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Under the light: high prevalence of haemoparasites in lizards (Reptilia: Squamata) from Central Amazonia revealed by microscopy

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Abstract: Blood samples from 330 lizards of 19 species were collected to investigate the occurrence of haemoparasites. Samplings were performed in areas of upland (*terra-firme*) forest adjacent to Manaus municipality, Amazonas, Brazil. Blood parasites were detected in 220 (66%) lizards of 12 species and comprised four major groups: Apicomplexa (including haemogregarines, piroplasms, and haemosporidians), trypanosomatids, microfilarid nematodes and viral or bacterial organisms. Order Haemosporida had the highest prevalence, with 118 (35%) animals from 11 species. For lizard species, *Uranoscodon superciliosus* was the most parasitised host, with 103 (87%; n = 118) positive individuals. This species also presented the highest parasite diversity, with the occurrence of six taxa. Despite the difficulties attributed by many authors regarding the use of morphological characters for taxonomic resolution of haemoparasites, our low-cost approach using light microscopy recorded a high prevalence and diversity of blood parasite taxa in a relatively small number of host species. This report is the first survey of haemoparasites in lizards in the study region. It revealed a high diversity of lizard haemoparasites and highlights the need to understand their impacts on hosts.

Key words: Biodiversity, blood parasites, Lacertilia, morphology, Neotropics.

INTRODUCTION

The protozoologist Dr. Ralph Lainson (1992) two decades ago in his work on neglected parasites in the Amazonia basin quoted a phrase from P.C.C. Garnham, his former advisor: “There is a serious danger that malarial parasites become extinct.” Since that time, very few efforts have been made to contain the threats to the diversity of these parasites and other organisms (Ferrante & Fearnside 2019). In fact, these threats have been aggravated by increased habitat destruction in recent years, particularly in tropical regions (INPE 2019). Extinction, alteration in the abundance or introduction of parasites can have profound impacts on the health of a large number of free-living species (Dobson et al. 2008), because parasites are ecologically involved in important mechanisms that regulate wildlife populations and structure communities (Tompkins & Begon 1999, Thomas et al. 2000). Moreover, they may influence their host biological processes, such as sexual selection (Ehman & Scott 2002, Megía-Palma et al. 2018), predation and competition dynamics (Schall 1992, Garcia-Longoria et al. 2015), as well as speciation and extinction processes (Anderson & May 1978, Poulin 1999, Prenter et al. 2004).

Reptiles are hosts for a wide variety of parasites, especially for diverse groups that parasitise blood cells (Davies & Johnston 2000, Telford 2009). These blood parasites may be intra- or extracellular organisms that range from protozoan kinetoplastids (Killick-Kendrick et al. 1986, Telford 1995) and apicomplexan parasites (Levine 1988, O'Donoghue 2017), to microfilarid nematodes (Thoisy et al. 2000, Halla et al. 2014) as well as viral and bacterial inclusions (Telford 2009). Except for the last two pathogens, whose transmission is not yet clear, the other three parasitic taxa share a common feature by using a range of haematophagous invertebrates as the main vectors for transmission between vertebrate hosts (Smallridge & Paperna 1997, Viana et al. 2012, Van As et al. 2015, Fermino et al. 2019). Furthermore, the haemoprotozoans of Phylum Apicomplexa Levine, 1970 are apparently the most studied of all and also represent the taxon with the largest number of species parasitising reptiles (Levine 1988). Only in lizards (Squamata: Sauria), approximately 14 genera were recorded (O'Donoghue 2017); haemogregarines and haemosporidians are the most frequently identified groups (Smith 1996, Perkins 2014).

Although Brazil is a megadiverse country and has the third richest reptilian fauna in the world (Costa & Bérnils 2018), approximately 795 species, knowledge about haemoparasite diversity in these hosts consists of mainly a few concentrated studies in the eastern Amazon region (Lainson 1992, 2012). These studies recorded a rich haematozoan fauna in lizards and also suggest that the Amazon biome has a great potential for the discovery of new haemoparasitic species in these vertebrates, as 29 (80%) of the 36 known protozoan species in the country occur in this region (Table I). However, these records are limited to a total of 20 lizard species (Table I), which represent 7% (n = 276) of the described Brazilian lizard fauna and 10% (n = 16/152) for the Amazon region (Costa & Bérnils 2018). This small number is probably due to the difficulties in collecting these hosts and also the lack of specialists interested in working with haemoparasites from herpetofauna.

Light microscopy is an important tool for diagnosing infections that has crossed centuries and generations of scientists, still being the fastest and most accessible technique for searching parasites (Halla et al. 2014). This is especially true for studies adopting horizontal approaches that aim to estimate parasitism in poorly known groups. In this sense, we sought to investigate using light microscopy the presence and diversity of haemoparasites in lizards from Central Amazonia.

MATERIALS AND METHODS

Study area

The study was conducted in four upland (*terra-firme*) forest sites in Brazilian Central Amazonia, all located in the State of Amazonas, Brazil (Figure 1). The first study area was the Federal University of Amazonas forest fragment campus (UFAM; 3º4'34"S, 59º57'30"W), located in the eastern part of the city of Manaus. The three remaining study areas were located, respectively, 38 km (UFAM Experimental Farm; 2°38'57.6"S, 60°3'11"W), 80 km (Biological Dynamics of Forest Fragments Project [BDFFP]; 2°25'S, 59°50'W), and 160 km (Agrovila Rio Pardo; 1°48'S, 60°19' W) north of Manaus. These sampling regions present a mean annual temperature of approximately 26°C with relative air humidity over 80% (Araujo et al. 2002). The yearly precipitation is over 2,000 mm and mostly concentrated in a rainy season that usually occurs from December to May (Marques-Filho et al. 1981). The vegetation of the sampling sites is mainly composed of a mosaic of upland Amazonian rainforest, which varies from primary and secondary forests to open areas. The average elevation is 40–160 m above sea level (Laurance et al. 2011). Some of these landscapes are relatively undisturbed (Deichmann et al.

