ECOSYSTEMS

Effects of the habitat on anuran blood parasites in the Eastern Brazilian Amazon

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Abstract: Biological interactions play an important role in regulating and maintaining natural populations. Like most interactions, parasitism may be influenced by environmental conditions. Therefore, changes caused by human activity may drastically affect the equilibrium of the assemblages of parasitized organisms (hosts). Herein, we described the composition of hemoparasites of anurans from two distinct environments: forest and oil palm plantations. We identified the most frequent groups of blood parasites, and whether infections differ between habitats (forest and plantation) and between microhabitats (arboreal or terrestrial). We collected 128 anurans, of which 46 (36%) were parasitized by hemoparasites. The genus *Trypanosoma* spp. was found in 30% (n = 37/128) of the infected anurans in both habitats, recorded mostly in terrestrial anurans in oil palm plantations. Apicomplexa hemoprotozoans were also found in 13% (n=17/128) of the anurans, which mainly were terrestrial species collected in oil palm plantations. There was no difference in parasitism between the two assemblies and between the studied microhabitats. This is the first study that has analyzed the ecological relationship between anurans as hosts and their blood parasites, in a region under intense anthropic pressure, in the Brazilian Amazon.

Key words: Amphibians, hemoparasites, Neotropics, oil palm plantation, parasitism.

INTRODUCTION

Biological interactions play a key role in the dynamics of natural populations. Like most interactions, parasitism can be influenced by environmental conditions (Ferreira 1973, Von Zuben 1997), which affects the development of both infection and disease (Ferreira et al. 2012). A wide variety of pathogens may cause parasitic infections, such as bacterias, fungi, helminths, and protozoans (Blaustein & Kiesecker 2002, Luz & Faccini 2013), with direct transmission between the hosts or through biological vectors (Telford 2009).

Environmental changes on habitats may increase parasitism (Hudson et al. 2006, Froeschke et al. 2013, Cable et al. 2017), with adverse effects on the hosts. Such effects can include consequences on the ability to prey and reproduce, and therefore, obtain resources to survive (Von Zuben 1997, Moretti et al. 2017). For this reason, parasites play an important role in natural ecosystems (Hudson et al. 2006), affecting the life of their hosts at different levels, from the individual to the population, and therefore influencing natural selection (Marcogliese et al. 2009, Thompson et al. 2010, Goater et al. 2013).

The Anura is one of the most diverse group of vertebrates in the world, including 7400 species described to date (Frost 2021). The Neotropics encompass the highest diversity (Duellman 1988, 1999, Buckley & Jetz 2007), and in Brazil, about 1144 species were described (SBH 2021), of which 329 occur in the Amazon

region (Censo-Museu-Goeldi 2019). Anurans are considered indicators of environmental quality due to their behavioral and physiological demands, as their dependence on water bodies and humid sites, especially during reproductive periods (Hyne et al. 2009). Besides, many environmental and anthropogenic pressures (e.g., climate change, diseases and deforestation) have caused population decline among many species (Eterovick et al. 2005). In an extreme scenario, the diseases can potentially give rise to local extinction (Silvano & Segalla 2005). For example, chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis* (Bd), and has led to the extinction of amphibian populations in many tropical areas (Valencia-Aguilar et al. 2015). The spread of this disease has been associated with changes in land use and deforestation, increasing the vulnerability of the amphibians to the pathogens (Marcogliese et al. 2009).

As the anurans inhabit various terrestrial and aquatic microhabitats, they are more susceptible to hematophagous vectors and, consequently, become infected by blood parasites (Bardsley & Harmsen 1973, Barta & Desser 1984). A wide variety of blood protozoans infect anurans (Desser & Yekutiel 1986, Leal et al. 2009, Netherlands et al. 2015, Ferreira et al. 2020) and are exclusively vector-transmitted (e.g., dipterans, ticks, leeches, and others). Protozoans from the phylum Apicomplexa are the most common blood parasites of anurans, especially those of the haemogregarine group (Levine et al. 1980). Flagellates of the genus *Trypanosoma* (Phylum Euglenozoa) are other parasites usually found in anurans, in single or mixed infections (Honigberg 1963, Woo & Bogart 1984, Leal et al. 2009), transmitted by mosquitoes, phlebotomies, tsetse flies, triatomines, and fleas (Simpson et al. 2006). In addition, Trypanosomatidae may be found infecting plants, a wide variety

of invertebrates, and vertebrates worldwide (Vickerman 1976, Moreira et al. 2004).

