



MINISTÉRIO DA  
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**MINISTÉRIO DA CIÊNCIA TECNOLOGIA E INOVAÇÃO  
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA -  
INPA PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA**

**INTERAÇÃO ENTRE HEMOPARASITOS EM ANUROS DA  
AMAZÔNIA CENTRAL: COINFECÇÃO, SUSCETIBILIDADE  
E FATORES DETERMINANTES**

**FRANCISCA JUÇARA CAVALCANTE DE ARAGÃO**

**MANAUS-AM  
MARÇO/2025**

FRANCISCA JUÇARA CAVALCANTE DE ARAGÃO

INTERAÇÃO ENTRE HEMOPARASITOS EM ANUROS DA AMAZÔNIA  
CENTRAL: SUSCETIBILIDADE, COINFECÇÃO E FATORES  
DETERMINANTES

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Dissertação apresentada ao Programa de  
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MANAUS-AM  
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## PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

### ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 31 dias do mês de março do ano de 2025, às 14:h00min, via videoconferência, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o Dr. **Adriano Teixeira de Oliveira**, do Instituto Federal do Amazonas – IFAM, a Dr<sup>a</sup>. **Jussara Santos Dayrell**, do Instituto Nacional de Pesquisas da Amazônia – INPA e a Dr<sup>a</sup>. **Luciana Frazão Luiz**, da Universidade de Coimbra – UC, sendo os suplentes o Dr. Pedro Aurélio Costa Lima Pequeno, do Sínteses da Biodiversidade Amazônica – INCT – SinBiAm e a Dr<sup>a</sup>. Amanda Maria Picelli, da Universidade Villanova, sob a presidência do orientador, a fim de proceder a arguição pública da **DISSERTAÇÃO DE MESTRADO** de **FRANCISCA JUÇARA CAVALCANTE DE ARAGÃO**, intitulada: **“INTERAÇÃO ENTRE HEMOPARASITOS E ANUROS DA AMAZÔNIA CENTRAL: SUSCETIBILIDADE, COINFEÇÃO E FATORES DETERMINANTES”**, orientada pelo Dr. William Ernest Magnusson, do Instituto Nacional de Pesquisas da Amazônia – INPA e coorientada pelo Dr. Igor Luis Kaefer, da Universidade Federal do Amazonas – UFAM.

Após a exposição, o(a) discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

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**Sinopse:**

Neste estudo, investigou-se a interação entre hemoparasitos e anuros da Amazônia Central, analisando a suscetibilidade diferencial das espécies à infecção, bem como a coinfecção por tripanossomas e hemogregarinas e a influência mútua entre esses parasitos. Além de avaliar a relação entre a abundância de hemoparasitos com as características do habitat e do hospedeiro, como a altura do poleiro e a proximidade de corpos d'água.

**Palavras-chave:** Anfíbios, interação parasito-hospedeiro, hemoparasitos, fatores intrínsecos e extrínsecos.

*“I love not man the less, but nature more”*

**Lord Byron**

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## RESUMO

Anfíbios anuros (Chordata, Amphibia) são hospedeiros de uma ampla variedade de parasitos, entre os quais se destacam os protozoários sanguíneos, como hemogregarinas (Apicomplexa, Adeleorina)–*Dactylosoma* spp. e *Hepatozoon* spp.– e tripanossomas (Euglenozoa, Trypanosomatidae)–*Trypanosoma* spp. A suscetibilidade dos anuros às infecções parasitárias pode variar de acordo com o comportamento, habitat e interações interespecíficas. No entanto, essas generalizações são baseadas principalmente em estudos sobre outros grupos taxonômicos e em sistemas ecológicos que não são Neotropicais. Nesse sentido, investigamos como fatores ecológicos e ambientais podem influenciar a interação entre hemoparasitos (tripanossomas e hemogregarinas) e seus hospedeiros anuros da Amazônia Central, Brasil. Para tanto, os animais foram coletados em duas localidades no estado do Amazonas: (i) a Reserva de Desenvolvimento Sustentável do Rio Negro (RDS-Rio Negro) no município de Iranduba; e (ii) a Reserva Florestal Adolpho Ducke (Reserva Ducke) em Manaus. Os anuros foram identificados, sexados, medidos no comprimento rostro-urostilar (CRU) e da tíbia, e sua massa corporal foi registrada. Foi medida também a altura do local (poleiro) onde cada anuro foi coletado. Amostras de sangue foram coletadas por punção cardíaca para a confecção de esfregaços, que foram fixados com metanol absoluto por 3 min e corados com Giemsa 10% por 40 min. Para detectar os hemoparasitos, os esfregaços sanguíneos foram examinados em microscópio óptico sob os aumentos 400x e 1000x. A quantidade de parasitos (abundância) foi estimada pela contagem do número de parasitos visualizados em 100 campos de 100 eritrócitos, totalizando 10.000 eritrócitos. Testes estatísticos foram aplicados para avaliar a suscetibilidade das diferentes espécies de anuros à infecção por hemoparasitos, e a associação entre infecção por tripanossomas e hemogregarinas. Modelos lineares generalizados foram utilizados para investigar a influência de características ambientais e do hospedeiro, como proximidade de corpos d'água e altura do poleiro, na abundância de parasitos. No total, foram amostrados 123 anuros de 14 espécies, dos quais 33,3% (n = 41) estavam positivos para hemoparasitos. Desses, 95,1% (n = 39/41) estavam infectados por tripanossomas e 53,6% (n = 22/41) por hemogregarinas. A ocorrência de coinfeção desses dois grupos de hemoparasitos foi de 16,3% (n = 20/123). Algumas espécies apresentaram maior suscetibilidade à

infecção do que outras, onde *Osteocephalus oophagus* foi a espécie mais parasitada. A coinfeção por hemoparasitos foi significativa, sugerindo interações entre tripanossomas e hemogregarinas dentro do hospedeiro. A proximidade de corpos d'água apresentou uma relação fraca com a abundância de hemoparasitos. No entanto, indivíduos da mesma espécie coletados em alturas maiores mostraram menor abundância de hemogregarinas, indicando que o estrato vertical pode apresentar diferentes níveis de exposição aos vetores, com alturas maiores sendo menos expostas. Este estudo foi o primeiro a abordar os aspectos ecológicos da relação entre hemoparasitos e seus hospedeiros anuros na Amazônia Central. Além disso, nossos resultados indicam que o habitat pode influenciar na transmissão e prevalência de hemoparasitos, mas as características individuais do hospedeiro podem ter um papel ainda mais determinante na dinâmica das infecções.

Palavras-chave: Anfíbios, interação parasito-hospedeiro, hemoparasitos, fatores intrínsecos e extrínsecos

## ABSTRACT

The susceptibility of anuran amphibians to parasitic infections can vary according to behavior, habitat and interspecific interactions. However, these generalizations are mainly based on studies of other taxonomic groups and ecological systems that are not Neotropical. In this sense, we investigated how ecological and environmental factors can influence the interaction between hemoparasites (trypanosomes and hemogregarines) and their anuran hosts in Central Amazonia. To detect the presence of hemoparasites, blood smears were examined using light microscopy to identify and quantify the parasites. Statistical tests were applied to assess the susceptibility of different anuran species to hemoparasite infection and to determine the association between trypanosome and hemogregarine infections. Generalized linear models were used to investigate the influence of environmental and host characteristics, such as proximity to water bodies and perch height, on the abundance of parasites. We found a hemoparasite prevalence of 33.3% (n = 41/123) and some species were more susceptible to infection than others, of which *Osteocephalus oophagus* was the most parasitized species. Co-infection by hemoparasites was significant, suggesting interactions between trypanosomes and hemogregarines within the host. Proximity to water bodies showed a weak relationship with the abundance of hemoparasites. However, individuals of the same species collected at higher elevations showed a lower quantity of hemogregarines in the blood smears examined, indicating that the vertical stratum may present different levels of exposure to vectors, with higher elevations being less exposed. Our results indicate that the habitat can influence the transmission and prevalence of hemoparasites, but the individual characteristics of the host can play an even more decisive role in the dynamics of infections.

Keywords: Anuran, parasite-host relationship, hemogregarines, intrinsic and extrinsic factors, trypanosomes.