Table I. Checklist of haematozoan parasite species occurring in Brazilian lizards.

Parasite species	Host species	Prevalence	Locality	Author/Year
Coccidia				
Hepatozoidae				
Hepatozoon ameivae	*Ameiva ameiva*	Not inf.	Minas Gerais	Carini & Rudolph 1912*
		Not inf.	Capanema/PA	Lainson et al. 2003
		1 of 10	São João da Barra/RJ	Sabagh et al. 2015
Hepatozoon cnemidophori	*Cnemidophorus* sp.	1 of 11	Porto Nacional/TO	Carini 1941a*
Hepatozoon missoni	*Tupinambis teguixin*	Not inf.	São Paulo/SP	Carini 1909*
Hepatozoon sinimbui	*Iguana iguana*	1 of 1	Porto Nacional/TO	Carini 1942*
Hepatozoon tupinambis	*Tupinambis teguixin*	2 of 3	Ilha do Governador/RJ	Laveran & Salimbeni 1909*
		Not inf.	São Paulo/SP	Carini 1909
		3 of 10	Ilha Solteira/SP	Pessôa et al. 1974
		3 of 7	Fortaleza/CE	Pessôa et al. 1974
Karyolysidae				
Hemolivia stellata	*Ameiva ameiva*	3 of 20	Capanema/PA	Lainson et al. 2007
Lankesterellidae				
Lainsonia iguanae	*Iguana iguana*	3	Exu/PE	Landau 1973*
		1	Belém/PA	Landau 1973*
Lainsonia legeri	*Tupinambis teguixin*	Not inf.	Belém/PA	Landau et al. 1974*
Schellackia landaue	*Polychrus marmoratus*	17 of 148	Capanema/PA	Lainson et al. 1976*
Haemospororida				
Garniidae				
Fallisia audaciosa	*Plica umbra*	4 of 235	Belém/PA	Lainson et al. 1975*
Fallisia effusa	*Neusticurus bicarinatus*	26 of 32	Capanema/PA	Lainson et al. 1974a*
Fallisia modesta	*Tropidurus oreadicus*	1 of 1	Belém/PA	Lainson et al. 1974a*
Fallisia simplex	*Plica umbra*	20 of 235	Belém/PA	Lainson et al. 1975*
Garnia gonatodi	*Gonatodes humeralis*	26 of 52	Belém/PA	Lainson et al. 1971
Garnia karyolytica	*Thecadactylus rapicauda*	1 of 4	Novo Repartimento/PA	Lainson & Naiff 1999*
Garnia morula	*Copeoglossum nigrupunctatum*	18 of 63	Belém/PA	Lainson et al. 1974b
Garnia multiformis	*Plica umbra*	41 of 235	Belém/PA	Lainson et al. 1975*
Garnia telfordi	*Ameiva ameiva*	1 of 1	Nova Xavantina/MT	Lainson et al. 1971*
Garnia uranoscodoni	*Uranoscodon superciliosus*	46 of 167	Belém/PA	Lainson et al. 1975*
Garnia utingensis	*Dactyloa punctata*	2 of 6	Belém/PA	Lainson et al. 1971*
Haemoproteidae				
*Haemocystidium catenatus***	*Enyalius catenatus*	1 of 1	Juquitiba/SP	Pessoa & Cavalheiro 1970*
Plasmodiidae				
Plasmodium carmelinoi	*Ameiva ameiva*	12 of 206	Pará	Lainson et al. 2010*
Plasmodium cnemidophori	*Cnemidophorus* sp.	2 of 4	Porto Nacional/TO	Carini 1941b*

Table I (continued)

Parasite species	Host species	Prevalence	Locality	Author/Year
	Ameiva ameiva	29 of 66	Belém/PA	Lainson & Shaw 1969a
Plasmodium dipoglossi	*Diploglossus fasciatus*	2 of 2	Xerém/RJ	Aragão & Neiva 1909*
	Copeoglossum nigrupunctatum	6 of 20	Belém/PA	Lainson & Shaw 1969a
Plasmodium kentropyxi	*Kentropyx calcarata*	31 of 39	Outeiro/PA	Lainson et al. 2001*
		36 of 55	Capanema/PA	Lainson et al. 2001*
		35 of 57	Belém/PA	Lainson et al. 2001*
Plasmodium minasense	*Copeoglossum nigrupunctatum*	Not inf.	Minas Gerais	Carini & Rudolph 1912*
	Polychrus acutirostris	1 of 1	Mateus Leme/MG	Cordeiro 1977
	Iguana iguana	Not inf.	Salvador/BA	Telford 1979
	Tupinambis teguixin	5 of 6	Belém/PA	Landau et al. 1973
Plasmodium neusticuri	*Neusticurus bicarinatus*	134 of 465	Belém/PA	Lainson & Paperna 1996*
Plasmodium rhadinurum	*Iguana iguana*	Not inf.	Porto Nacional/TO	Carini 1945
		3 of 3	Codajás/AM	Walliker 1966
Plasmodium tropiduri	*Tropidurus torquatus*	1 of 1	Bicudos/MG	Aragão & Neiva 1909*
		4 of 39	Jacobina/BA	Pessôa & Lopes 1963
		10 of 51	Davinolândia/SP	Silva & Rodrigues 1974
		2 of 31	Pinhal/SP	Silva & Rodrigues 1974
		6 of 49	Águas da Prata/SP	Silva & Rodrigues 1974
		1 of 1	Porto Nacional/TO	Carini 1941c
		10 of 89	Belo Horizonte/MG	Hernandes-Córdoba & Braga 2019
		50 of 87	Ouro Preto/MG	Hernandes-Córdoba & Braga 2019
Plasmodium tropiduri	*Tropidurus itambere*	3 of 12	Ibitipoca/MG	Nunes et al. 2010
	Copeoglossum nigrupunctatum	8 of 20	Belém/PA	Lainson & Shaw 1969a
	Kentropyx calcarata	20 of 151	Pará	Lainson et al. 2001
Plasmodium vacuolatum	*Plica umbra*	31 of 235	Belém/PA	Lainson et al. 1975*
Plasmodium vautieri	*Urostrophus vautieri*	1 of 1	São Paulo/SP	Pessôa & de Biasi 1973*
Saurocytozoon mabui	*Copeoglossum nigrupunctatum*	2 of 31	Ananindeua/PA	Lainson et al. 1974b*
Saurocytozoon tupinambi	*Tupinambis teguixin*	5 of 6	Belém/PA	Lainson & Shaw 1969b*
	Crocodilurus amazonicus	1 of 1	Bacarena/PA	Lainson et al. 1974b
Kinetoplastea				
Trypanosomatidae				
Trypanosoma plicae	*Plica umbra*	27 of 235	Belém/PA	Lainson et al. 1975*
Trypanosoma superciliosae	*Uranoscodon superciliosus*	Not inf.	Codajás/AM	Walliker 1965*
Trypanosoma rudolphi	*Copeoglossum nigrupunctatum*	Not inf.	Minas Gerais	Carini & Rudolph 1912*