Oil palm monoculture has been one of the booming activities in tropical regions (Bengtsson et al. 2000, Koh & Wilcove 2008). The conversion of forests into oil palm monocultures has been one of the threats to biodiversity in the Amazon (Faruk et al. 2013, Cunha et al. 2015, Vijay et al. 2016, Mendes-Oliveira et al. 2017). These drastic changes in land use have affected frogs in a number of ways, including their relationship with their parasites. In this study, we evaluated the differences between the parasites and the prevalence of blood parasites in the frog communities of different habitats, including oil palm plantations, forest areas, comparing arboreal and terrestrial environments.

MATERIALS AND METHODS

Study area

The study was carried out in various forest fragments and areas of oil palm plantations (*Elaeis guineensis*) within the property of AGROPALMA Company (02º13'00''S to 02º43'00''S and 48º54'00''W to 48º28'00''W), in the municipality of Tailândia, State of Pará, Brazil (Figure 1). The forest fragments are distributed amongst 64000 ha of the property and are covered mostly by dense upland rainforest (called terra-firme forest), with canopy trees up to 30 m in height. Small trees and lianas dominate the understory. In addition, there are permanent water bodies up to 20m in width, while temporary water bodies are formed during the rainy season. The oil palm plantations cover 39000 ha of the property, and in these areas, the understory is less complex than in the forests, with lower microhabitat diversity, dominated by gramineans and herbaceous plants, and water bodies that are formed after heavy rains. The local climate is humid equatorial,
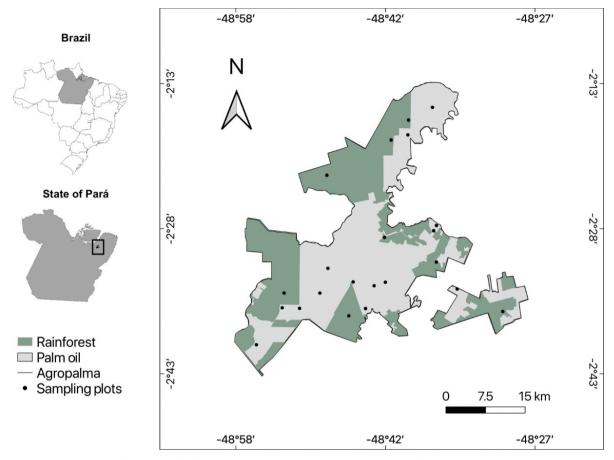
Figure 1. Map of the sampling sites within the property of AGROPALMA Company, in eastern Amazon. Black dots represent sites of forest and oil palm plantations where data was collected.

with a mean annual rainfall of 2344 mm, and a marked rainy season between December and May (mean peak of 427 mm), and a period of reduced precipitation, reaching a maximum of 54 mm in September (Albuquerque et al. 2010). Mean annual air temperature is approximately 26ºC, and mean relative humidity of the air is approximately 85% (Oliveira et al. 2000).

Data collection

We collected the anurans in forest habitats and oil palm plantations in two field expeditions, carried out in April 2016 and 2017, during the rainy season. We installed 12 plots of 30 x 70 m within the forest fragments and ten plots, of the same size, in oil palm plantations. All plots had a water body inside and had a minimum distance of 1 km between them. The forest plots were located at 1 Km from the forest edge in order to avoid the influence of the as edge effects (Correa et al. 2015). Surveys for the presence of anurans were made through active visual searches. We moved through the plot searching for anurans on different substrates, such as litter, leaf surfaces, trunks, tree holes and water bodies. In each plot, we conducted the surveys by three experienced researchers during the day, between 2:00 pm and 5:00 pm, and at night between 7:00 pm and 10:00 pm, the inspections took place in the same plots in the two years.