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## Introdução geral

Os parasitos podem ser definidos como qualquer organismo que obtém benefícios de viver às custas de outro (hospedeiro), gerando um custo para esses indivíduos (NORTHROP-CLEWES & SHAW, 2000). Embora sejam conhecidos pelos prejuízos causados em seus hospedeiros, ecologicamente os parasitos são relevantes por conseguirem exercer efeitos importantes na cadeia trófica (HUDSON et al., 2006), sendo que alguns podem atuar no controle populacional ao interferir diretamente no ajustamento evolutivo (aptidão) dos seus hospedeiros (MARCOGLIESE, 2009).

Quando um patógeno altera o comportamento ou a imunidade, ele compromete o ajustamento individual do hospedeiro, potencialmente modificando a estrutura de populações e de comunidades (POULIN, 1999, 2007). Apesar disso, alguns aspectos que estruturam essa dinâmica parasito-hospedeiro ainda são desconhecidos, dentre os quais se destacam os padrões de propagação e a sua heterogeneidade distribuída dentro de espécies (CAMPIÃO, 2020, ENGELSTÄDTER, 2006).

Parte dessa lacuna decorre dos distintos papéis ecológicos que os parasitos podem exercer, os quais se refletem em ciclos de vida variados e, por vezes, altamente especializados. Por desempenharem diferentes papéis ecológicos, parasitos frequentemente possuem ciclos de vida bastante complexos, os quais podem envolver um ou mais hospedeiros, caracterizando ciclos monoxeno e heteroxeno, respectivamente. No caso deste último, os hospedeiros que participam desse ciclo heteroxeno são caracterizados de acordo com a fase de desenvolvimento do parasito, sendo o hospedeiro definitivo aquele que abriga as fases sexualmente ativas do parasito, enquanto o hospedeiro intermediário possui as formas não-sexuais do parasito (SCHMID-HEMPEL, 2021). Além disso, parasitos utilizam-se de diferentes estratégias de transmissão entre seus hospedeiros, dentre as quais há aquela que depende de um organismo (vetor) que atue na dispersão de um hospedeiro para outro (POULIN & RANDHAWA, 2015). Vetores, geralmente, são hospedeiros invertebrados que transmitem o patógeno enquanto se alimentam não letalmente dos fluidos internos de outro hospedeiro (WILSON *et al.*, 2017).

Devido ao interesse econômico, estudos sobre relações ecológicas parasitárias são direcionados a poucos grupos de hospedeiros, tais como humanos

pelo interesse médico ou alguns animais silvestres pelo potencial zoonótico (SPODAREVA *et al.*, 2018). Entretanto, embora subestimada, a riqueza de parasitos sanguíneos (hemoparasitos) registrada em anfíbios e répteis é maior do que em outros grupos de vertebrados como aves e mamíferos (SOUZA *et al.*, 2021, TELFORD, 2009). Dentre os protozoários que podem ser encontrados em anfíbios anuros (sapos, rãs e pererecas) está o filo Apicomplexa, o qual inclui as hemogregarinas, e o gênero *Trypanosoma* que possui a maior riqueza registrada em anfíbios anuros do que outros grupos de vertebrados, sendo descritas mais de 60 espécies desses tripanossomatídeos (SOUZA *et al.*, 2021, PINHO, 2018).

Neste trabalho, investigamos se todas as espécies de anuros são igualmente suscetíveis à infecção por hemoparasitos em duas localidades na Amazônia central, e se a infecção por um grupo de hemoparasito (*trypanosoma* ou hemogregarina) influencia a infecção pelo outro grupo de hemoparasito. Também avaliamos se características intrínsecas ou extrínsecas dos anuros influenciam a abundância de hemoparasitos nos hospedeiros.

## Objetivos

### Objetivo geral

Investigar a relação parasito-hospedeiro, interação entre hemoparasitos e fatores determinantes na ocorrência e abundância de tripanossomas e hemogregarinas na Amazônia Central.

### Objetivos específicos

- I. Comparar a taxa de infecção por tripanossomas e hemogregarinas entre diferentes espécies de anuros, identificando possíveis padrões de suscetibilidade;
- II. Investigar a relação entre a coinfeção por diferentes grupos de hemoparasitos, analisando possíveis interações sinérgicas ou competitivas dentro dos hospedeiros;
- III. Avaliar a influência de características dos hospedeiros e do habitat, como a altura do poleiro e a proximidade de corpos d'água, na abundância e prevalência de hemoparasitos nos anfíbios anuros.

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**CAPÍTULO ÚNICO**

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INTERACTION BETWEEN HEMOPARASITES AND ANURANS IN CENTRAL AMAZON:  
SUSCEPTIBILITY, CO-INFECTION AND DETERMINING FACTORS

## INTERACTION BETWEEN HEMOPARASITES AND ANURANS IN CENTRAL AMAZON: SUSCEPTIBILITY, CO-INFECTION AND DETERMINING FACTORS

Francisca J. C. de Aragão, Matheus G. B. Rosa, Keillen M. M. Campos, Lina M. P. Cortes, Amanda M. Picelli, Claudia M. R. Velasquez, Felipe A. C. Pessoa, Igor L. Kaefer, William E. Magnusson

### ABSTRACT

The susceptibility of anuran amphibians to parasitic infections can vary according to behavior, habitat and interspecific interactions. However, these generalizations are mainly based on studies of other taxonomic groups and ecological systems that are not Neotropical. In this sense, we investigated how ecological and environmental factors can influence the interaction between hemoparasites (trypanosomes and hemogregarines) and their anuran hosts in Central Amazonia. To detect the presence of hemoparasites, blood smears were examined using light microscopy to identify and quantify the parasites. Statistical tests were applied to assess the susceptibility of different anuran species to hemoparasite infection and to determine the association between trypanosome and hemogregarine infections. Generalized linear models were used to investigate the influence of environmental and host characteristics, such as proximity to water bodies and perch height, on the abundance of parasites. We found a hemoparasite prevalence of 33.3% ( $n = 41/123$ ) and some species were more susceptible to infection than others, of which *Osteocephalus oophagus* was the most parasitized species. Co-infection by hemoparasites was significant, suggesting interactions between trypanosomes and hemogregarines within the host. Proximity to water bodies showed a weak relationship with the abundance of hemoparasites. However, individuals of the same species collected at higher elevations showed a lower quantity of hemogregarines in the blood smears examined, indicating that the vertical stratum may present different levels of exposure to vectors, with higher elevations being less exposed. Our results indicate that the habitat can influence the transmission and prevalence of hemoparasites, but the individual characteristics of the host can play an even more decisive role in the dynamics of infections.

Keywords: Anuran, parasite-host relationship, hemogregarines, intrinsic and extrinsic factors, trypanosomes.

## 1. INTRODUCTION

The ecology of anurans (Chordata, Amphibia) and their interactions with parasites are topics of growing interest in conservation biology and disease epidemiology, and have gained notoriety with the increasing cases of chytridiomycosis that have drastically reduced anuran populations all over the world (BERGER *et al.*, 1998, HUDSON *et al.*, 2006, SCHEELE *et al.*, 2017). Although they are better known for the damage caused to their hosts, parasites can also exert important ecological effects on the ecosystems (HUDSON *et al.*, 2006). However, many fundamental questions remain open, especially for anurans in hyperdiverse regions such as Amazonia, including the differential susceptibility of anuran species to infection by these organisms and the interaction between different groups of parasites in the host, particularly concerning hemoparasites transmitted by vectors.

The premise that all species are equally susceptible to parasite infection lacks consensus. This is particularly true given that individual responses vary significantly, depending on factors such as immune system function, diet, habitat, and the probability of encountering infected vectors (BROWNE *et al.*, 2006, CATTADORI *et al.*, 2008, FREELAND, 1983, FORBES *et al.*, 2017, MEDEIROS-SOUSA *et al.*, 2013, MOTA, 2005). This variation leads to differences in parasite exposure and in the way each species responds to infections. Furthermore, host diversity can influence parasite interactions. For instance, Anderson and Gordon (1982) suggested that heterogeneity in host susceptibility is a key factor driving parasite aggregation.

Parasite-host interactions also reflect the diversity of parasite groups. If species sharing the same niche compete for resources without some form of discrimination, one species may experience a decline in abundance (DOBSON, 1985). Previous studies indicate that parasite co-infection can be influenced by factors such as resource competition within the host, host specificity towards parasites (allowing coexistence), and immune system modulation which can either facilitate or inhibit the establishment of different parasite species (FREELAND, 1983, MIDEO, 2009, HARTGERS & YAZDANBAKHS, 2006). This suggests that infection dynamics are not isolated but interconnected. These interconnections extend beyond interspecific interactions, encompassing more complex factors such as host characteristics and environmental variables.

