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Short note

New anuran amphibian host for *Hemolivia* sp. (Adeleorina: Karyolysidae) in the Neotropical region

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ABSTRACT. This study aims to identify and report a new anuran host for parasitic infection by *Hemolivia* sp. in the Neotropical region, providing data on morphology and morphometry of this hemoparasite, and data on infection intensity, prevalence, and abundance. The collection of amphibians was conducted on the banks of two tributaries of the Amazon River. The average intensity of infection was 20,769 parasites/ml (min: 5,000 parasites/ml, max: 60,000 parasites/ml). Significant differences were identified between the width of infected and uninfected erythrocytes. In the Neotropical region, aspects about parasitic diversity and information about parasite-host relationships and their vectors are little clarifying.

Keywords: parasite-host relationship, Brazil, hemoparasites, Brazilian Amazon, *Rhinella major*

Introduction

The effects of parasitism on hosts can vary, depending on climate variations or changes in environmental conditions, having an impact on the development of certain infections and facilitating the onset of diseases originated from several pathogens [1,2]. Research on intra-erythrocytic parasites in recent years has focused on those of veterinary or medical importance. On the other hand, literature on ectothermic wildlife hemoparasites is extremely limited and data deficient, especially that of amphibians [3,4].

The description of the impacts of parasites on the dynamics and ecology of host populations is important for understanding the role of these organisms in ecological interactions and the regulation of populations in natural environments. However, few blood infections of fish, amphibians and reptiles have proven pathogenicity, in contrast to the many intra-erythrocytic parasites of mammals and some birds that can cause severe damage to hosts and can lead to death [5,6].

Parasites of the phylum Apicomplexa, belonging to the order Eucoccidiorida and subclass Coccidiasina are known as Haemogregarinas and comprise six known genera: *Cyrilia* Lainson, 1981; *Desseria* Siddall, 1995; *Haemogregarina* (sensu stricto) Danilewsky, 1885; *Hemolivia* Petit et al., 1990; *Hepatozoon* Miller, 1908 and *Karyolysus* Labbé, 1894. These parasites have a complex biological cycle, involving hematophagous vectors (ticks, mosquitoes, fleas, and lice) and have already been reported parasitizing amphibians, reptiles, mammals, and birds [7,8].

Hemolivia is one of these genera registered in amphibians, reptiles, and ticks. It presents sporogony processes occurring in the blood of vertebrate hosts (intermediate hosts). Fertilization, formation of oocysts and sporocysts with sporozoites, occurs in the tissues of tick vectors (definitive hosts) [9–11].

The anuran *Rhinella major* (Müller and Helmich, 1936) of the Bufonidae family, has a wide