The captured frogs were taken to the laboratory, and then euthanized by the application of anesthetic in the form of an

ointment, xylocaine, in the ventral region. We followed the procedures established by the Federal Council of Veterinary Medicine (CFMV) (Ordinance 1000, 2012). In each individual, we made a ventral incision to collect blood samples directly from the heart with the aid of a syringe to make of at least two smears. All individuals were preserved in 10% formaldehyde, for 24 hours, transferred to 70% alcohol and deposited in the temporary Herpetological Collection of the Federal University of Pará and will soon be deposited at the "Museu Paraense Emilio Goeldi", as they are being used for other studies of natural history, which are linked to a larger project, the Conservation International Brazil and AGROPALMA Company, under the license 8716-1 (process: 12420-2). For each individual, the following data were recorded: date of collection, field number, species, sampling plot, tissue sample and blood smear for parasitism analysis, substrate, microhabitat, weight, and size.

The smears were fixed in cold methanol for one minute. Subsequently, they were stained for 30 minutes, using Giemsa diluted in buffered water. To confirm the presence of hemoparasites, blood smears were analyzed using an Olympus BX41 microscope at 100x total magnification, observing 20 fields of each slide. The images were captured with a CCD Camera (AxioCam MR monochrome) coupled to a Zeiss Axiophot microscope controlled by the Axiovision 3.0 software. The characterization of hemoparasites was carried out through the morphological analysis of each parasitized sample. Molecular analysis was not possible. The parasites of the genus *Trypanosoma* were evaluated from the morphology analysis following the classification proposed by Diamond (1965), Desser (2001) and Zickus (2002), including five groups: Group A: Flat and leaf-shaped body (Figure 2: 1, 2); Group B: Body, in frontal view, flat, but not in the form of a leaf; laterally, it presents a wide anterior
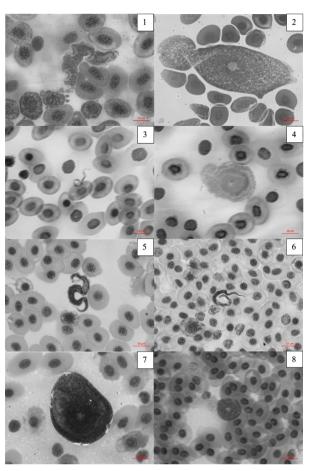

Figure 2. Characterization of *Trypanosoma* parasites found in anurans from forest areas and oil palm plantations in the municipality of Tailândia, Pará, Brazil. 1) Group A (*Osteocephalus oophagus*); 2) Group A (*Scinax* gr. *ruber*); 3) Group B (*Leptodactylus fuscus*); 4) Group C (*Physalaemus ephippifer*); 5) Group D1 (*Leptodactylus macrosternum*); 6) Group D2 (*L. macrosternum*); 7) Group F (*Boana geographica*); 8) Group F (*L. macrosternum*). Scale = 10 µm. Species between parentheses = Anurans.

region, starting from the kinetoplast, which narrows later (Figure 2: 3); Group C: Robust body, frontally compressed and ovoid; laterally robust, elliptical, about twice as long as it is wide (Figure 2: 4); Group D: Elongated body, narrow extremities; D1: Kinetoplast closer to the nucleus than to the posterior end (Figure 2: 5); D2: Kinetoplast closer to the posterior end than to the nucleus (Figure 2: 6); Group F: Spherical body (Figure 2: 7, 8). Apicomplexa hemoprotozoans
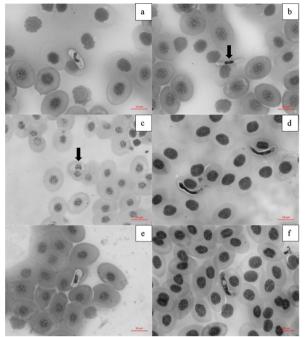
Figure 3. Hemoparasites of the phylum Apicomplexa in the peripheral blood of the anurans sampled in forest and oil palm plantations, Eastern Amazon, Brazil: a (*Leptodactylus pentadactylus*); b (*L. pentadactylus*); c (*L. macrosternum*); d (*L. pentadactylus*); e (*L. pentadactylus*); f (*L. pentadactylus*). Scale = *10 µm.* Species between parentheses = Anurans.

were characterized with the help of specialists of the Parasitology laboratory of the Federal University of Pará and following Telford (2009).