Among intrinsic factors, perch height (for host species that perch) may indicate which individuals are more likely to be targeted by hematophagous invertebrates (vectors) and, consequently, the infections they can harbor (GOMES & FORATTINI, 1990, SERVICE, 1997). Extrinsic factors influencing parasite occurrence include the physical structure of the habitat, and changes in the habitat can affect the transmission of parasitic diseases among organisms (KEESING *et al.*, 2010). The presence of nearby water bodies is also considered potentially relevant, as many vectors, such as mosquitoes, exhibit a strong association with aquatic environments (ALMEIDA *et al.*, 2020).

These interconnected questions are fundamental to understanding the dynamics of infection in anurans, and have significant implications for the conservation of endangered species and the health of the ecosystems they inhabit. Therefore, investigating the relationship between susceptibility to hemoparasites, the interdependence between groups of hemoparasites and the ecological characteristics of anurans is essential to elucidate the complex mechanisms that govern the interactions between hosts and pathogens.

In this study, we investigated whether anuran species are equally susceptible to infection by hemoparasites in two localities in Central Amazonia, and whether infection by one hemoparasite group (trypanosome or hemogregarine) influences infection by the other hemoparasite group. We also evaluated whether intrinsic or extrinsic characteristics of anurans influence the abundance of hemoparasites in their hosts.

## **2. MATERIAL AND METHODS**

### **2.1 Study area**

The study was conducted between May 2023 and January 2024, in two sampling modules of the Biodiversity Research Program (PPBio). The first area was the Rio Negro Sustainable Development Reserve (RDS-Rio Negro) in the municipality of Iranduba, Amazonas, Brazil. The RDS-Rio Negro has an area of 103,000 ha, with a predominance of white sand soil phytophysionomies, classified as campinas and campinaranas (ADENEY *et al.*, 2016). The climate is Afi - Tropical Rainy, with average annual temperatures of 25.6 °C and rainfall of around 2,300 mm (INMET,

2014). The driest periods occur between June and November and the wettest between December and May (VICENTINI, 2001).

The configuration of the sampling module in the RDS-Rio Negro followed the RAPELD method (acronym for Rapid Surveys and Long-term Ecological Research). The RAPELD plot system consists of  $5 \times 1$  km grids, where 250 m long terrestrial and riparian plots are distributed. Of the 20 plots, 10 are evenly distributed plots arranged 1 km apart, 6 are riparian plots built from the availability of streams with 1.5 m spacing from the other adjacent plots (MAGNUSSON *et al.*, 2005, MAGNUSSON *et al.*, 2013). Four plots distributed campina vegetation and the center lines of all plots followed the altitudinal contours. Each plot consists of 25 continuous 10-meter segments, totaling 250 meters.

The second site is in the Adolpho Ducke Forest Reserve (Ducke Reserve), which covers 10,000 ha of forest on the outskirts of Manaus and is located on the divisor between two drainage basins, one of which drains to the Negro River and the other to the Amazon River (PPBio, 2012). The Ducke Reserve is located on clay plateaus, with the clay composition gradually decreasing on the slopes until it reaches the lowlands, where it is more sandy (OLIVEIRA *et al.*, 2008). It has a humid tropical climate with an average annual temperature of 26 °C, annual rainfall of between 1,700 and 2,500 mm and relative humidity of between 75% and 86% (data from the period 2000 to 2006). The rainy season runs from November to May, with March and April having the highest rainfall, and the dry season runs from June to October, with September being the driest month (OLIVEIRA *et al.*, 2008).

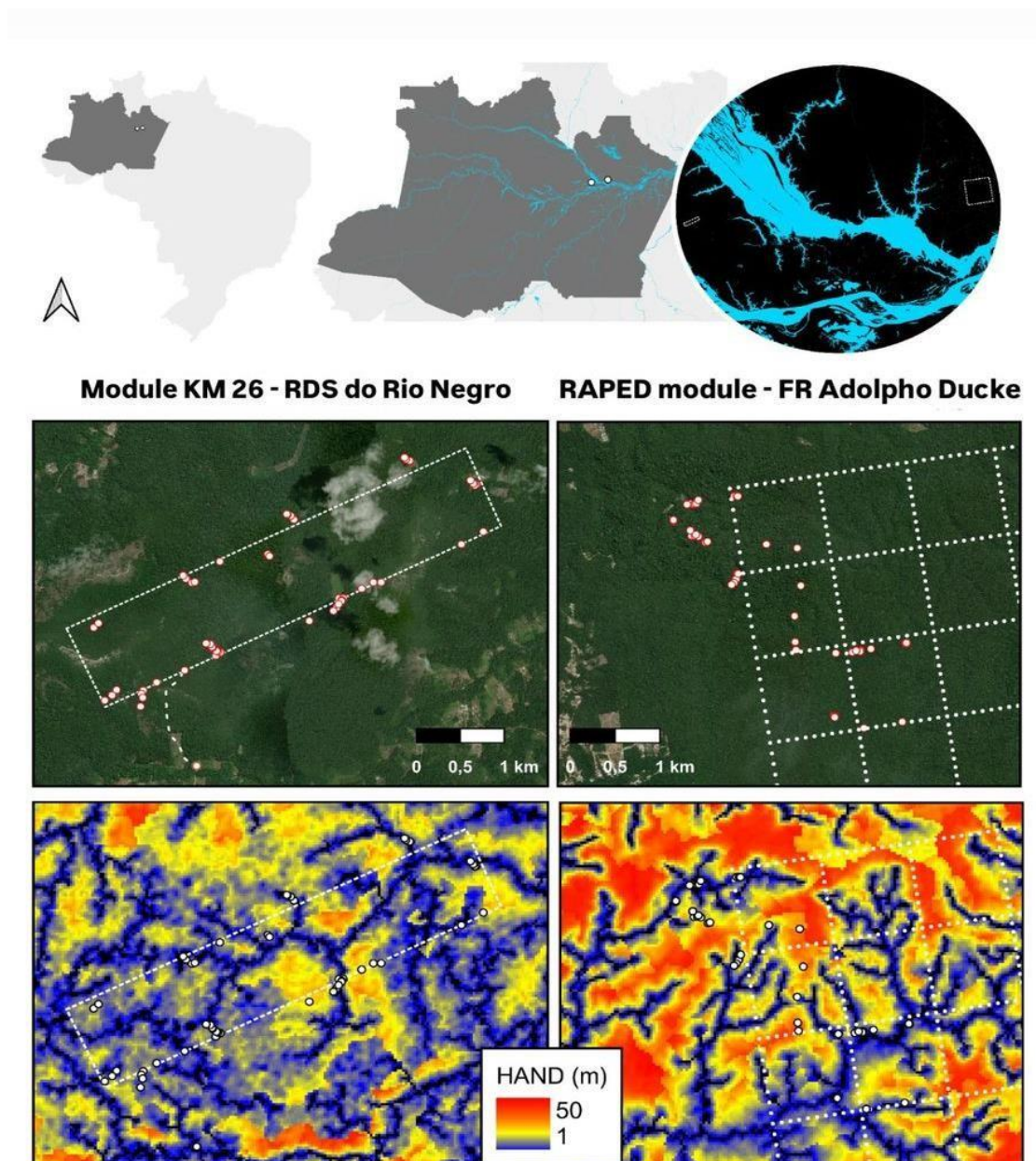


Figure 1. Location of the PPBio module in the Rio Negro Sustainable Development Reserve and the RAPELD grid in the Adolpho Ducke Forest Reserve, Amazonas, Brazil.

## 2.2 Management of Anuran Amphibians and Blood Sampling

The collection was carried out in the 20 plots in the RDS-Rio Negro and on some of the main trails in the Ducke Reserve, using visual and auditory searches (HEYER *et al.*, 1994, CRUMP & SCOTT, 1994, ZANI & VITT, 1995) at night. The individuals were captured by hand and transported individually in moistened plastic bags to avoid dehydration. In the RDS, the module and plot in which the individuals were collected were noted. From there, the geographical location of the module and plot

was acquired from the PPBIO website, via a GEOPACKAGE file ([https://ppbio.inpa.gov.br/en/sites/RDS\\_Rio\\_Negro](https://ppbio.inpa.gov.br/en/sites/RDS_Rio_Negro), last accessed in August 2024). GPS coordinates of anuran sampling points in Reserva Ducke were recorded.