***Species description studies. **Originally described as *Haemoproteus catenatus*.**

2010, Rojas-Ahumada et al. 2012), but most exhibit anthropogenic alterations (Rocha et al. 2004, Ramos et al. 2014).

Lizard and blood sampling

A total of 330 lizards from 19 species distributed in 17 genera and 10 families were sampled between 2016 and 2019 (Table II). Animals were captured using several methods, such as active search (Doan 2003) and traps, i.e., pitfalls with drift-fences (Jenkins et al. 2003), funnels made out of PVC pipes (Abrahão et al. 2019) and live-traps (Vieira et al. 2015). Lizards were identified through specialised literature (Ávila-Pires 1995, Vitt et al. 2008), and taxonomic nomenclature was adopted following Costa & Bérnils (2018). The blood samples were obtained by tail or cardiac puncture using a sterile insulin syringe (Samour et al. 1984). A portion of collected blood was used to make smears, which were fixed with absolute methanol and stained with 10% Giemsa. The other portion was applied to a filter paper for molecular analyses. Lizards were released within 24 h of capture, but in the case of cardiac puncture, the blood was collected after euthanasia (via injection of 2% lidocaine). Specimens were preserved in 10% formalin and deposited in the Zoological Collections of the National Institute of Amazonian Research (INPA) and UFAM in Manaus, Brazil.

Lizard sampling and access to the genetic data were authorised by the Brazilian Ministry of the Environment (SISBIO n° 53851-4 and SISGEN AA6199D, respectively). All procedures were approved by the ethics committee on animal use from Universidade Federal do Amazonas (protocol number 012/2016).

Microscopic analyses

Blood smears were examined for up to 20 min under a Leica DM4B microscope (Leica Microsystems, Heerbrugg, Switzerland) at × 400 and × 1000 total magnification. The slides with parasites were carefully examined and images were captured with an attached Leica DMC4500 digital camera and processed with LAS V4.8 (Leica Microsystems Suiza Limited 2015). Morphometric measurements were taken with this same system. However, they will not be presented here in this work, as they are part of ongoing taxonomic studies. Haematozoan parasites were taxonomically identified by comparing their morphologies to the descriptions from the guides of Telford (2009) and Lainson (2012), besides original description articles. Additionally, to confirm the identification of some haemosporidian species, we compared our material with that of the collection of Dr. Ralph Lainson, deposited at the Evandro Chagas Institute (IEC) in Belém, Brazil.

RESULTS

Haemoparasite infections were detected in 220 (66%) out of 330 lizards of 12 species distributed among seven families (Table II). Mixed infections occurred in 91 positive specimens. For sampling sites, BDFFP had 78% (n = 156/200) of the infected lizards, UFAM Experimental Farm had 68% (n = 13/19), Agrovila Rio Pardo had 47% (n = 50/105) and UFAM urban forest fragment had 16% (n = 1/6). Parasites were grouped into four major groups (Figure 2), with the following prevalence: (i) intracellular apicomplexan parasites at 173 (52%) individuals; (ii) trypanosomatids at 84 (25%); (iii) microfilarial worms at 38 (11%); (iv) unidentified viral or bacterial inclusions at 30 (9%).

Among the positive lizards, Tropiduridae and Teiidae were the families that showed the highest prevalence, with 86% (n = 112/130) and 66% (n = 90/135) positive animals, respectively. With regard to lizard species, *Uranoscodon*