Data analysis

We used a Mann-Whitey U test (Zar 2010) and chi-square test ($\chi 2$) with Yates' correction and significance level P ≤ 0.05 to analyze whether there is any difference in the prevalence of infection with blood parasites (hemogregarines and trypanosomes) in anurans between forest and oil palm plantations. We used the same test to analyze whether there is any difference in the prevalence of infection with blood parasites in anurans between microhabitats (arboreal and terrestrial). The analyses were run in R Project software (R Development Core Team 2017).

RESULTS

We made blood smears from 128 anurans representing 27 species of five families and 13 genera, of the total species, 21 occurring in the forest, 14 from oil palm plantations, and 8 occurring in common in the two areas. We collected 65 individuals from forest fragments and 63 from oil palm plantations. Sixteen arboreal anuran species (68 individuals) and 12 terrestrial species (60 individuals). We found 36% (n = 46/128) of the individuals with hemoparasites (Table I), 28% (n = 18/65) of which were found in forest fragments and 44% (n = 28/63) in oil palm plantations. Terrestrial anurans had prevalence varying from 31% (n = 8/26) to 62% (n = 21/34), in forest and oil palm plantation areas, respectively. For arboreal anurans the proportion of infected hosts was similar for both habitats, 24% (n = 7/29) in oil palm plantations, and 26% (n = 10/39) in forest sites. Regarding the anuran species, the most infected in oil palm plantations were *Leptodactylus fuscus* (100%; n = 4/4) and *Leptodactylus macrosternum* (82%; n = 9/11), and in forest fragments were *Trachycephalus typhonius* (100%; n = 2/2) and *Leptodactylus pentadactylus* (71%; n = 5/7). The host species that showed the lowest prevalence were *Boana multifasciata* (10%; n = 1/10) in forest, and *Physalaemus ephippifer* (20%; n = 1/5) in oil palm plantation (Table I).

Among positive individuals from both environments, we found 80% (n = 37/46) were infected with trypanosomes (Figure 2) and 37% (n = 17/46) with apicomplexan hemoparasites (Figure 3). Mixed infections by these two parasites groups were observed at a prevalence of 6.2% (n = 8/128). *Leptodactylus pentadactylus* was the only species to harbor mixed infections in the forest area, with a prevalence of 6.1% (n = 4/65). In oil palm plantations, 6.3% (n = 4/63) of the individuals, all terrestrial species (*Leptodactylus macrosternum* and *Leptodactylus mystaceus*), showed mixed infections.

Table I. Anurans with hemoparasites infections in forest fragments and oil palm plantation areas within the property of AGROPALMA Company, in Tailândia, Pará, Brazil. Api = Apicomplexa hemoprotozoans; Tryp = *Trypanosoma*.

Habitat type	Species	Arboreal	Terrestrial	Hemoparasites/ Anurans
Forest	*Boana cinerascens*	7		-
	Boana geographica	3 of 7		Tryp (3)
	Boana multifasciata	1 of 10		Tryp (1)
	Dendropsophus leucophyllatus	1		-
	Dendropsophus minutus	1 of 1		Tryp (1)
	Leptodactylus fuscus		1 of 3	Tryp (1)
	Leptodactylus macrosternum		1	-
	Leptodactylus pentadactylus		5 of 7	Api (5); Tryp (4)
	Leptodactylus petersii		2	-
	Osteocephalus leprieurii	1		-
	Osteocephalus oophagus	1 of 1		Tryp (1)
	Phyllomedusa bicolor	1 of 3		Tryp (1)
	Physalaemus ephippifer		5	-
	Pithecopus hypochondrialis	1 of 3		Tryp (1)
	Rhaebo guttatus		1	-
	Rhinella gr. *margaritifera*		1 of 4	Tryp (1)
	Rhinella marina		2	-
	Rhinella mirandaribeiroi		1 of 1	Tryp (1)
	Scinax nebulosus	2		-
	Trachycephalus typhonius	2 of 2		Tryp (2)
	Vitreorana oyapiensis	1		-
Total	**21**	**10 of 39**	**8 of 26**	**Api (5); Tryp (17)**
Oil palm plantation	*Boana multifasciata*	3 of 6		Api (2); Tryp (1)
	Leptodactylus fuscus		4 of 4	Api (1); Tryp (3)
	Leptodactylus macrosternum		9 of 11	Api (4); Tryp (7)
	Leptodactylus mystaceus		2 of 4	Api (1); Tryp (2)
	Leptodactylus paraensis		2 of 2	Api (1); Tryp (1)
	Physalaemus ephippifer		1 of 5	Tryp (1)
	Pithecopus hypochondrialis	7		-
	Rhinella marina		1 of 1	Api (1)

Table I. Continuation.