The anurans were photographed and identified using specialized literature (LIMA *et al.*, 2021, LIMA *et al.*, 2012). Subsequently, they were sexed based on morphological characters (presence of a developed vocal sac, size, vocalization activity, presence of eggs in the belly). Their snout-urostyle length (SUL) and tibial length were also measured using a caliper accurate to 0.2 mm, and their body mass was weighed using a PESOLA precision scale (accurate to 0.2 or 0.5 g). In order to prevent recaptures, the captured frogs were marked by cutting off the last segment on a digit following the system of Hero (1989). Using a sterile insulin syringe, 0.3 to 1 mL of blood was collected via cardiac puncture or from the orbital bell (ARIKAN & CICEK, 2014). Part of the blood sampled was used to make thin smears, which were fixed with absolute methanol and stained with 10% Giemsa.

After blood collection, the specimens were returned to the capture site within 24 hours. However, some individuals were collected as testimonial material. These specimens were euthanized using a lidocaine-based anesthetic (CONCEA, 2018), fixed in 10% formaldehyde and stored in vials containing 70% ethanol. The biological material collected was deposited in the Amphibian and Reptile Collection of the Instituto Nacional de Pesquisas da Amazônia-INPA (INPA-H) in Manaus, Amazonas, Brazil. The data acquired in this study was deposited in the data repository of the Biodiversity Research Program (PPBio/INPA - <https://ppbio.inpa.gov.br/>). All procedures involving the animals were authorized by the Brazilian Ministry of Environment (SISBIO 85033-2; SISGEN A27DF5B; and DEMUC/SEMA nº 01/2021 process nº 01.01.030101.003046/2021-07).

The presence of hemoparasites was detected in the blood smears by using a light microscope under at 400x and 1000x magnification. The parasitic forms were photographed with an attached digital camera (Zeiss Axioplan 2). The parasites were identified by comparing their morphologies with the descriptions found in the literature (FONSECA and REYNOLDS, 2021, ÚNGARI, 2023, COELHO, 2020). The quantity of parasite (abundance) was estimated by counting the number of parasites seen in 100 microscope fields (each field containing approximately 100 blood cells) at 400x and 100x magnification (GODFREY *et al.*, 1987, BUSH *et al.*, 1997).

### 2.3 Data analysis

To know the differential susceptibility of species, the chi-squared test was used to compare the distribution of trypanosome or hemogregarine infection between species. In this analysis, only anuran species with more than 5 individuals sampled were considered. The hypothesis tested was that the number of infected individuals was higher in some species than in others. As it is not possible to affirm that individuals identified as *Leptodactylus cf. pentadactylus* (Laurenti, 1768) and *Rhinella cf. proboscidea* (Spix, 1824) in the two localities belong to the same species, the taxa from each locality were treated as different units.

The chi-squared test was also employed to check whether there is a tendency for individuals infected by one group of hemoparasites to be infected by the other group. In this analysis, only the anuran species that had individuals co-infected with trypanosome and hemogregarine were considered. The hypothesis tested is that the occurrence of one group of hemoparasites in an anuran influences the occurrence of another group.

To test whether the intrinsic or extrinsic characteristics influence the abundance of hemoparasites in the hosts, three anuran species –*L. pentadactylus*, *Osteocephalus oophagus* (Jungfer & Schiesari, 1995), *Osteocephalus vilarsi* (Melin, 1941)– with the highest number of infected individuals were selected. Generalized linear models (GLM) were fitted for each species of interest. As this was count data, the Negative Binomial distribution family, implemented with the gam package, was selected. Before testing the fit of the models, all the predictor variables were standardized to have a mean equal to zero and a standard deviation equal to one, using the scale function. The standardization of the predictor variables was carried out in order to remove the effect of the unit difference, making the IRR (Incidence Rate Ratio) estimates comparable between the models. The IRR is a parameter estimated by negative binomial regression models, which reflects the change in the incidence rate of abundance as a result of an increase in the value of a continuous predictor, while keeping the other factors in the model constant. IRR values between 0 and 1 indicate a reduction in abundance as the value of the predictor increases, while IRR values  $> 1$  indicate an increase in abundance as the value of the predictor increases.

The hypotheses tested were based on the life habits of anurans and their interaction with the vectors that transmit hemoparasites. It is expected that individuals with a perching habit (i.e., *Osteocephalus* spp.) should show higher abundance if they are closer to the ground and near water bodies, where vectors are generally more abundant, while individuals of a species found mainly near water bodies (i.e., *L. pentadactylus*), but does not have a perching habit, should show higher abundance of hemoparasites the closer they are to water bodies.

To test the effect of proximity to the ground, the height of the perch was measured in the field, i.e. the height of the place where the anuran was collected. To test the effect of proximity to water bodies, the vertical distances above the nearest drainage course were extracted (Figure 1), using the layers of a HAND model developed for the Amazon basin (BANON & NOVO, 2018). HAND has a strong correlation with the depth of the water table (NOBRE *et al.*, 2011), making it possible to identify areas of the Amazon where the water table may rise to the surface during the rainiest period. Thus, low HAND values should indicate rivers, streams, perennial or intermittent drainage courses, as well as waterlogged areas that may contain temporary water pools (Figure 1). For the two species with a perching habit (*O. vilarsi* and *O. oophagus*), perch height and HAND were considered predictor variables. For the species that is mainly found near water bodies (*L. pentadactylus*), only HAND was considered, as all the individuals of this species were collected on the ground (or close to the ground on rocks and logs). All the analyses were implemented in R software version 4.4.0.

### 3. RESULTS

A total of 140 anuran individuals were sampled (67 in Ducke Reserve and 73 in the RDS-Rio Negro) belonging to 16 different species, of which 123 individuals were selected based on the criteria for the data analysis. The microscopic search revealed that 33.3% (n = 41/123) were positive by hemoparasites, of which 95.1% (n = 39/41) were infected by trypanosomes and 53.6% (n = 22/41) by hemogregarines (Table 1). The occurrence of co-infection of these two groups of hemoparasites was 16.3% (n = 20/123). Different morphologies of trypanosomes (Fig. 2) and hemogregarines (Fig. 3) were encountered in *O. oophagus* in Ducke Reserve, as well as in *L. pentadactylus* in the RDS-Rio Negro (Fig. 4).