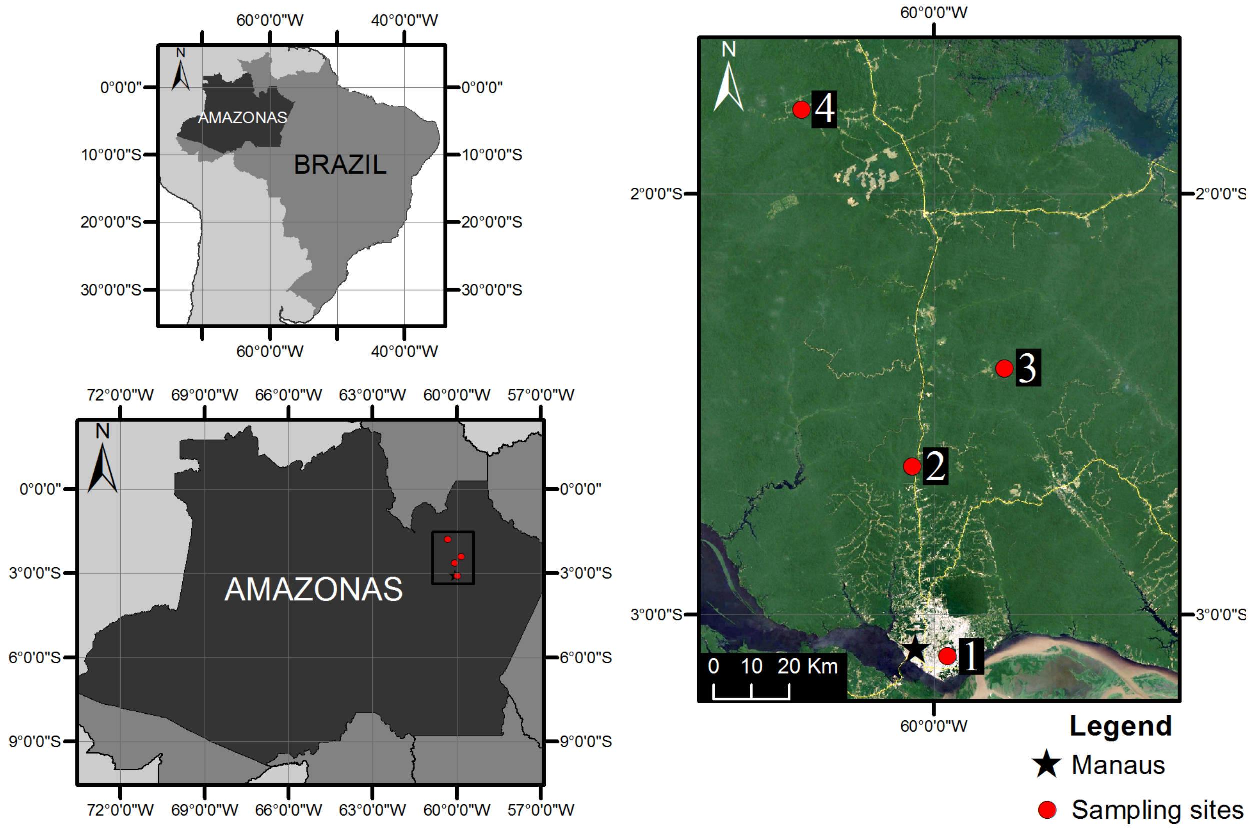

Figure 1. Sampling areas in Central Amazonia: (1) Campus of the Federal University of Amazonas (UFAM); (2) UFAM Experimental Farm; (3) Biological Dynamics of Forest Fragments Project (BDFFP) Reserve; (4) Agrovila Rio Pardo.

superciliosus Linnaeus, 1758 stood out for presenting a high prevalence, with 87% (n = 103/118) of infected individuals, and also because it was the species with the greatest diversity of parasites, with the occurrence of six different taxa: Haemohormidiidae, Plasmodiidae, Garniidae, Trypanosomatidae, microfilarial worms and unidentified inclusions.

Parasites of phylum Apicomplexa (Table III) were found in all infected lizard species; 14 species from five families were identified. Two morphotypes of the genus *Hepatozoon* (Hepatozoidae) were observed in 40 *Ameiva ameiva* Linnaeus, 1758 (55%; n = 72) and one was identified as *Hepatozoon ameivae* Carini & Rudolph, 1912 (Figure 2a). *H. ameivae* was recorded overlapping the nucleus of the parasitised cells, whereas the other morphotype caused lateral displacement of the nucleus to one end of the red blood cell (Figure 2b). Both parasites were restricted to erythrocytes. *Sauroplasma*-like (Haemohormidiidae) infections (Figure 2c) appeared in 14% (n = 49/330) of individuals from six lizard species (Table III). Notably, *U. superciliosus* had the highest number of parasite occurrences, with 32 (27%; n = 118) positive specimens.

Haemosporidian parasites presented the highest prevalence, with 35% (n = 118/330) animals infected and, except for *Alopoglossus angulatus* Linnaeus, 1758, all positive host species were parasitised by malaria. Based on blood stage morphology, 13 species from two families, Plasmodiidae and Garniidae, were identified (Table III; Figure 2d-j). It is important to note that despite some authors (e.g., Levine

Table II. Prevalence of haemoparasites in lizards from Central Amazonia.

Species	No. examined	No. infected	No. (%) lizards infected			
			Apicomplexa parasites	Trypanosomes	Microfilarial worms	Viral or bacterial inclusions
Alopoglossidae						
Alopoglossus angulatus	1	1	1 (100)	—	—	—
Dactyloidae						
Dactyloa punctata	3	2	2 (66)	—	—	—
Norops fuscoauratus	1	—	—	—	—	—
Norops ortonii	1	—	—	—	—	—
Norops planiceps	9	2	2 (22)	—	1 (11)	—
Gekkonidae						
Hemidactylus mabouia	1	—	—	—	—	—
Gymnophthalmidae						
Arthrosaura reticulata	11	2	1 (9)	—	—	2 (18)
Cercosaura argulus	1	—	—	—	—	—
Loxopholis sp.	1	—	—	—	—	—
Loxopholis percarinatum	6	—	—	—	—	—
Neusticurus bicarinatus	3	2	2 (66)	—	—	—
Iguanidae						
Iguana iguana	1	—	—	—	—	—
Mabuyidae						
Copeoglossum nigropunctatum	8	6	6 (75)	—	2 (25)	—
Phyllodactylidae						
Thecadactylus rapicauda	10	—	—	—	—	—
Sphaerodactylidae						
Gonatodes humeralis	8	3	3 (37)	—	—	—
Teiidae						
Ameiva ameiva	72	60	58 (80)	—	27 (37)	3 (4)
Cnemidophorus sp.	2	—	—	—	—	—
Kentropyx calcarata	36	24	23 (63)	—	2 (5)	—
Tupinambis teguixin	25	6	6 (24)	—	—	3 (12)
Tropiduridae						
Plica umbra	12	9	7 (58)	1 (8)	—	1 (8)
Uranoscodon superciliosus	118	103	62 (52)	83(70)	6 (5)	21 (18)
Total	330	220	173 (52)	84 (25)	38 (11)	30 (9)

1988, Telford 2009), here we recognise the family Garniidae as well as the genera *Garnia* and *Fallisia* as valid taxa diagnosed by absence of pigment and ultrastructural characteristics (Lainson et al. 1971, Boulard et al. 1987).