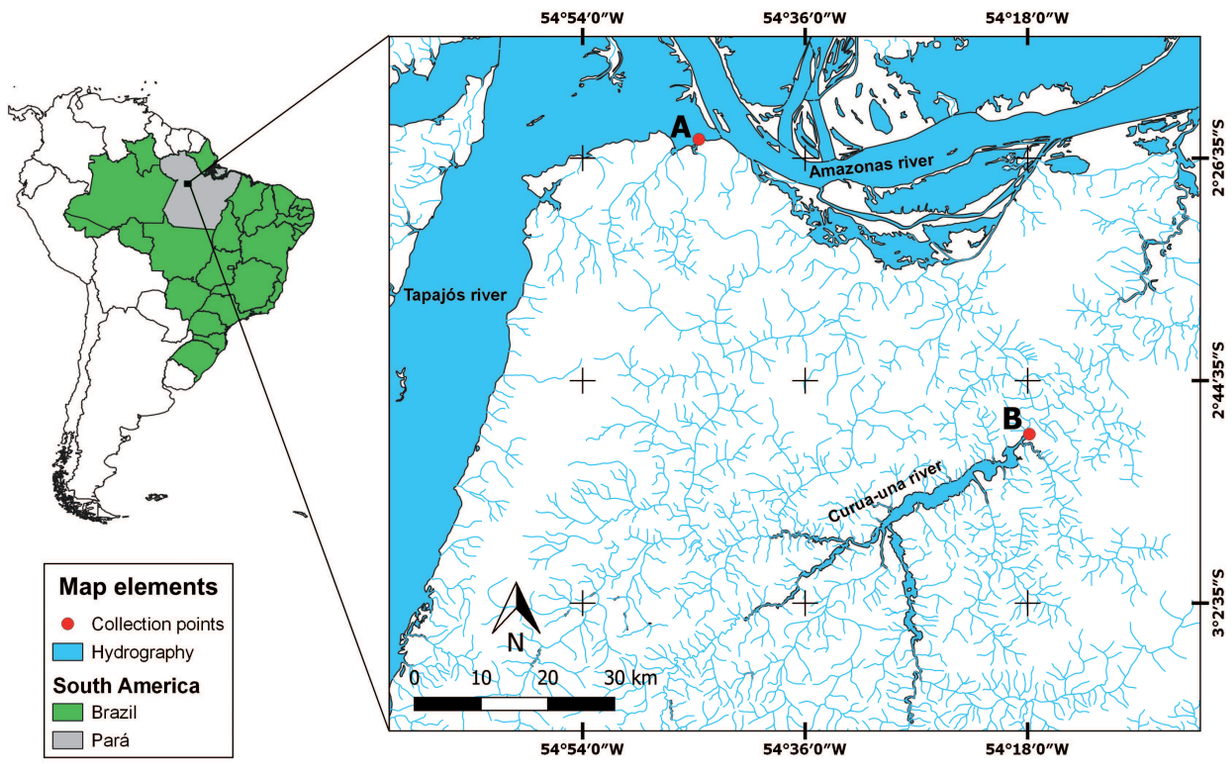

Figure 1. Collection points for specimens of *Rhinella major*, Santarém, Pará, Brazil. (A) Tapajós Campus of the Universidade Federal do Oeste do Pará (UFOPA) on the right bank of the Tapajós River, (B) Curuá-Una hydroelectric plant on the right bank of the Curuá-Una river

distribution in South America, with records known in Brazil, Argentina, Paraguay, and Bolivia. These anurans are characterized by having a small parotid gland, warty skin and well-developed cranial ridges [12]. Considering the lack of information about parasitism for several species of anurans [13], this study aims to identify and report a new anuran host for parasitic infection by *Hemolivia* sp. in the Neotropical region, providing data on morphology and morphometry of this hemoparasite, and data on infection intensity, prevalence and abundance.

Materials and Methods

Between the months of May 2017 and June 2018, 92 specimens of *R. major* were collected at two points located in the municipality of Santarém, west of the state of Pará, Brazil, on the banks of two tributaries of the Amazon River system. The first point is located on the right bank of the Tapajós River at the limits of the Tapajós Campus of the Universidade Federal do Oeste do Pará (UFOPA) (2°25'2.82"S, 54°44'35.28"W). The second point is in the area of direct influence of the Curuá-Una hydroelectric plant (2°48'54.03"S, 54°17'50.82"W) (Fig. 1).

Amphibians were collected using the active search method, separated in individual plastic bags, and sent to sample collection procedures. In laboratory environment, body mass (g) and snout-vent length (SVL; in millimeters) of the specimens were measured, after being anesthetized and euthanized with Lidocaine Hydrochloride (2%), according to the methodological protocol suggested by [14] and [15]. Blood samples were obtained by cardiac puncture using syringes containing EDTA (10%). Following, blood extensions were made in duplicate and stained by the Fast-Panoptic method (Laborclin® Brazil).