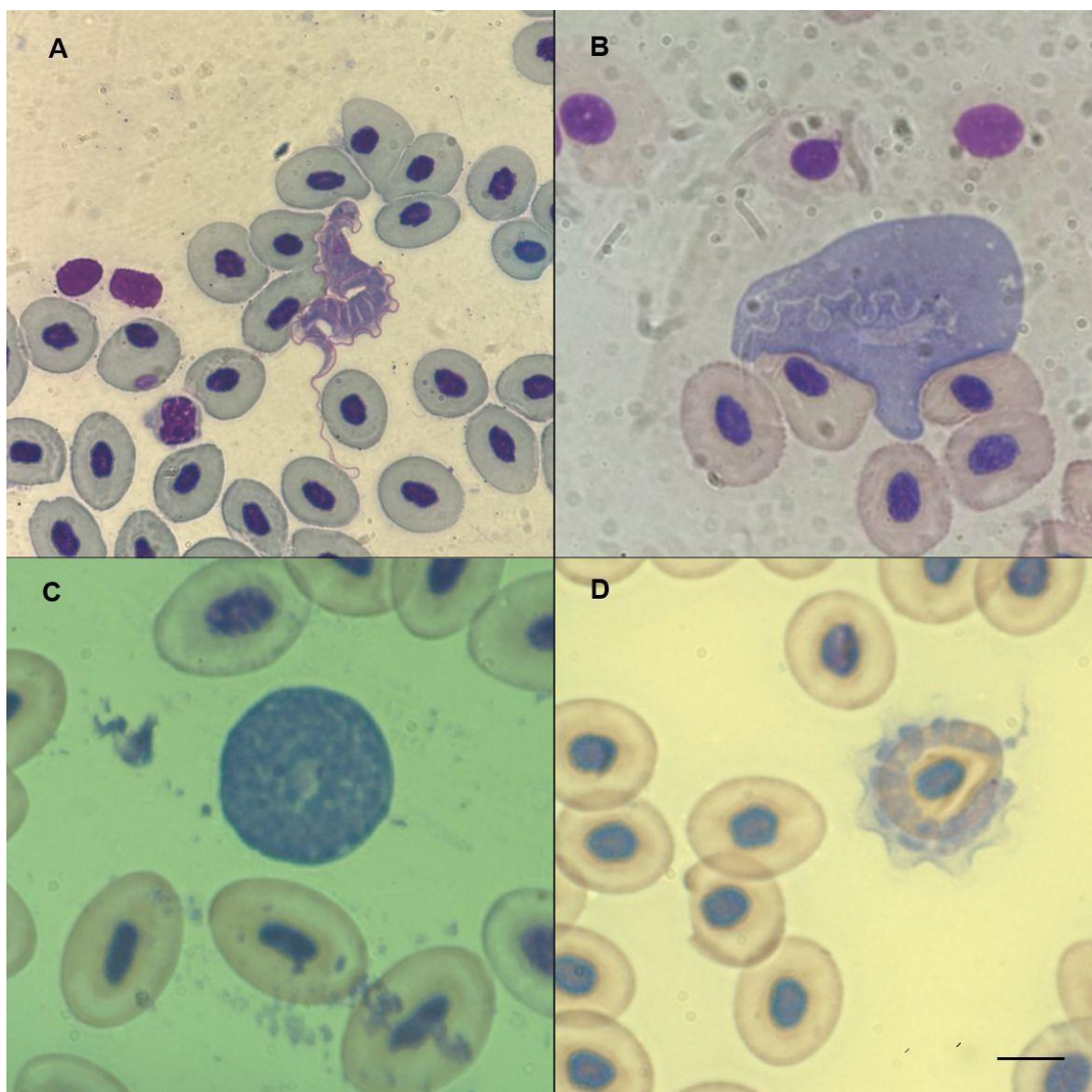


Figure 2 (A-D) Different forms of trypanosome observed in *Osteocephalus oophagus* in the Ducke Reserve. Black arrow indicating the parasite, scale at 10  $\mu\text{m}$ .

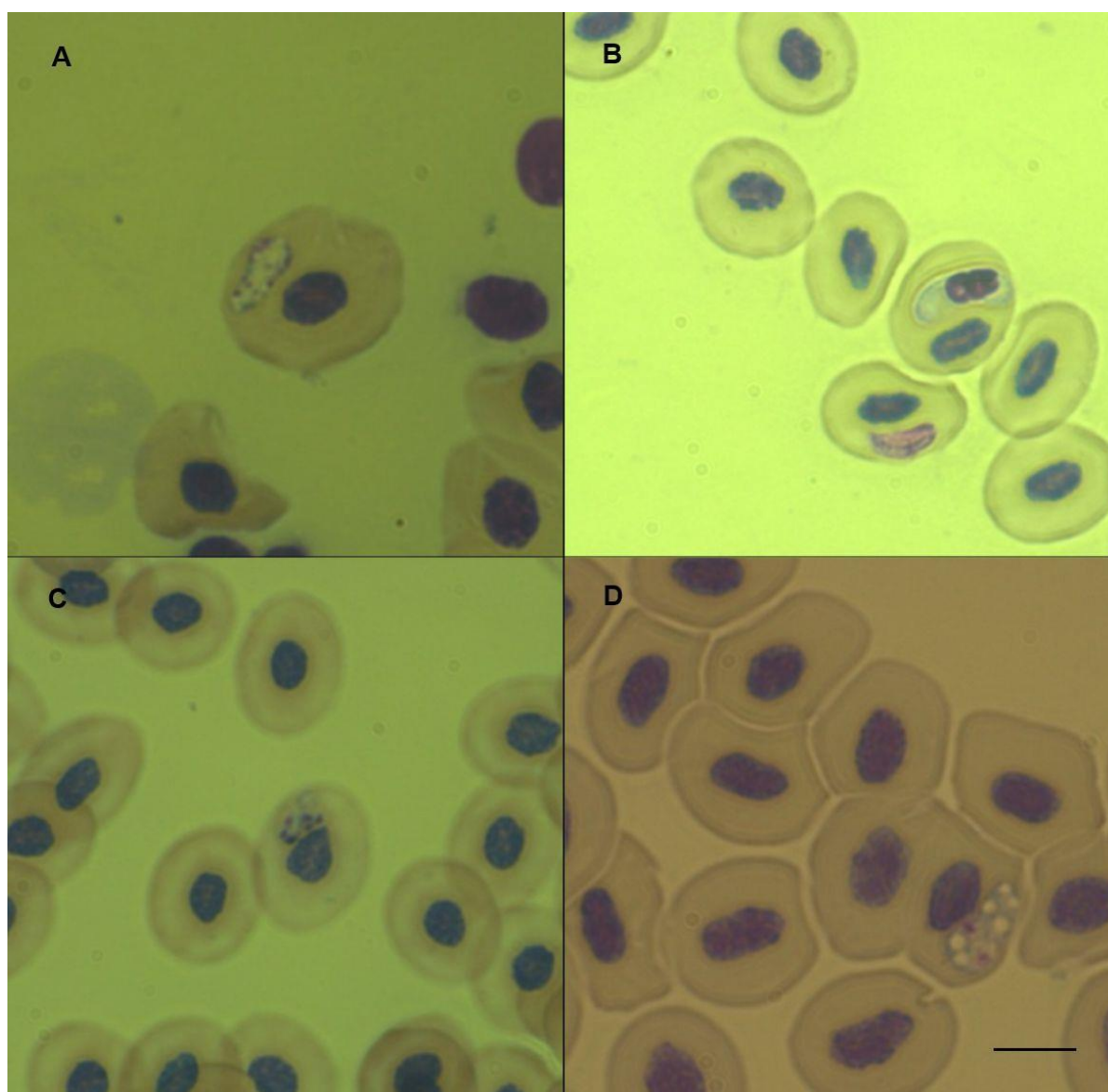


Figure 3 (A-D) Hemogregarine observed in *Osteocephalus ophagus* in the Ducke Reserve. Black arrow indicating the parasite, scale at 10  $\mu\text{m}$ .

