Podarcis vaucheri*	Morocco	HQ734793
Hepatozoon sp.	*Algyroides marchi*	Spain	JX531941
Hepatozoon sp.	*Atlantolacerta andreanskyi*	Morocco	HQ734798
Hepatozoon sp.	*Tarentola angustimentalis*	Spain	KU680423
Hepatozoon sp.	*Podarcis vaucheri*	Morocco	HQ734792
Hepatozoon sp.	*Tarentola mauritanica*	Morocco	KU680463
Hepatozoon sp.	*Tarentola mauritanica*	Morocco	KU680462
Hepatozoon felis	*Panthera leo*	India	KX017290
Hepatozoon felis	*Felis catus*	Spain	AY620232
Hepatozoon americanum	*Amblyomma maculatum*	United States	AF176836
Hepatozoon americanum	*Dusicyon thous*	Brazil	AY461377
Hepatozoon canis	*Canis lupus familiaris*	Sudan	DQ111754
Hepatozoon canis	*Vulpes sp.*	Spain	AY150067

Hepatozoon sp.	*Dromiciops gliroides*	Chile	FJ719814
Hepatozoon sp.	*Dromiciops gliroides*	Chile	FJ719813
Hepatozoon sp.	*Amblyomma fimbriatum* from *Varanus panoptes*	Australia	EU430234
Hepatozoon sp.	*Aponomma varanense* from *Ophiophagus hannah*	Thailand	JQ670908
Hepatozoon sp.	*Hemidactylus mabouia*	Brazil	KM234616
Hepatozoon ayorgbor	*Python regius*	Ghana	EF157822
Hepatozoon sp.	*Tarentola deserti*	Algeria	KU680459
Hepatozoon sp.	*Tarentola deserti*	Morocco	KU680460
Hepatozoon sp.	*Tarentola mauritanica*	Morocco	HQ734806
Hepatozoon sp.	*Tarentola mauritanica*	Morocco	KU680466
Hepatozoon sp.	*Tarentola mauritanica*	Morocco	KU680465
Hepatozoon sp.	*Akodon* sp.	Brazil	KU667308
Hepatozoon sp.	*Oplurus* sp.	Madagascar	KM234650
Hepatozoon caimani	*Caiman crocodilus yacare*	Brazil	MF322539
Hepatozoon caimani	*Caiman crocodilus yacare*	Brazil	MF322538
Hepatozoon sp.	*Boiga* sp.	Australia	AF297085
Hepatozoon sp.	*Ornithodoros atacamensis*	Chile	MH174343
Hepatozoon sp.	*Phyllopezus periosus*	Brazil	KM234614
Hepatozoon massardii	*Crotalus durissus terrificus*	Brazil	KC342526
Hepatozoon cevapi	*Crotalus durissus terrificus*	Brazil	KC342525
Hepatozoon fitzsimonsi	*Kinixys zombensis*	South Africa	KR069084
Hepatozoon sp.	*Phyllopezus pollicaris*	Brazil	KM234613
Hepatozoon sp.	*Hemidactylus mabouia*	Brazil	KM234618
Hepatozoon musa	*Philodryas nattereri*	Brazil	KX880079
Hepatozoon musa	*Epicrates crassus*	Brazil	MF497767

Hepatozoon cuestensis	*Crotalus durissus terrificus*	Brazil	KC342524
Hepatozoon cuestensis	*Crotalus durissus*	Brazil	MF497769
Hepatozoon sp.	*Boa constrictor*	Brazil	MF497768
Hepatozoon sp.	*Phyllopezus pollicaris*	Brazil	KM234612
Hepatozoon sp.	*Varanus scalaris*	Australia	AY252108
Hepatozoon cecilhoarei	*Philothamnus natalensis natalensis*	South Africa	MG519504
Hepatozoon angeladaviesae	*Philothamnus hoplogaster*	South Africa	MG519501
Hepatozoon sipedon	*Nerodia sipedon sipedon*	Canada	JN181157
Hepatozoon sp.	*Rana esculenta*	France	HQ224960
Hepatozoon catesbianae	Bullfrog	Canada	AF176837
Hepatozoon ixoxo	*Sclerophrys* sp.	South Africa	KP119772
Hepatozoon theileri	*Amietia quecketti*	South Africa	KP119773

Table 3. Measurements (μm) of gamonts of *Hepatozoon lainsoni* sp. nov. and *Hepatozoon* sp., infecting lizards from Brazil.