Plasmodium spp. (Figure 2d-g) were detected in 64 (19%; n = 330) lizards from nine species, with the highest number of positive specimens seen in *A. ameiva* (36%; n = 72). At least 11 morphotypes were visualised, and five *Plasmodium* species could be recognised (Table III). Gametocytes of *Saurocytozoon* cf. *tupinambi* Lainson & Shaw, 1969b were observed in leucocytes (Figure 2h) from five (20%; n = 25) *Tubinambis teguixin* Linnaeus, 1758. Non-pigmented malaria parasites from the genera *Fallisia* (Figure 2i) and *Garnia* (Figure 2j) were found in four (1%; n = 330) and 46 (14%; n = 330) lizards, respectively (Table III). Two *Fallisia* species were detected in *Plica umbra* Linnaeus, 1758, *Fallisia* cf. *simplex* Lainson et al., 1975 and *Fallisia* cf. *audaciosa* Lainson et al., 1975. In *Neusticurus bicarinatus* Linnaeus, 1758, we found *Fallisia* cf. *effusa* Lainson et al., 1974a. Parasites of the genus *Garnia* were mainly recorded in *U. superciliosus* (22%; n = 118). We also detected four unidentified morphotypes and four species of this genus (Table III).

Extracellular parasites of the family Trypanosomatidae (Table II) were found in 83 *U. superciliosus* (70%; n = 118) and one *P. umbra* (8%; n = 12): each tropidurid species had one *Trypanosoma* morphotype. The trypanosome of *U. superciliosus* had an elongated body and diffuse nucleus (Figure 2k), while the observed *P. umbra* had a rounded shape and compact nucleus (Figure 2l). Microfilarial worms (Nematoda) occurred in five lizard species (Table II), with higher prevalence in *A. ameiva* with 37% (n = 27/72) positive specimens. These blood parasites exhibited highly variable sizes and shapes (Figure 2m-n) and were very similar to the genus *Piratuba*. However, accurate diagnoses of filarial worms is mainly based on morphological features of adult worms. Thus, identification of this group in the present study remains indeterminate.

The last of the four major groups, inclusions of uncertain nature (Figure 2o-p), were detected in erythrocytes of five lizard species and showed little morphological variation. They consisted of a large spherical shape with a rarely darker stained margin. These vacuoles resemble rickettsial parasites recorded for other reptilian hosts, although without ultrastructural study it was not possible to confirm this identification.

DISCUSSION

We observed a high prevalence of blood parasites among lizards from Central Amazonia: more than half of the sampled individuals and species were infected. We also demonstrated that lizards are the hosts for a wide variety of haemoparasites. Indeed, we observed great parasite richness in a small number of host species and in a limited sampling area. This finding reinforces that the neotropical region holds a rich haemoparasite fauna, as shown by studies conducted in other localities across the Amazon Basin (Renjifo et al. 1952, Telford 1970, 1973, 1980, Ayala et al. 1973, Lainson 1992, Thoisy et al. 2000, Matta et al. 2018). Furthermore, it is important to note that we sampled lizard species with diversified microhabitat use, ranging from terrestrial (e.g., *A. ameiva*), semi-aquatic (e.g., *Neusticurus bicarinatus)*, scansorial (e.g., *P. umbra*) to arboreal (e.g., *U. superciliosus*) (Vitt et al. 2008). This environmental diversity may imply determinant characteristics for the composition of the haemoparasite assemblages found in these lizards because different species of vectors, including mosquitoes, sandflies and