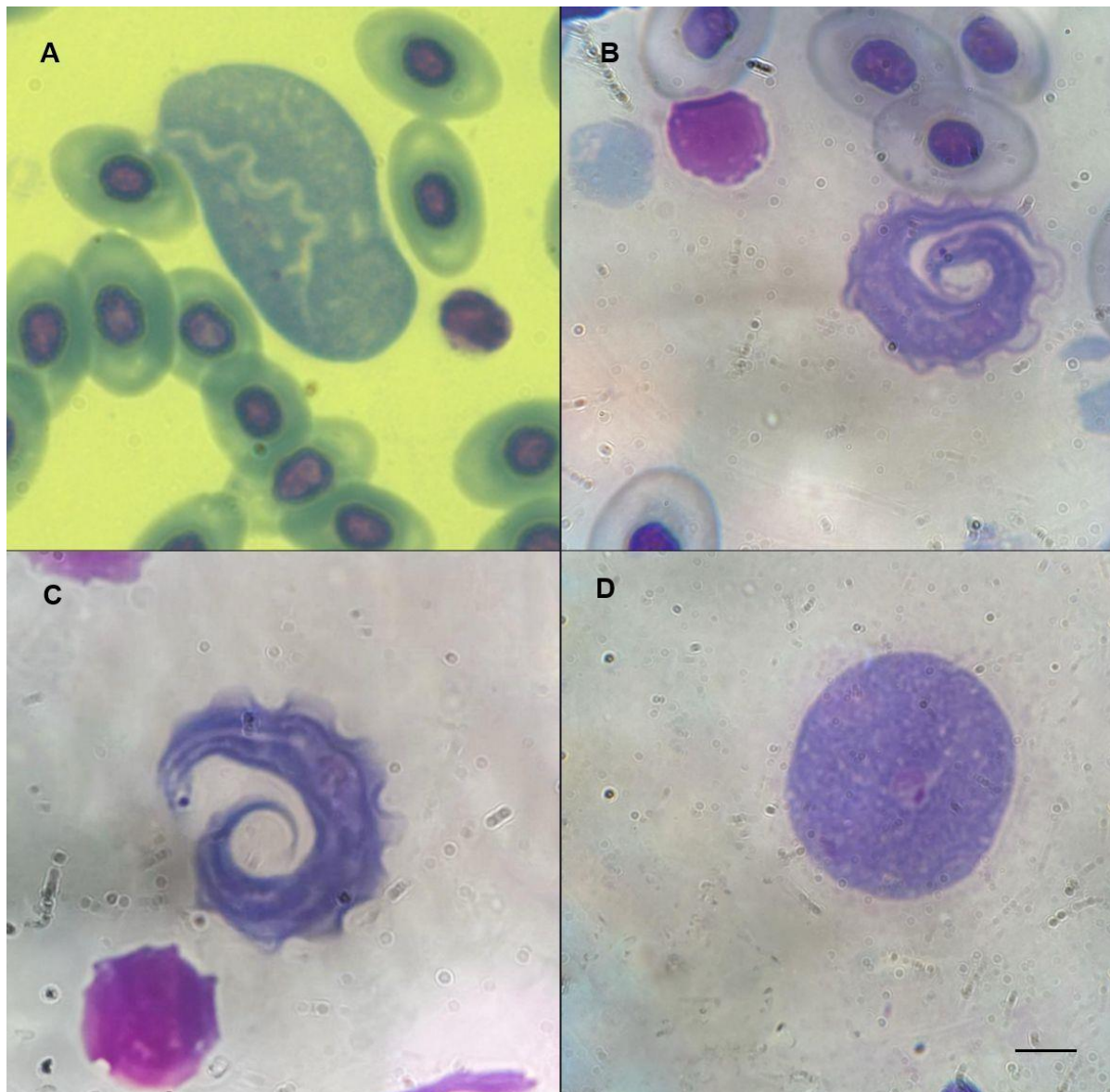


Figure 4. (A-D) Different forms of trypanosome observed in *Leptodactylus pentadactylus* in the Rio Negro Sustainable Development Reserve (RDS). Black arrow indicating the parasite, scale at 10  $\mu$ m.

Certain anuran species exhibit a significantly higher prevalence of individuals infected with trypanosomes ( $\chi^2 = 39.72$ ;  $df = 8$ ;  $p = 0.000004$ ) or hemogregarines ( $\chi^2 = 25.17$ ;  $df = 8$ ;  $p = 0.0015$ ) compared to others. Among the nine species examined, five presented infections with both trypanosomes and hemogregarines (Table 1). *O. oophagus* was the species with the highest prevalence of trypanosomes (78.9%,  $n = 15/19$ ; Fig. 2) and hemogregarines (47.3%,  $n = 9/19$ ; Fig. 3). Conversely, *Pristimantis fenestratus* (Steindachner, 1864) (Ducke Reserve), *Rhinella proboscidea* (Ducke Reserve and RDS-Rio Negro), and *Leptodactylus rhodomystax*

(Boulenger, 1884) (RDS-Negro) showed no detectable infections with either trypanosomes or hemogregarines (Table 1).

Table 1. Number of individuals infected and not infected by trypanosomes and hemogregarines.

Species	Site	Not infected	Infected	Total individuals	Parasite Type
<i>Leptodactylus pentadactylus</i>	RDS	6	5	11	Trypanosomes
<i>Leptodactylus pentadactylus</i>	RDS	8	3	11	Hemogregarines
<i>Leptodactylus pentadactylus</i>	DUCKE	10	7	17	Trypanosomes
<i>Leptodactylus pentadactylus</i>	DUCKE	11	6	17	Hemogregarines
<i>Leptodactylus rhodomystax</i>	RDS	11	0	11	Trypanosomes
<i>Leptodactylus rhodomystax</i>	RDS	11	0	11	Hemogregarines
<i>Osteocephalus oophagus</i>	DUCKE	4	15	19	Trypanosomes
<i>Osteocephalus oophagus</i>	DUCKE	10	9	19	Hemogregarines
<i>Osteocephalus taurinus</i>	DUCKE	6	4	10	Trypanosomes
<i>Osteocephalus taurinus</i>	DUCKE	9	1	10	Hemogregarines
<i>Osteocephalus vilarsi</i>	RDS	19	8	27	Trypanosomes
<i>Osteocephalus vilarsi</i>	RDS	24	3	27	Hemogregarines
<i>Pristimantis fenestratus</i>	DUCKE	7	0	7	Trypanosomes
<i>Pristimantis fenestratus</i>	DUCKE	7	0	7	Hemogregarines
<i>Rhinella proboscidea</i>	RDS	8	0	8	Trypanosomes
<i>Rhinella proboscidea</i>	RDS	8	0	8	Hemogregarines
<i>Rhinella proboscidea</i>	DUCKE	13	0	13	Trypanosomes
<i>Rhinella proboscidea</i>	DUCKE	13	0	13	Hemogregarines

The incidence of one type of hemoparasite was associated with the incidence of the other ( $p < 0.05$  in the three species in which there were cases of co-infection; Tables 2-5).

Table 2. Number of individuals of *Osteocephalus oophagus* infected simultaneously by trypanosome and hemogregarine in DUCKE ( $\chi^2 = 4.56$ ,  $p = 0.033$ ).

		Trypanosome	
		Not infected	Infected
Hemogregarine	Not infected	4	6
	Infected	0	9

Table 3. Number of individuals of *Osteocephalus vilarsi* infected simultaneously by trypanosome and hemogregarine in the RDS ( $\chi^2 = 8.015$ ,  $p = 0.0054$ ).

		Trypanosome	
		Not infected	Infected
Hemogregarine	Not infected	19	5
	Infected	0	3

Table 4. Number of individuals of *Leptodactylus pentadactylus* infected simultaneously by trypanosome and hemogregarine in DUCKE ( $\chi^2 = 6.80$ ,  $p = 0.009$ ).

		Trypanosome	
		Not infected	Infected
Hemogregarine	Not infected	9	2
	Infected	1	5

Table 5. Number of individuals of *Leptodactylus pentadactylus* infected simultaneously by trypanosome and hemogregarine in the RDS ( $\chi^2 = 4.95$ ,  $p = 0.026$ ).

		Trypanosome	
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		Not infected	Infected
<b>Hemogregarine</b>	Not infected	6	2
	Infected	0	3

There was strong evidence (Table 6) that, in *O. oophagus*, individuals collected at greater heights (i.e. further from the ground) showed lower levels of hemogregarine abundance (Figure 5). In addition, although much weaker and not statistically significant (Table 6), there was a tendency for individuals with high HAND (i.e. further from bodies of water) to show lower hemogregarine abundance (Figure 5).

Table 6. Estimates of the Incidence Rate Ratio (IRR) of hemogregarine parasitemia in *Osteocephalus oophagus*, with predictors on a normalized scale.

Predictors	IRR	CI (95%)	p-value
(Intercept)	2.62	0.70 – 9.75	0.152
<b>Perch height</b>	<b>0.21</b>	<b>0.05 – 0.87</b>	<b>0.032</b>
HAND	0.26	0.05 – 1.37	0.112

Observations: 18  
Deviance explained: 21.9%

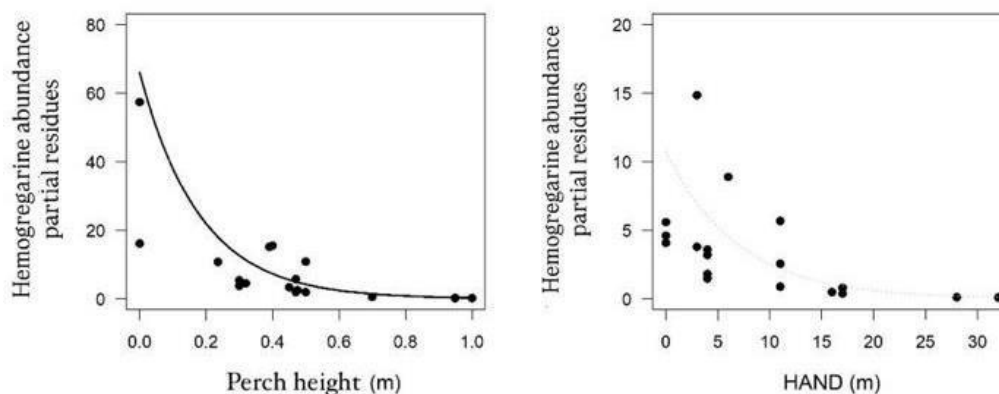


Figure 5 - Partial regression of hemogregarine abundance in *Osteocephalus oophagus* individuals as a function of perch height (left) and as a function of HAND (right).