	Rhinella mirandaribeiroi		2 of 7	Tryp (2)
	Scinax boesemani	1		-
	Scinax gr. *ruber*	2 of 8		Api (1); Tryp (2)
	Scinax x-signatus	1		-
	Sphaenorhynchus lacteus	1		-
	Trachycephalus typhonius	2 of 5		Api (1); Tryp (1)
Total	**14**	**7 of 29**	**21 of 34**	**Api (12); Tryp (20)**

We identified six of the seven proposed morphological groups of trypanosomes (Table II), showing a high morphological diversity of trypomastigote forms. In addition, only in forest, we found 17 out of 65 individuals with trypanosomes in their peripheral blood, most of them arboreal (*Dendropsophus minutus, Boana geographica, Boana multifasciata, Osteocephalus oophagus, Phyllomedusa bicolor, Pithecopus hypochondrialis* and *Trachycephalus typhonius*) (Table II). In oil palm plantations, 17 out of 20 individuals infected by trypanosomes were terrestrial (*Leptodactylus fuscus, Leptodactylus macrosternum, Leptodactylus mystaceus, Leptodactylus paraensis, Physalaemus ephippifer* and *Rhinella mirandaribeiroi*) (Table II).

The second group of parasites observed was apicomplexan hemoprotozoans (Figure 3), occurring in 13% (n = 17/128) of the sampled hosts. Among these infected anurans, 19% (n = 12/63) were found in the oil palm plantation area and 7.6% (n = 5/65) in the forest area, mainly in terrestrial anurans 82% (n = 14/17) from six species (Table II), while in arboreal anurans they were discovered in only three individuals all within the oil palm plantation.

We analyzed whether there is any difference in parasitism between individuals in forest areas 28% (n = 18/65) and oil palm plantations 44% (n = 28/63) and found no difference (X^2 = 3.206, P = 0.073). We made the same comparison using only

individuals infected with trypanosomes in forest areas 26% (n = 17/65) and oil palm plantations 33% (n = 21/63) and once again observed no difference (X^2 = 0.483, P = 0.486).

Considering the forest plots, three of them were positioned close to the edge. To check if these plots could be influencing the results, so a test was carried out, which showed no differences (X^2 = 0.113, P = 0.737) between the infected anurans collected inside the forest (n = 13/54) and the infected anurans collected close to the forest edge (n = 4/11). Also, there was no difference when randomly selecting the three plots within the forest (n = 5/19) and comparing them with the three plots close to the edge (X^2 = 0.312, P = 0.575).

The analyses of the Mann-Whitney U test showed no differences between the prevalence of parasitism in anurans found in the forest and oil palm plantations (P = 0.329). We also tested for differences in the prevalence of parasitism according to the habit of anurans (arboreal and terrestrial) in each habitat type. We found that in oil palm plantations 62% (n = 21/34) there were more parasitized terrestrial anurans than in forest areas 31% (n = 8/26) (X^2 = 4.494, P = 0.034), whereas the prevalence of parasitism in arboreal anurans did not differ between the forest 26% (n = 10/39) and oil palm plantations 28% (n = 8/29) (X^2 = 0, P = 1.00).

In the forest and oil palm plantations we also found differences in the distribution of

hemoparasites, with more individuals infested with trypanosomes 26% (n = 17/65) than apicomplexa hemoprotozoans 7% (n = 5/65) in the forest (X^2 = 6.620, P = 0.010), while there was no difference between infections with trypanosomes 33% (n = 21/63) and apicomplexa hemoprotozoans 17% (n = 11/63) in oil palm plantations (X^2 = 3.393, P = 0.065).

