



MICROBIOLOGY

First record of *Trypanosoma* sp. (Kinetoplastea: Trypanosomatidae) parasiting *Rhinella major* in the Brazilian Amazon

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Abstract: *Rhinella major* is one of the component species of the *Rhinella granulosa* group and is distributed in the neotropical region of the South American continent, being found in anthropized environments. The occurrence of trypanosomes in anurans involves a yet unknown diversity of species and hosts. In the present study, we aimed to describe the first record of *Trypanosoma* sp. at *R. major* as a new host. Of the species captured, four were positive for trypomastigote forms of *Trypanosoma* sp. The morphometry of the trypomastigote forms revealed parasitemia by only one morphotype. There is a need for reports and studies of parasite-host relationships in anurans and a lack of records regarding hemoparasite diversity linked to the *Rhinella granulosa* group.

Key words: Amphibians, Brazil, hemoparasites and trypanosomatids.

INTRODUCTION

Amphibians are animals that live in the most diverse habitats, playing an important role in the food webs, contributing to the regulation of invertebrates in natural environments and anthropic areas. Furthermore, they are hosts of a variety of parasites, showing no signs of disease (O'Rourke & Rosenbaum 2015). The invertebrate hosts and vectors of anuran trypanosomes are related to the habitat of these animals, with hirudinea and hematophagous arthropods reported as the main vectors (Bailey 1962, Anderson & Ayala 1968, Siddall & Desser 1992). Relatively, little is known about vectors of trypanosomes of amphibians (Bardsley & Harmsen 1973, Martin & Desser 1991, Johnson et al. 1993). The descriptions of all the records of *Trypanosoma* species found parasitizing anurans are based on morphological aspects observed in the trypomastigote form in the peripheral blood

(Martin et al. 2002). Due to the plasticity of these hemoflagellates, the species may differ in body size and shape, in the position of the nucleus and the kinetoplast, as well as in the degree of development of the undulating membrane and flagellum (Martin et al. 2002).

Rhinella major, Müller and Helmich, 1936 is a component species of the *Rhinella granulosa* group, Spix, 1824 and is distributed in the Neotropical region of the South American continent. These anurans are characterized by their small parotid gland, warty skin and well developed cranial ridges (Narvaes & Rodrigues 2009). Adult males have a variable length of 35.8 to 75.8 mm while females range from 33.9 to 81.1 mm, have a dorsum with dark spots scattered or nearby, forming a mosaic, with a cream-coloured and non-pigmented belly (Narvaes & Rodrigues 2009).

Despite the lack of knowledge about the pathological effects of the parasite-host

relationship involving anuran trypanosomes, most reports suggest a nonpathogenic relationship (Desser 2001). However, high rates of parasitemia can damage the health of hosts, especially in the early forms of their life cycle (Bardsley & Harmsen 1973). Studies in the European and North American continents have shown that the introduction of exotic trypanosomes into new habitats has potential lethality for native amphibian populations (Bardsley & Harmsen 1973). In this study, we describe the morphology and morphometry of the first record of *Trypanosoma* sp. parasitizing the *R. major* species in the Brazilian Amazon, also addressing data on prevalence, intensity and abundance of infection.

MATERIALS AND METHODS

Between June 20 and 22 2018, 44 specimens of *R. major* were collected by an active search in the area of direct influence of the Curuá-Una Hydroelectric Power Plant, in the municipal region of Santarém, in the west of the state of Pará, Brazil, at the coordinates 2°48'52.00"S, 54°17'56.66"O.

The collected specimens were individualized in plastic bags and sent to the Curuá-Una scientific base of the Universidade Federal do Oeste do Pará (UFOPA), for night-time blood sampling and collection procedures (Johnson et al. 1993). In a laboratory environment, the subjects were anaesthetized and euthanized using (2%) Lidocaine Hydrochloride and their weight and snout-vent length (SVL) in millimetres was measured. The blood was collected by cardiac puncture using syringes containing EDTA (10%). It was used to make panchromatic-stained blood extensions using Fast Panoptic (Laborclin® Brazil), to evaluate the Trypanosomatidae morphotypes. Individuals

of *R. major* were deposited in the Herpetology Collection of the Laboratory of Ecology and Animal Behavior - LECAN of UFOPA for use in studies involving endoparasites, ecology and taxonomy (access numbers #UFOPA-H 1295-1335).

The blood extensions were examined for trypanosomes with optical microscope. The parasites were photographed using a Zeiss Axioplan optical microscope with an Axiocam ERc 5S camera. The Zen Blue 2 software package was used to determine the morphometric characteristics of trypanosomes were measured according to Desser (2001) and Borges et al. (2016) (Figure 1).