Hepatozoon species	Vertebrate host	Length (μm)	Width (μm)	Host cell type	References
H. tupinambis	*Tupinambis teguixim*	16	6	Erythrocyte	Laveran and Salimbeni, 1909
H. missoni	*Tupinambis teguixim*	9	3	Erythrocyte	Carini, 1909
H. cnemidophori	*Cnemidophorus sp.*	17	4	Erythrocyte	Carini, 1941
H. sinimbui	*Iguana iguana*	NI	NI	Erythrocyte	Carini, 1942
H. ameivae	*Ameiva sp.*	13	4	Erythrocyte	Carini and Rudolph, 1912
H. lainsoni	*Ameiva ameiva*	12	6	Monocyte	Present study

NI: not informed

Table 4. Measurements (µm) of host cells and gamonts of *Hepatozoon lainsoni* sp. nov. observed in infected *Ameiva ameiva* lizards from Pará State, Brazil.

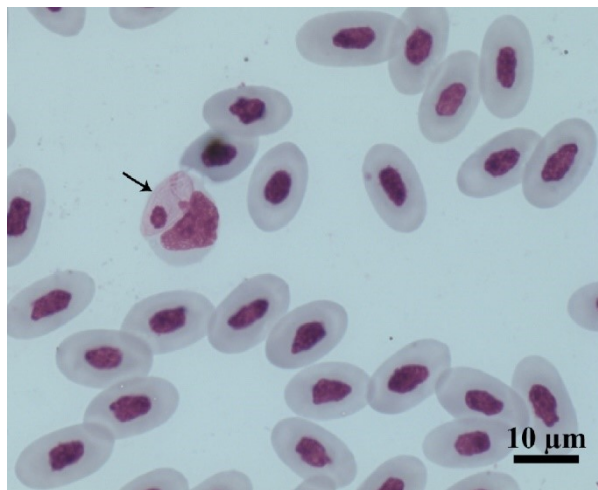|	n	Area (µm²)	Length (µm)	Width (µm)
Parasite	50	36.9-74.1 (±8.2)	10.0-13.5 (±0.8)	4.5-7.7 (±0.6)
Parasite nucleus	*50*	3.2-10.1 (±1.7)	1.9-5.4 (±0.78)	1.5-4.0 (±0.52)
Infected monocyte	*50*	87.2-225.6 (±31.0)	11.3-20.9 (±2.1)	8.9-16.0 (±7.0)
Infected monocyte nuclei	*50*	32.5-88.4 (±10.2)	7.3-14.3 (±1.6)	2.4-8.9 (±1.4)
Uninfected monocyte	*50*	67.6-186.3 (±24.9)	9.6-17.2 (±1.6)	8.3-15.3 (±1.5)
Uninfected monocyte nuclei	*50*	31.6-86.4 (±13.2)	7.8-12.3 (±1.1)	4.4-10.3 (±1.4)

± standard deviation

Figure 1. Intramonocytic gamonts (arrows) of Hepatozoon lainsoni sp. nov. from peripheral blood of the lizard A. ameiva stained with Giemsa. Bar: 10 μm.
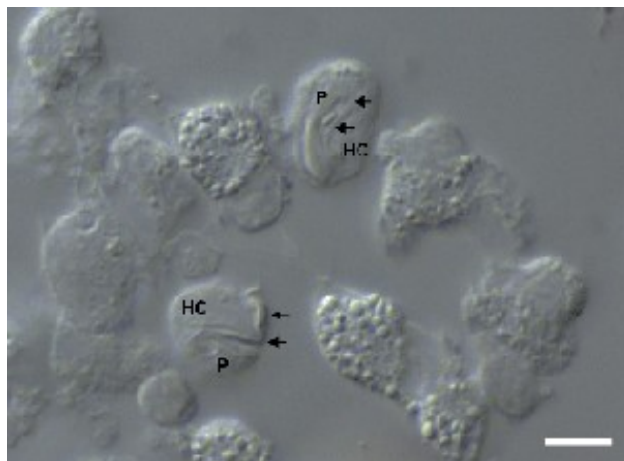