Light microscopy was used to locate the hemoparasites. The identified parasites were photographed with an Axiocam ERc 5S camera coupled to a Zeiss Axioplan optical microscope. The Zen Blue 2 software package was used to determine the morphometric characteristics of intracellular shapes following [16]. Intensity of the infection (parasites/ml), was determined by methodology described by [17]. The method consists of counting all the parasites found in 100 microscopic fields with a magnification of 1000×, being recorded and calculated as follows: It is assumed that 100

Table 1. Prevalence, mean intensity, and mean abundance of *Hemolivia* sp. infection in *Rhinella major* from Santarém, Pará, Brazil. Values inside the parenthesis represent 95 % confident intervals.

	General	Males		Females	
		Curuá-Una river	Tapajós river	Curuá-Una river	Tapajós river
Prevalence (%)	14.1 (0.077–0.229)	8.6 (0.018–0.230)	20.0 (0.057–0.436)	11.8 (0.014–0.364)	20.0 (0.057–0.436)
Mean abundance	0.60 (0.30–1.10)	0.40 (0.06–1.49)	0.95 (0.20–3.15	0.41 (0.00–1.06)	0.75 (0.10–1.65)
Mean intensity	4.23 (2.92–6.46)	4.67 (2.00–7.33)	4.75 (2.00–9.50)	3.50 (3.00–3.50)	3.75 (1.00–4.75)

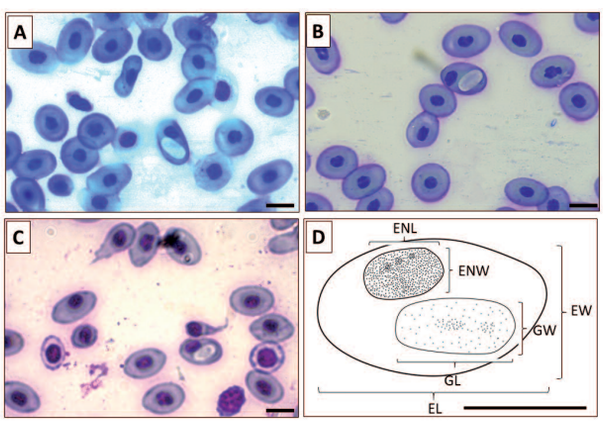

Figure 2. Intracellular gametocytes of *Hemolivia* sp. inside *Rhinella major* erythrocyte from Santarém, Pará, Brazil (A, B, C), and measurements (D), (WG) Width of gamonts, (LG) Length of gamonts, (WE) Width of erythrocyte, (LE) Length of erythrocyte, (LEN) Length of erythrocyte nucleus, and (WEN) Width of erythrocyte nucleus. Scale bars = 10µm.

microscopic fields are equivalent to 0.2 µl of blood (intensity of infection = (N°: number of parasites × 5) × 1,000 = (parasites/ml)). The ecological terms used, and the parameters of prevalence, abundance and average intensity were calculated according to those proposed by [18]. Parameters were calculated using Quantitative Parasitology 3.0 software [19]. All data were previously evaluated based on normality assumptions using Shapiro-Wilk test. The Mann-Whitney nonparametric test was used to assess possible differences between infected and not infected erythrocytes and to compare the intensity of parasitism between hosts' sexes. Spearman's correlation coefficient (rs) was used to verify possible correlations of parasite abundance with SVL and body mass [20]. Values were considered statistically significant when $p<0.05$.

Results

After the hemoparasite analyses, intracellular forms were identified inside erythrocytes with a diagnosis compatible with Haemogregarina *Hemolivia* sp. (Fig. 2), with a total prevalence of 14.1% in the examined amphibians. The values of prevalence, mean abundance and mean intensity for amphibians collected on the right bank of the Tapajós river and on the right bank of the Curuá-Una river for both sexes of the hosts, are shown in Table 1.