Trypanosome abundance levels in *O. oophagus* had a non-statistically significant tendency (Table 7) for individuals collected at greater heights to have

lower abundance levels (Figure 6), while there was no evidence (Table 7) for an effect of HAND (Figure 6).

Table 7. Estimates of the Incidence Rate Ratio (IRR) of trypanosome parasitemia in *Osteocephalus oophagus*, with predictors on a normalized scale.

Predictors	IRR	CI (95%)	p-value
(Intercept)	39.44	17.81 – 87.34	<0.001
Perch height	0.47	0.21 – 1.08	0.074
HAND	1.01	0.44 – 2.30	0.984
Observations:	18		
Deviance explained:	15.7%		

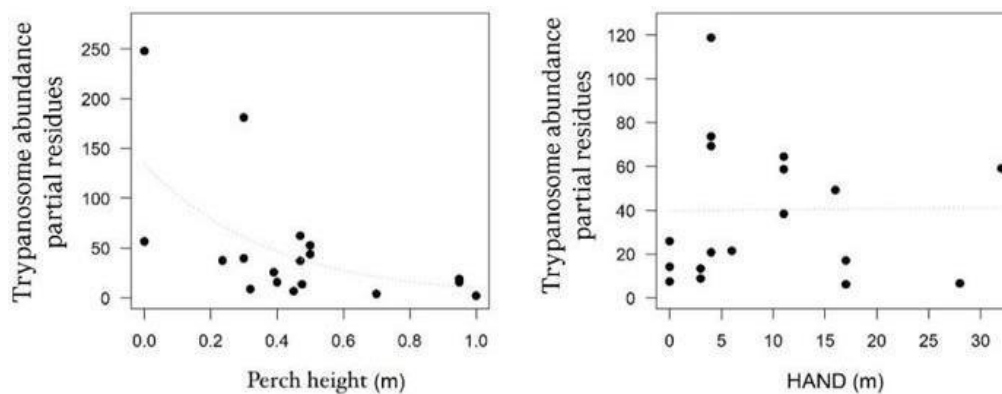


Figure 6 - Partial regression of trypanosome abundance in *Osteocephalus oophagus* individuals as a function of perch height (left) and as a function of HAND (right).

In *O. vilarsi*, although the number of anurans collected was higher, the number of individuals infected with trypanosomes was much lower (approximately 30% of individuals were infected with trypanosomes). The model estimates point to very similar patterns to the other species of the same genus (Table 8, Figure 7), but much weaker compared to the model with individuals of *O. oophagus*, in which approximately 79% of the individuals were infected by trypanosome. Only 3 individuals of *O. vilarsi* showed hemogregarine abundance, and the model fit was inadequate due to the excess of zeros and insufficient information (result not shown).

Table 8. Estimates of the Incidence Rate Ratio (IRR) of trypanosome parasitemia in *Osteocephalus vilarsi*, with predictors on a normalized scale.

Predictors	IRR	CI (95%)	p-valor
(Intercept)	7.73	1.62 – 36.98	0.010
Perch height	0.25	0.04 – 1.59	0.142
HAND	0.64	0.12 – 3.42	0.604
Observations:	26		
Deviance explained:	8.67%		

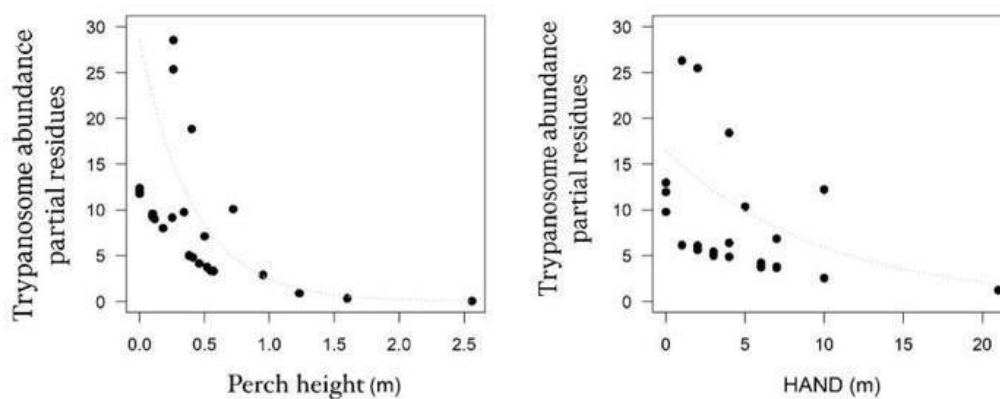


Figure 7. Partial regression of trypanosome abundance in *Osteocephalus vilarsi* individuals as a function of perch height (left) and as a function of HAND (right).

Although *L. pentadactylus* individuals with high HAND values tended to have lower hemogregarine abundance, the result was not statistically significant (Table 9) and the explained variance was low (Figure 8). There was no evidence of an effect of HAND (Table 10) on trypanosome abundance (Figure 9).

Table 9. Estimates of the Incidence Rate Ratio (IRR) of hemogregarine parasitemia in *Leptodactylus pentadactylus*, with the normalized scale predictor.

Predictors	IRR	CI (95%)	p-valor
(Intercept)	5.96	1.57 – 22.59	0.009
HAND	0.42	0.11 – 1.68	0.221
Observations:	27		
Deviance explained:	2.44%		

Figure 8. Regression of hemogregarine abundance in *Leptodactylus pentadactylus* individuals as a function of HAND.

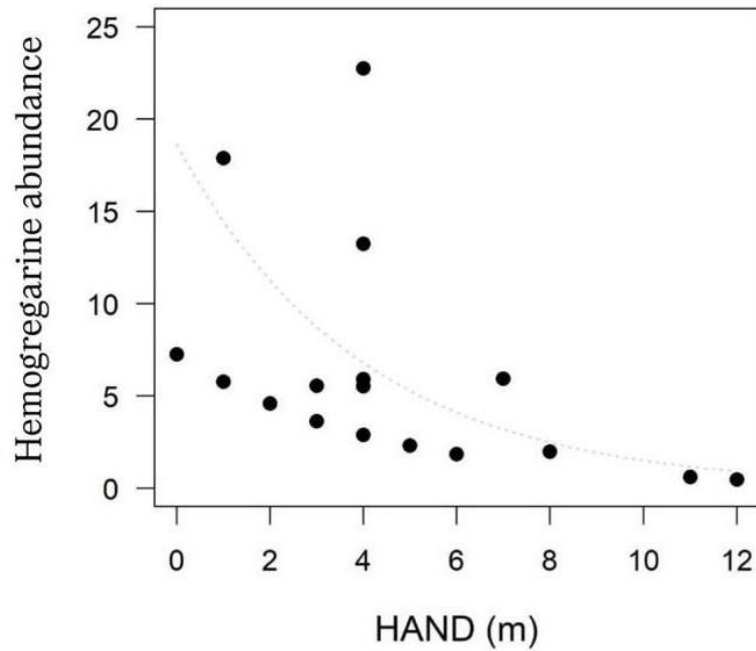


Table 10. Estimates of the Incidence Rate Ratio (IRR) of trypanosome parasitemia in *Leptodactylus pentadactylus*, with the normalized scale predictor.

Predictors	IRR	CI (95%)	p-value
(Intercept)	8.63	3.10 – 24.03	<0.001
HAND	1.01	0.36 – 2.86	0.987
Observations:	27		
Deviance explained:	0.0014%		

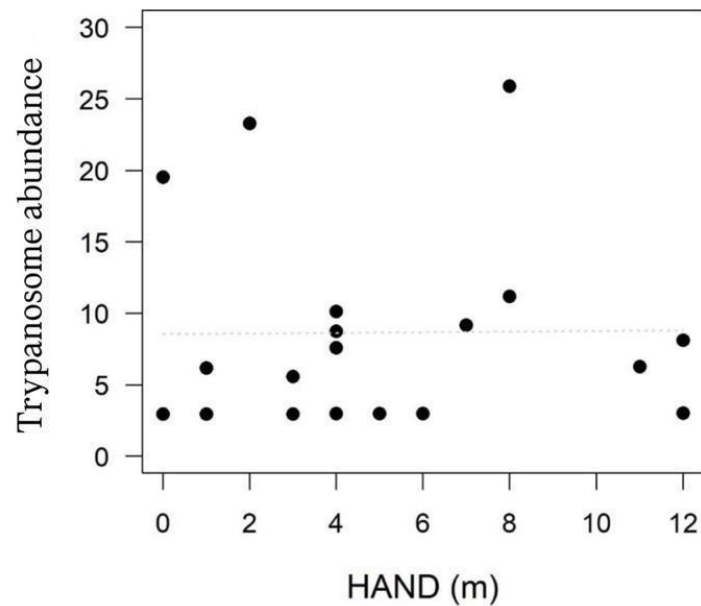


Figure 9. Regression of trypanosome abundance in *Leptodactylus pentadactylus* individuals as a function of HAND.