Figure 2. Intramonocytic gamonts (arrows) of Hepatozoon lainsoni sp. nov. from peripheral blood of the lizard A. ameiva observed by differential interference contrast microscopy (DIC). HC: host cell; P: parasite. Bar: 10 μm.
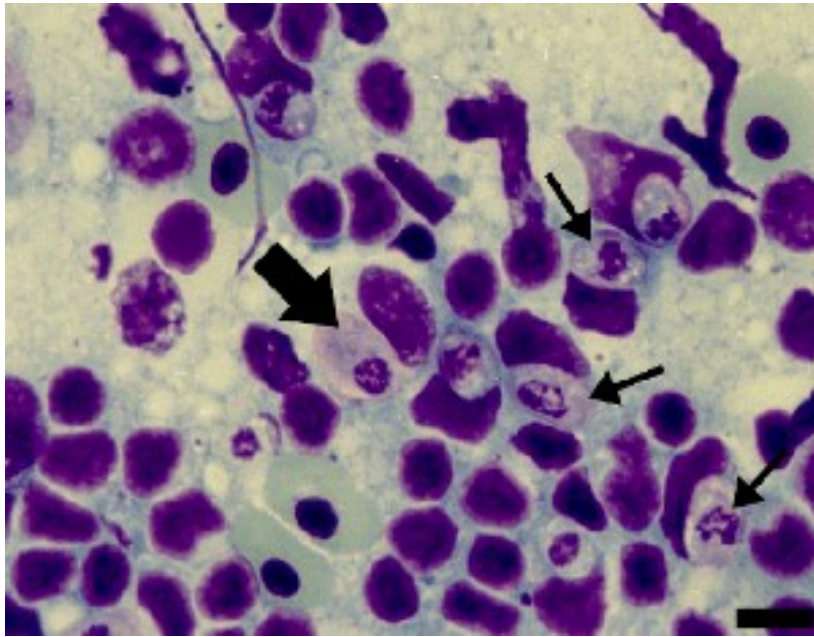
Figure 3. Cells from the spleen imprints stained with Giemsa from A. ameiva lizards infected with the parasite. Immature gamonts (arrows) and mature gamonts (big arrow). Bar: 10 μm.
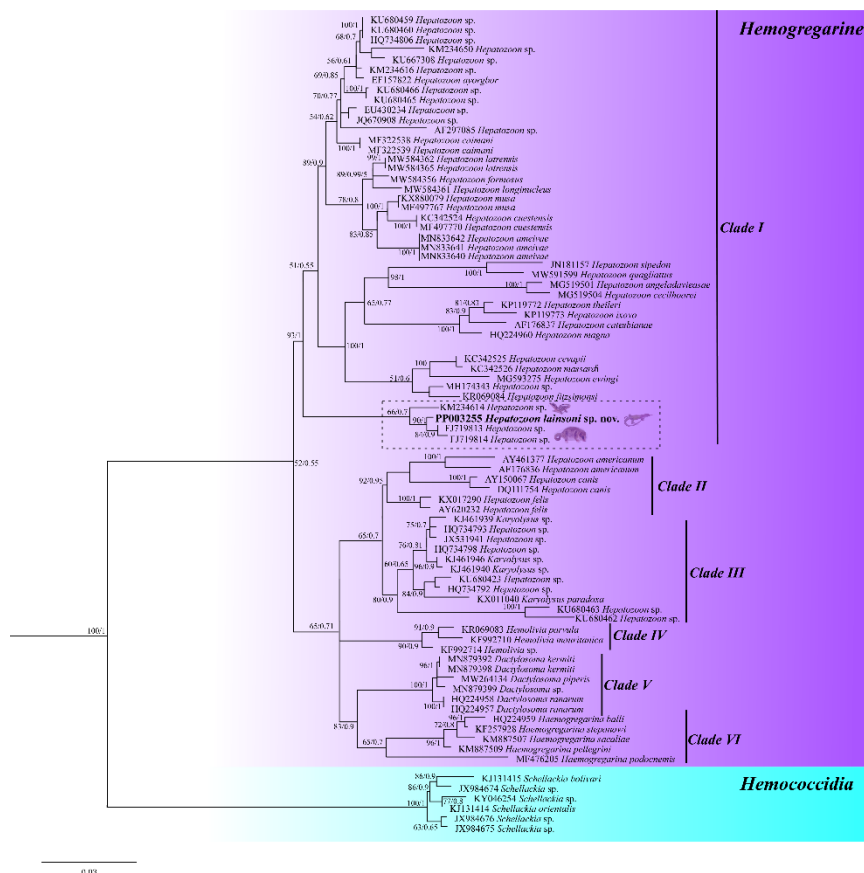
Figure 4. Phylogenetic tree of Hepatozoon spp. based on 18S rRNA region gene (1052bp —sequence length). The topology was identical across maximum likelihood (ML) and Bayesian interference (BI) with supported node values clades.