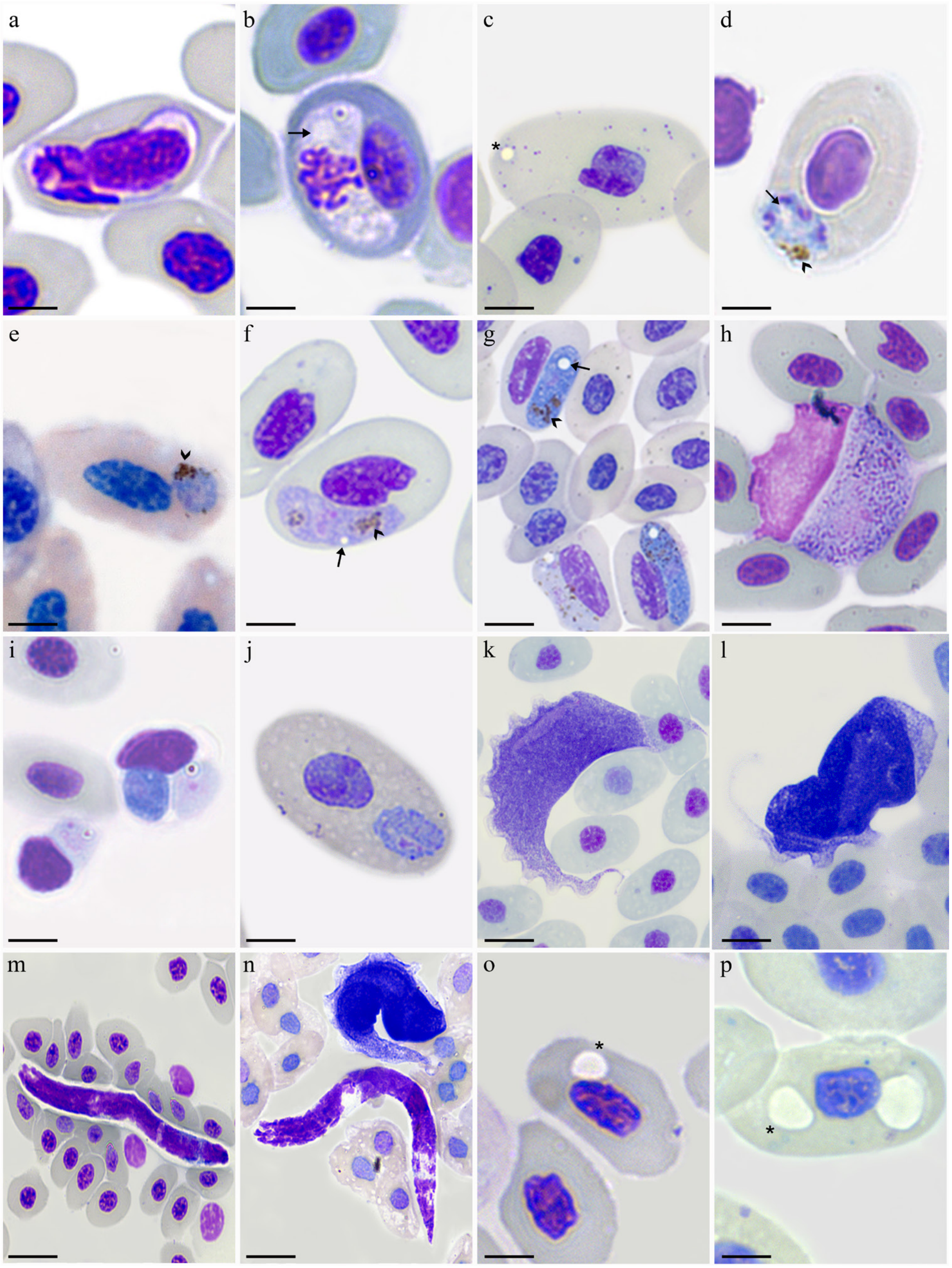

Figure 2. Parasites and inclusions found in lizards from Central Amazonia. Gametocytes of (a) *Hepatozoon ameivae* and (b) *Hepatozoon* sp. in *Ameiva ameiva.* (c) *Sauroplasma*-like infection in *Uranoscodon superciliosus.* (d) Trophozoite with nuclear division of *Plasmodium carmelinoi* from *A. ameiva.* (e) Trophozoite and mature (f) gametocyte of *Plasmodium* sp. in *Norops planiceps.* (g) Macrogametocytes and microgametocyte of *Plasmodium kentropyxi* in *Kentropyx calcarata.* (h) Gametocyte of *Sarocytozoon tupinambi* in a lymphocyte from *Tupinambis teguixin.* (i) *Fallisia simplex* in *Plica umbra*, showing single and double gametocyte infections in the thrombocytes. (j) Gametocyte of *Garnia uranoscodoni* from *U. superciliosus. Trypanosoma* spp. infections in (k) *U. superciliosus* and (l) *P. umbra.* (m) Microfilaria in *A. ameiva* and in (n) mixed infection in *U. superciliosus.* Vacuole-like inclusions in erythrocytes from (o) *U. superciliosus* and (p) *A. ameiva*. Arrow heads indicate pigment granules; black arrows indicate parasite vacuoles and asterisks indicate inclusions. Micrographs are from Giemsa-stained thin blood films. Scale bar is 10 µm.

Table III. Infections of Apicomplexa parasites in 12 lizard species sampled in this study.

Host species (N)	Parasites	n infected (% infected)
Alopoglossidae		
Alopoglossus angulatus (1)	*Sauroplasma*-like	1 (100)
Dactyloidae		
Dactyloa punctata (3)	*Garnia* cf. *utingensis*	1 (33)
	Plasmodium sp.	1 (33)
Norops planiceps (9)	*Plasmodium* sp.	2 (22)
	Sauroplasma-like	1 (11)
Gymnophthalmidae		
Arthrosaura reticulata (11)	*Plasmodium* sp.	1 (9)
Neusticurus bicarinatus (3)	*Fallisia* cf. *effusa*	2 (66)
Mabuyidae		
Copeoglossum nigropunctatum (8)	*Plasmodium* sp.	2 (25)
	Plasmodium cf. *tropiduri*	3 (37)
	Sauroplasma-like	1 (12)
Sphaerodactylidae		
Gonatodes humeralis (8)	*Garnia* sp.	3 (37)
Teiidae		
Ameiva ameiva (72)	*Hepatozoon ameivae*	40 (55)
	Hepatozoon sp.	11 (15)
	Garnia cf. *telfordi*	10 (13)
	Garnia sp.	4 (5)
	Plasmodium cf. *carmelinoi*	4 (5)
	Plasmodium cf. *cnemidophori*	1 (1)
	Plasmodium sp.	21 (29)
	Sauroplasma-like	13 (18)
Kentropyx calcarata (36)	*Plasmodium* cf. *kentropyxi*	12 (33)
	Plasmodium cf. *tropiduri*	2 (5)
	Plasmodium sp.	6 (16)
	Sauroplasma sp.	13 (36)
Tupinambis teguixin (25)	*Plasmodium* sp.	1 (4)
	Saurocytozoon cf. *tupinambi*	5 (20)
Tropiduridae		
Plica umbra (12)	*Fallisia* cf. *audaciosa*	1 (8)
	Fallisia cf. *simplex*	1 (8)
	Garnia cf. *multiformes*	2 (16)
	Plasmodium cf. *vacuolatum*	4 (33)
Uranoscodon superciliosus (118)	*Garnia* cf. *uranoscodoni*	16 (13)
	Garnia sp.	10 (8)
	Plasmodium sp.	2 (1)
	Sauroplasma-like	32 (27)

ticks, are likely distributed along the gradient occupied by these hosts.