#### 4. DISCUSSION

Our study provided the first ecological assessment between anurans and their hemoparasites in Central Amazonia. Some species showed a greater tendency to infection than others, suggesting that the incidence of infection by trypanosomes and hemogregarines may be associated with the type of host, probably in response to the characteristics of the individuals and the habit of the species. Aspects related to the immune system, the presence of specific defense mechanisms, temperature and diet can influence resistance to infection (BOWER *et al.*, 2019, FERGUSON & SMITH, 2012, FIELD *et al.*, 2002, VARGA *et al.*, 2019). In addition, some species may have developed immunological adaptations that confer greater resistance to certain parasites, while others may be more susceptible due to environmental factors (BIELBY *et al.*, 2015, BOWER *et al.*, 2019).

Congeneric species showed differences in infection, while individuals of *L. pentadactylus* and *R. proboscidea* were infected, *L. rhodomystax* and *Rhinella marina* (Linnaeus, 1758) did not show infection. Considering the individual characteristics related to the species choice of habitat, feeding and even predation, it is not possible to confirm whether phylogeny is associated with infection patterns, but this question could be used as a basis for future research.

The prevalence, abundance, and diversity of hemoparasites in our study were relatively similar to those observed in other studies conducted in Brazil. In the Eastern Amazon, Pinho *et al.* (2021) reported anurans infected with *Trypanosoma* and Hemogregarina, with *Trypanosoma* being more frequent (80%; n = 37/46) than Hemogregarina (37%; n = 17/46). Also in the Amazon region, Coelho *et al.* (2021) recorded *Leptodactylus* and *Rhinella* species infected with hemoparasites. In addition, Úngari (2023) identified *Trypanosoma*, *Dactylosoma* and *Hepatozoon*, with 33% of anurans testing positive in the Central-West and Southeast regions. There are few studies on Hemogregarina in anurans in Brazil, both in terms of their diversity and ecology (COELHO *et al.*, 2021), making it difficult to compare these results. However, across different biomes (Amazon, Cerrado, and Atlantic Forest), a significant occurrence of hemoparasites was observed, indicating that much remains to be explored.

Co-infections can result in a competitive dynamic between parasites, where one may dominate the other, depending on host and environmental conditions (HENRICHS *et al.*, 2016, GALEN *et al.*, 2019). However, in this study, we found evidence that the infection by one hemoparasite group is associated with infection by another. Hemoparasite co-infections in their hosts may be associated with synergistic or antagonistic factors, influencing the parasite pathogenicity within the host (PEDERSEN & FENTON, 2007).

Due to the lack of significance of the predictors in most models, it is possible that important variables for understanding the effect of habitat were not included in the analysis. However, the abundance and occurrence in the two locations were different. Anderson (1981) points out that white sand forests are poorer in tree species than terra-firme forests, which are known for their high biodiversity and heterogeneity (PITMAN *et al.*, 2001). In the study by Chaves *et al.* (2011) landscape heterogeneity proved to be the best predictor of vector richness and diversity, with more heterogeneous landscapes containing a greater number of species. This may explain the differences in abundance between the two study locations.

Encounters between frogs and vectors, and consequently hemoparasites, often requires a set of specific conditions to initiate an interaction. Perch height and the HAND model appear to be related to the abundance of hemogregarines and trypanosomes in anurans. Our results indicated that perch height has a significant impact on hemogregarine abundance in *O. oophagus*, whose individuals at greater

heights showed lower abundance. This suggests that perching behavior may serve as a defense mechanism, reducing exposure to vectors, which may be more abundant close to the ground. This relationship indicates that specific behavioral characteristics, such as microhabitat choice, can directly influence the risk of infection (LOPES *et al.*, 2022).

Despite HAND was not statistically related to infection in *O. oophagus*, there was a weak tendency for lower levels of hemogregarine abundance to be related to greater distances from water bodies. For *L. pentadactylus*, which is predominantly terrestrial and usually stays close to water bodies, there was a trend towards lower levels of hemogregarine abundance with increasing HAND values, although this was also not statistically significant.

Trypanosome abundance in *L. pentadactylus* and *O. oophagus* was shown to be poor influenced by distance from water bodies, with statistically non-significant effects in both species analyzed. In anurans, trypanosomes are often transmitted by mosquitoes and sandflies, while hemogregarines are transmitted by leeches, ticks and mosquitoes (FONSECA & REYNOLDS, 2021). This may explain this result, since some vectors are more closely associated with bodies of water than others.

In fact, there is a great difficulty in knowing whether there are distribution patterns of parasite individuals in their hosts, as there is no defined occurrence model (POULIN, 2007). However, the characteristics of the hosts can be an important premise for elucidating possible interactions between these parasites (PRICE, 1990, VON ZUBEN, 1997).

The results of this study fill some gaps, but raise other questions, which can only be answered by longitudinal studies to assess seasonality and fluctuations in abundance levels; more detailed analyses of the composition and abundance of vectors in different habitats; and molecular investigations to identify possible differences in genetic susceptibility between species and individuals.

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## CONCLUSÃO

Neste estudo detectamos fortes evidências de que a suscetibilidade dos anuros à infecção por hemoparasitos na Amazônia Central varia entre espécies. Os resultados também indicam que a presença de um grupo de hemoparasitos pode estar associada à ocorrência do outro, sugerindo interações sinérgicas ou competitivas dentro do hospedeiro. Contudo, essas interações ainda necessitam de investigações mais detalhadas para compreender melhor os mecanismos subjacentes. Além de evidenciarmos que características individuais, como a altura do poleiro, influenciam a carga parasitária de hemogregarina em *Osteocephalus oophagus*, com indivíduos encontrados em maiores alturas apresentando menor

parasitemia. No entanto, a proximidade de corpos d'água mostrou-se um fator de influência menos expressivo para todas as espécies analisadas.

Concluimos que fatores intrínsecos dos hospedeiros e características do habitat podem influenciar a ocorrência e a abundância de hemoparasitos em anuros. No entanto, as diferenças na taxa de infecção entre espécies, associadas a características intrínsecas, como a altura do poleiro, sugerem que a infecção está mais fortemente relacionada a aspectos próprios dos hospedeiros do que às características do habitat.

Porém, a complexidade das interações parasitárias reforça a necessidade de estudos futuros que incorporem variáveis sazonais, análises moleculares e a ecologia dos vetores para uma compreensão mais abrangente da dinâmica das infecções em ambientes tropicais.

## SUPPLEMENTARY MATERIAL

Table A1. Total number of individuals sampled and number of individuals infected with some type of hemoparasite in the Rio Negro Sustainable Development Reserve (May 2023/January 2024) and the Adolpho Ducke Forest Reserve (December 2023).

Species	DUCKE	RDS	Total	Infected individuals
<i>Osteocephalus oophagus</i>	19	0	19	15
<i>Leptodactylus pentadactylus</i>	17	11	28	13
<i>Osteocephalus vilarsi</i>	0	27	27	8
<i>Osteocephalus taurinus</i>	10	0	10	5
<i>Boana sp</i>	0	1	1	1
<i>Rhinella marina</i>	0	4	4	1
<i>Boana lanciformis</i>	0	3	3	0
<i>Callimedusa tomopterna</i>	0	2	2	0
<i>Leptodactylus rhodomystax</i>	1	11	12	0
<i>Leptodactylus riveroi</i>	0	2	2	0
<i>Leptodactylus sp</i>	0	1	1	0
<i>Phyllomedusa vaillanti</i>	0	1	1	0
<i>Pristimantis sp</i>	7	2	9	0
<i>Rhinella proboscidea</i>	13	8	21	0