The morphometric analyzes showed gametocytes measuring 10.75 µm±1.23 µm long by 5.15 µm±0.71 µm wide, with parasitized erythrocytes measuring 17.95 µm±1.18 µm long by 10.23 µm±0.67 µm wide, with nucleus of 6.34 µm±0.62 µm long by 4.20 µm±0.48 µm wide. Non-parasitized erythrocytes were 11.47 µm±0.20 µm long by 15.82 µm±0.46 µm wide, with nucleus measuring 7.11 µm±0.34 µm long by 4.82 µm±0.18 µm wide. The Mann-Whitney test showed significant differences between the width of the infected and uninfected erythrocytes where infected erythrocytes wider, considering the central region of the cell ($W=15.0$; $p=0.011$); as well as longer cells ($W=40.0$; $p=0.012$). The mean intensity of infection was 20,769 parasites/ml. (min: 5,000 parasites/ml, max: 60,000 parasites/ml). No differences were identified between intensity of infection and host sex ($p>0.05$). The Spearman correlation (rs), did not show significant results ($p>0.05$), with no correlations between parasitic abundance and SVL, nor with as the individuals' body mass.

Discussion

Reports of hemoparasites of the genus *Hemolivia* are mostly described on reptiles (lizards and Testudines) [6,10,21–24]. However, *Hemolivia* is also considered as an amphibian parasite [9]. The only record of parasitism of *Hemolivia* in anurans in the Neotropical region is by *Hemolivia stellata*, described by [9], in *Rhinella marina* (Linnaeus, 1758) (syn. *Bufo marinus*) collected in the city of

Belém, in the State of Pará, Brazil. Studies that report infection in amphibians by *Hemolivia* are scarce, therefore, data on prevalence and intensity of parasitism are reported only in studies conducted with reptiles [6,21–23]. In our study, we are reporting a new case of infection with *Hemolivia* sp. in the Neotropical region, in *R. major*, with prevalence ranging from 8.6 to 20% were found in males and from 11.8 to 20% in females. Encapsulated gametocyte forms were found in the blood of these hosts, corroborating the description by [9] for *Hemolivia* genus in amphibians.

The transmission of *Hemolivia,* occurs through the ingestion of an infected invertebrate, or even by a potential infected vertebrate host as the infection takes place in the definitive host's tissue [3,25]. According to [9], in his descriptive study of the cycle of *H. stellata*, he considers the tick species *Amblyomma rotundatum* Koch, 1844 as a vector. [10] demonstrated the absence of parasitic specificity for *H. stellata* in the lizard *Ameiva ameiva* (Linnaeus, 1758) and the *R. marina*, by using macerated ticks infected with sporozoites of the pathogen to infect these organisms. Thus, it concluded the participation of ticks of the genus *Amblyomma* in the cycle of these parasites in the Amazon region.

In a study conducted with the same sample set of *R. major* from the banks of the Curuá-Una river, [26] demonstrated the existence of a parasitic relationship between *R. major* and *Amblyomma dissimile* Koch, 1844 larvae and nymphs, with a prevalence of infection of 36.53%, which can enable the transmission of hemoparasites. However, this study did not describe the vectorial capacity of these hematophagous invertebrates or the diversity of hemoparasites that can be transmitted.

Recently, [27] found 24 specimens of *R. major* infested with 16 larvae of ticks of the genus *Amblyomma* and 15 nymphs of *A. dissimile* in the western region of the State of Pará; no ticks were found in adulthood. Thus, the mechanisms of transmission of these hemoparasites in the Amazon region have also not been clarified since little is known about the specificity of hemoparasites in their invertebrate and vertebrate hosts.

In the Neotropical region, aspects about parasitic diversity and information about parasite-host relationships and their vectors are little clarifying. We are aware that with more elaborate analyzes, we could characterize and possibly even find new species of *Hemolivia*. However, in Brazil and especially in the Amazon region, the interest of parasitology in amphibians in a natural environment is precarious, due to the considerable decline in funding, scientific support, and attention to this neglected taxonomic group. Therefore, that taxonomic studies, of molecular biology and parasitic ecology are still extremely necessary and important to help accurately quantify these host-parasite associations in amphibians in this region.

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