Parasite and host checklists are crucial in expanding our knowledge on species distribution. Nonetheless, surveys and descriptive studies of haemoparasite species on lizards conducted in the Amazonian biome have decreased considerably in recent years. In Brazil, it has been 10 years since a haematozoan species from a lizard has been described (Lainson et al. 2010). Most access to this haemoparasitic diversity in the Neotropics departs from the classical approach by using light microscopy to investigate prevalence and parasite identity. The exclusive use of morphological attributes for the diagnosis of species has been strongly criticised as unreliable because molecular tools have advanced in solving taxonomic problems (Pineda-Catalan et al. 2013, Perkins 2014). In fact, molecular biology constitutes a modern and acurate tool in parasitology, but its use still faces financial and technical limitations —i.e. difficulties in developing protocols and molecular markers—, especially in megadiverse and developing countries such as Brazil (Perkins et al. 2011, Morand 2018). Nevertheless, in comprehensive multi-species approaches like ours, whose main objective is not to solve systematic and phylogenetic questions, observations of blood smears under a microscope still prove to be a feasible method to access the prevalence and parasite diversity hidden in these hosts, despite some taxonomic limitations.

Among the 12 infected lizard species, haemoparasites were recorded for the first time in three of them: *Arthrosaura reticulata* O'Shaughnessy, 1881, *Norops planiceps* Troschel, 1848 and *A. angulatus*. However, we did not find blood parasites in nine lizard taxa (Table II), even though parasites have already recorded in some of these hosts in other localities (Table I). This discrepancy may simply reflect unequal sampling efforts. The methods we used for capture were diversified and effective for certain hosts groups, such as Teiidae and Tropiduridae, but are limited for many lizard species, mainly those that access subterranean microhabitats (Faria et al. 2019). Indeed, Teiidae, Tropiduridae and a lizard species, *U. superciliosus,* were the hosts with highest parasite prevalence. However, with the myriad known problems in obtaining samples (Perkins et al. 2011)—financial, technical and logistical difficulties in accessing remote areas—and the need to move forward on other parasitology research fronts, such as vectors and life cycle, landscape and epizootiology studies, those abundant taxa may be an interesting choice to be included in ecological parasitic systems as model organisms. Additionally, for many reasons lizards are considered model organisms (Huey et al. 1983, Camargo et al. 2010), as they respond very well when testing ecological and evolutionary hypotheses (Schall 1996).

Most of the parasites found in this study belong to phylum Apicomplexa. Indeed, all host species had some representative of this group. One of them was the genus *Hepatozoon*, relatively common parasite in reptiles and, despite the great diversity of lizards sampled in this study, was found exclusively infecting *A. ameiva*. *Hepatozoon ameivae* was described by Carini & Rudolph 1912 in *A. ameiva* in the State of Minas Gerais and later recorded in the municipality of São João da Barra, State of Rio de Janeiro, both in southeastern Brazil (Carini & Rudolph 1912, Sabagh et al. 2015). Lainson et al. (2003) also probably recorded *H. ameivae* in lizards from the municipality of Capanema, State of Pará, northern Brazil. This parasite has an outstanding feature: its gametocytes are found in the erythrocyte nucleus, a relatively uncommon developmental pattern in the

Apicomplexa that can lead to severe distortion and even lysis of the infected cell nucleus (Telford 2009). It is important to note that *H. ameivae* found here was morphologically and molecularly characterized, and the analysis of its phylogenetic position clearly showed that this parasite belongs to the genus *Hepatozoon* (Picelli et al. 2020).

Our results showed a relatively low prevalence for *Sauroplasma*-like and we thought that positive lizard species were not previously recorded for piroplasms (Table III). *Sauroplasma* infections are common in lizards, even though there are only three species described for these hosts: *Sauroplasma thomasi* du Toit, 1938, *Sauroplasma zonurum* Pienaar, 1962, and *Sauroplasma boreale* Svahn, 1976 (Telford 2009, Halla et al. 2014). In Brazil, these parasites were recently recorded in the freshwater turtle *Podocnemis expansa* (Picelli et al. 2016). Morphologically, they are small (2.5-4 µm) vacuole-shaped intraerythrocytic parasites with chromatin granules associated (Halla et al. 2014, Picelli et al. 2016). These morphological features mislead many authors to identify *Sauroplasma*-like inclusions as *Chelonoplasma*, *Nuttalia* or *Pirhemocyton* (Bardi et al 2019). They can also be overlooked as artefacts or bacterial and viral infections (Telford 2009). Parasitologists always pay attention to this conflicting taxonomic situation, but no molecular data is yet known for this genus.

Haemosporidian were the most predominant and richest taxon detected on lizards, mainly from Plasmodiidae parasites. It is well known that malaria parasites are widely distributed geographically, ubiquitous for most lizard families and are morphologically diverse, with over 100 species reported to infect reptiles (Schall 1996, Telford 2009). In the Eastern Brazilian Amazonia, 21 species of lizard malaria are known, and 13 (61%) of them were found in our research. For some of these (*Garnia* cf. *uranoscodoni* Lainson et al. 1975, *Garnia* cf. *multiformes* Lainson et al. 1975, *Garnia* cf. *utingensis* Lainson et al. 1971, *Fallisia* cf. *audaciosa* and *F.* cf. *effusa*), this finding is the first occurrence record away from their type localities. Recently, Matta et al. (2018) reported the presence of *Plasmodium kentropyxi* Lainson et al., 2001 and *Plasmodium carmelinoi* Lainson et al., 2010 in Teiidae lizards, at a low prevalence, in the Colombia Orinoco-Amazon basin. The difference between our findings is that here *P.* cf. *kentropyxi* was found at a relatively high prevalence only in its type host, *Kentropyx calcarata* Spix, 1825. Another interesting species seen in our study is *Plasmodium* cf. *tropiduri* Aragão & Neiva, 1909. It was the only haemosporidian species found in two different host species: *K. calcarata* and *Copeoglossum nigropunctatum* Spix, 1825. This haemoparasite was one of the world's first reptilian malaria parasites, described by Aragão & Neiva (1909) in the lizard *Tropidurus torquatus* Wied-Neuwied, 1820. Since then, it was observed across many lizard families and can be considered one of the most widespread saurian malaria species in South America (Telford 2009). In fact, most of the haemosporidians present here were previously reported in other Amazonian locations (Telford 2009, Matta et al. 2018), evidence that malaria species rediscovered here may be widely distributed throughout the biome. Phylogenetic and phylogeographic studies that involve samples from different Amazonian localities may provide insights regarding the diversification and evolution of this group.