DISCUSSION

In this study, the prevalence was relatively high and contrasts with other results observed in anurans in modified areas. In Brazil, Leal et al. (2009) found 20% of the 40 anurans collected in São Paulo (Atlantic Forest) and Mato Grosso do Sul (Pantanal) were infected with hemoparasites. Similarly, in northern South Africa, Netherlands et al. (2015) observed that only 20% of the 436 anurans were infected in three different landscapes: reserve, rural activity and sugar cane agriculture. These differences can be explained by the different levels of human disturbance that occur in these locations. However, it is necessary to emphasize that the Amazon region, where our study was conducted, is recognized for harboring a great diversity of host fauna and their hemoparasites (Lainson et al. 1992, Picelli et al. 2020), although this last one is little known in anurans.

Regarding blood parasites in anurans, trypanosomes were the most prevalent in both habitats. These protozoans are commonly found in anurans blood worldwide (Bardsley & Harmsen 1973), with a large number of hematophagous vectors (Simpson et al. 2006). We observed that 16 out of 17 species in both habitats (forest and oil palm plantations) were infected by trypanosomes, except *Rhinella marina*. However, Attias et al. (2016) observed that this species is susceptible to trypanosome

infections too. Recently, Úngari et al. (2021) carried out a study in the state of Mato Grosso and captured three host species in common to our survey, *L. petersii, R. mirandaribeiroi* and *T. typhonius*, but without finding hemoparasites in the last two anuran species as we did here.

Besides the morphological diversity of trypanosomes infecting anurans, one single *Trypanosoma* species may have a high degree of polymorphism when it infects different anuran species (Scorza & Dagert 1958, Spodareva et al. 2018). These morphological differences may be caused by the differential expression of the same genome or by infection from several lineages of the same parasite (Ferreira et al. 2007). Despite the lack of molecular analysis, we found a high morphological diversity, based on Diamond (1965), together with other studies (Desser 2001, Zickus 2002), which allowed us to identify six morphological groups of trypanosomes. Our results show that most infections of trypanosomes were caused by morphotypes A and D, indicating a wide distribution of those flagellates in both habitats (forest and oil palm plantations). In another study, Zickus (2002) identified four morphotypes that were attributed to *Trypanosoma rotatorium*. These morphotypes were found in the peripheral blood of the anurans from Lithuania in the same prevalence in both habitat types of the study (Zickus 2002).

Similarly, infections of apicomplexan hemoprotozoans occurred more often in oil palm plantations, where 12 individuals 43% (n = 12/28) were infected. In forest areas we found only five terrestrial anurans 28% (n = 5/18), all of which *L. pentadactylus* were infected with apicomplexa hemoprotozoans. However, they appeared less frequently in the total of analyzed anurans. Our findings differ, for example, from those of other studies conducted in Africa, where hemogregarines are more frequent in the

Table II. Parasites of the genus *Trypanosoma* in anurans from forest areas and oil palm plantations in the municipality of Tailândia, Pará, Brazil.

Individuals/Habitat	Habit	*Trypanosoma* groups
Forest	Arboreal	D2
Dendropsophus minutus		
Boana multifasciata	Arboreal	A
Boana geographica	Arboreal	A; F
Boana geographica	Arboreal	A; D1
Boana geographica	Arboreal	D1
Leptodactylus fuscus	Terrestrial	D2
Leptodactylus pentadactylus	Terrestrial	D1
Leptodactylus pentadactylus	Terrestrial	A; D1; D2; F
Leptodactylus pentadactylus	Terrestrial	D1
Leptodactylus pentadactylus	Terrestrial	D1
Osteocephalus oophagus	Arboreal	A
Phyllomedusa bicolor	Arboreal	A; C
Pithecopus hypochondrialis	Arboreal	A
Rhinella mirandaribeiroi	Terrestrial	F
Rhinella gr. *margaritifera*	Terrestrial	F
Trachycephalus typhonius	Arboreal	A
Trachycephalus typhonius	Arboreal	A
Oil palm plantations	Arboreal	D2
Boana multifasciata		
Leptodactylus fuscus	Terrestrial	B; D1; D2
Leptodactylus fuscus	Terrestrial	A; D1; D2
Leptodactylus fuscus	Terrestrial	A; F
Leptodactylus macrosternum	Terrestrial	A; D1; D2
Leptodactylus macrosternum	Terrestrial	A
Leptodactylus macrosternum	Terrestrial	D2
Leptodactylus macrosternum	Terrestrial	D1; F
Leptodactylus macrosternum	Terrestrial	F
Leptodactylus macrosternum	Terrestrial	A
Leptodactylus macrosternum	Terrestrial	D1; D2
Leptodactylus macrosternum	Terrestrial	A
Leptodactylus mystaceus	Terrestrial	A
Leptodactylus paraensis	Terrestrial	F
Physalaemus ephippifer	Terrestrial	C
Rhinella mirandaribeiroi	Terrestrial	D2
Rhinella mirandaribeiroi	Terrestrial	D2