Parasites of the genus *Trypanosoma* were restricted to two lizard species and at a low prevalence when compared to protozoans of the phylum Apicomplexa, which parasitised more than half of the captured lizards. However, these flagellates were found in several *U. superciliosus.*

Both *P. umbra* and *U. superciliosus* already had trypanosomatids recorded by Walliker (1965) and Lainson et al. (1975), respectively. The first author provided a poor morphological description of *Trypanosoma superciliosae* Walliker, 1965 without reporting their prevalence in *U. superciliosus* from the municipality of Codajás, Amazonas State. Interestingly, Lainson et al. (1975) mentioned that they searched, in Pará state, for this parasite in a large number of *U. superciliosus* individuals but were unsuccessful. Nevertheless, the same authors described *Trypanosoma plicae* Lainson et al., 1975 in *P. umbra.* Besides these species, there is only one other species described for this genus on Brazilian lizards: *Trypanosoma rudolphi,* recorded just once in *C. nigropunctatum* (Carini & Rudolph 1912). This low species richness is probably due to the lack of studies conducted on these parasites in Brazilian lizards. Indeed, trypanosome species have been reported worldwide in lizards more than in any other reptilian group (Fermino et al. 2019). Although trypanosomes have a unique stage of their life cycle by circulating in the blood of reptiles, trypomastigote forms exhibit high polymorphism and plasticity (Spodareva et al. 2018). Therefore, it is not possible to confirm that we found the same species described for those hosts, even with some morphological similarities.

Our data revealed a low prevalence of microfilaria, which are larval stages from nematodes of the superfamily Filaroidea. These vector-borne parasite larvae are commonly found in the peripheral blood of vertebrates and here, except for *U. superciliosu*s, all lizard species that we found positive for these parasites already had records for adult worms from many Onchocercidae species in other locations (Ávila & Silva 2010, Macedo et al. 2017). For *U. superciliosu*s, the occurrence of microfilariae has been vaguely reported in eastern Amazonia and these studies did not provide morphological characterisation of these nematodes (Lainson et al. 1975). In reptiles, Oswaldofilariinae, a onchocercid subfamily, stands out as the main filarid group that parasitise these hosts. Some genera that infected lizards include *Oswaldofilaria*, *Piratuboides* and *Piratuba* (Pereira et al. 2010). Adult worms from this taxon are recognised by the long distance between the head and vulva, and a series of other characters are used for species identification (Pereira et al. 2010). Given that there is scarce information on their larval morphology and we did not collect data related to the adult phase of these helminths, we are unable to advance the identification of this group in this study.

One of the most intriguing findings of our work was the intraerythrocytic inclusions of an uncertain nature. These vacuole-like inclusions appeared at a low prevalence and resembled some bacterial infections, caused by *Rickettsia*, and also to the viruses of the Lizard Erythrocytic Virus (LEV) group, such as *Pirhemocyton* (Telford & Jacobson 1993, Telford 2009). In fact, pirhemocytonosis are commonly found in lizards, mainly green iguanas (*Iguana iguana*), as white square vacuole-like cytoplasmic inclusions (Harr et al. 2001, Halla et al. 2014). Viral or bacterial infections have been reported in many amphibians and reptiles across the world and some of them can cause diseases in these hosts (Davies & Johnston 2000, Ariel 2011). However, these organisms are poorly studied and their diagnosis can be complex because it involves several approaches, including electron microscopy, serological surveys and molecular tools (Ariel 2011). Unfortunately, our knowledge about these inclusions and its occurrence throughout the Amazonian biome is very limited, and therefore we were unable to deepen in their identification.

Parasites commonly co-occur in the same host (Vaumourin et al. 2015, Galen et al. 2019), and we detected a high prevalence of this interaction. Indeed, we observed the co-occurrence of very distinct groups of haemoparasites in terms of life cycles, evolutionary history and in the exploitation of their hosts. The presence of an infracommunity in a host may be the result of a random occurrence of these parasites or a consequence modulated by the existence of a previous infection (Vaumourin et al. 2015, Hernandes-Córdoba & Braga 2019). Meanwhile, there are several challenges to understanding these interactions. Most previous studies ignored them, and only recently has the importance of such multiparasitism been recognised (Vaumourin et al. 2015). For lizards, parasitic ecological systems are frequently based on the one-on-one interactions and focus mainly on ecology of coccidian or malarial parasitism (Schall 1996, Amo et al. 2005, Hernandes-Córdoba & Braga 2019, Megía-Palma et al. 2020). From our perspective, there is still a long and curious path to explore until we can better understand haemoparasites and their lizard hosts.

This study is the first multi-species haemoparasite survey performed on lizard assemblages in Central Amazonia. We also present the most complete and updated list of haematozoan species described for these hosts in this region. Furthermore, our low-cost investigation using light microscopy demonstrates that Central Amazonia has a high prevalence and significant diversity with potential for new records of haemoparasites, especially malaria species. These findings might support future taxonomic characterisation of the parasites reported here, as well as further studies in parasite ecology and evolution. At last, our work emphasizes the importance of screening parasites in wildlife animals to allow a better understanding of the biodiversity of this biome.

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AMP, LAV, FAC and ILK conceived and designed the study. AMP, ACR and GSM performed the fieldwork. AMP and ACR processed the data and performed the microscopic analysis. AMP interpreted the results and worked on the manuscript. LAV, FAC and ILK contributed to critical reading of the manuscript and supervised the findings of this work. All authors took part on the preparation, revised and approved the final version of the manuscript.