Table II. Continuation

Scinax gr. *ruber*	Arboreal	A; D1
Scinax gr. *ruber*	Arboreal	A; D1
Trachycephalus typhonius	Arboreal	D2
Total = 37		

blood of anurans than other protozoans (Readel & Goldberg 2010, Netherlands et al. 2015).

Comparing the number of infections between forest and oil palm plantations, we observed that, in most cases, infections were by *Trypanosoma* and more frequent in oil palm plantations. Anurans in disturbed areas are more susceptible to interactions with hematophagous vectors of these parasites (Borkent 2008, Bernal & Pinto 2016). Dipterans of the genus *Corethrella* are known to be vectors of trypanosomes (Johnson et al. 1993, Ferguson & Smith 2012, Aihara et al. 2016) and benefit from changes in habitat, which in turn increases the chances of interactions with anurans and the transmission of blood parasites (Ambrozio-Assis et al. 2018).

In the human population, a similar effect can be observed through the transmission of pathogens, where changes in climatic factors associated with a high level of disturbances may affect the transmission of vector-transmitted pathogens (Cable et al. 2017). As a consequence, both transmission and occurrence of parasites may increase or decrease due to human activities. In other words, although parasitism is a natural interaction, its effects differ, and this interaction may cause diseases with habitat changes (Bower et al. 2019). Therefore, the composition and diversity of the parasite community may vary, and indicating environmental stress (Marcogliese 2005). Parasites of amphibians are indicators of ecosystem health and animal biodiversity, acting as indicators for conservation (King et al. 2010).

Homogeneous and less complex environments, such as monocultures, usually have a significant loss of biodiversity. Concerning oil palm plantations, this environment supports a much smaller diversity of anurans than preserved forests (Correa et al. 2015). In addition to the decrease in the diversity of anurans, it was observed that animals infected with blood parasites were mainly terrestrial species, indicating that anurans are more susceptible to stress due to habitat conversion (McMahon et al. 2017) and pathogen vectors (Ferguson & Smith 2012). Therefore, preserved forests are essential in maintaining a great diversity of microhabitats and the fauna that exploits these microhabitats.

In our study, we carried out the detection of parasites by microscopic observation. In wild animals, the incidence of parasites in a population can be calculated using three techniques, which are not mutually exclusive: blood culture, molecular diagnosis, or microscopic observation of peripheral blood samples. The isolation of these parasites by means of culture in axenic media is limited to studies of extracellular parasites, preventing the performance of the procedure with intra-erythrocyte parasites (Ferreira et al. 2007, Attias et al. 2016). On the other hand, microscopic observation allows the detection of various parasites belonging to different taxa, but their sensitivity is compromised in infections with a low parasitic load (Leal et al. 2009, Rajabi et al. 2017, Picelli et al. 2020). Finally, the use of molecular tools (PCR) allows both the detection of parasites with high sensitivity and the sequencing for phylogeny studies (Ferreira et al. 2020). However, the intrinsic specificity

of molecular techniques can compromise the detection of parasites with high diversity. In addition, studies that combine morphology and molecular analysis are considered more complete (de Oliveira et al. 2018, Isaak-Delgado et al. 2020).

The morphological observation allowed us to conclude that hemoparasites are highly diverse in both types of habitat. In this scenario, new studies are extremely important and recommended in our region, using principally molecular techniques, as well and also the characterization of possible vectors, which may assist researchers to recognize and understand the diversity and understand the degree of alterations in the transmission of pathogens of anurans in altered environments.

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SRCP, SRM and MCSC started and designed the study. The fieldwork was carried out by SRCP, IBS, FSC and MCSC. Microscopic analysis, images, map and final data were performed by SRCP, IBS, RLO and SRM. SRCP and FSC interpreted the results and the organization of the manuscript. All authors approved the revisions and the final version.