The parameters of prevalence, mean abundance and mean intensity were calculated according to Bush et al. (1997). The Spearman coefficient (r_s) was used to determine possible correlations between parasite abundance and SVL and weight (Zar 2010). The Bray-Curtis similarity test and distance indices were used with the Past. 3.0 program to verify possible morphological similarities with the

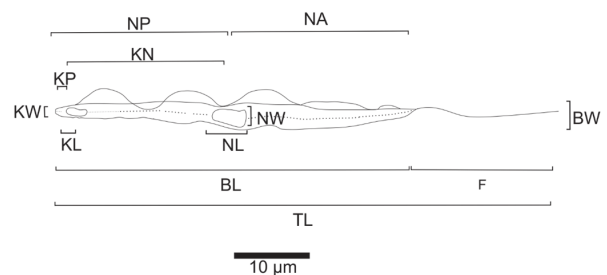


Figure 1. Morphometric localization of the regions of *Trypanosoma* sp. measured, with values in μm , of total body length with flagellum (TL), body length along the midline (BL), body width at the center of the nucleus (BW), length of the free flagellum (F), nucleus length (NL), nucleus width in the central portion (NW), distance from the center of the nucleus to the anterior extremity (NA), distance from the center of the nucleus to the posterior extremity (NP), distance from the center of the kinetoplast to the center of the nucleus (KN), kinetoplast length (KL), kinetoplast width (KW) and distance from the center of the kinetoplast to the posterior extremity (KP).

Trypanosoma species validated by França 1925, Woo 1969, Martin & Dessler 1990, Ferreira et al. 2007, Lemos et al. 2008, Malysheva 2009, Ferreira Jigdas et al. (2015) and Bernal & Pinto (2016).

Ethical responsibilities of authors

The collection of anurans for this study was authorized by the Brazilian government through the Biodiversity Authorization and Information System – (SISBIO nº 52610-1). This study was approved by the Animal Use Committee of the Federal University of Western Pará (Authorization nº 0320180024). All the procedures performed are by the principles adopted by the Colégio Brasileiro de Experimentação Animal - COBEA.

RESULTS

Of the 44 captured specimens of *R. major* (35 males and 9 females), four males (9.09%) presented positivity for trypomastigote forms, with mean weight (\bar{x} = 13.5; $s = \pm 1.7$ g) and mean SVL (\bar{x} = 51.9; $s = \pm 1.7$ mm). Parasite mean abundance was 0.31 and with mean intensity of 3.5 parasites per infected host. Spearman

analyses revealed no correlation between host SVL and parasite abundance ($p > 0.05$), nor with host weight and parasite abundance ($p > 0.05$).

When comparing morphometry with valid amphibian trypanosomes species using the Bray-Curtis similarity index, the trypomastigote forms found in the present study (Table I) showed a similarity of 96% to *Trypanosoma fallisi* (Martin & Dessler 1990). Grouping in an isolated class (Figure 2), providing evidence for a morphometric identification. Despite the morphometric similarity, it is still not possible to associate at the species level, due to the morphological and morphometric parasite plasticity (Spodareva et al. 2018).

For the morphological study, twenty specimens of the only morphotype found of *Trypanosoma* sp. were analyzed, presenting a body that is longer than wide, from two to three flexions, with shapes varying from S and C. The present flagellum represents on average one-third of the total length, with two to four flexions. The cytoplasm presents granulations with two to three vacuoles in the anterior region and the nucleus presents an oval shape, positively displaced to the posterior

Table I. Matrix of morphological similarity between *Trypanosoma* sp. and valid species of *Trypanosoma* spp. anuran parasites in accordance with Ferreira et al. (2007, 2008) and Bernal & Pinto (2016).

	<i>T. tungare</i>	<i>T. sp*</i>	<i>T. fallisi</i>	<i>T. chattoni</i>	<i>T. rotatorium</i>	<i>T. ranarum</i>	<i>T. neveulemairei</i>	<i>T. mega</i>
<i>T. tungare</i>	1	92.803	94.076	62.111	78.920	87.388	66.866	75.807
<i>T. sp*</i>	92.803	1	96.034	62.778	79.282	85.711	60.363	78.512
<i>T. fallisi</i>	94.076	96.034	1	63.439	79.824	85.119	63.689	80.010
<i>T. chattoni</i>	62.111	62.778	63.439	1	67.107	58.601	33.218	61.064
<i>T. rotatorium</i>	78.920	79.282	79.824	67.107	1	75.239	66.530	59.786
<i>T. ranarum</i>	87.388	85.711	85.119	58.601	75.239	1	56.424	81.494
<i>T. neveulemairei</i>	66.866	60.363	63.689	33.218	66.530	56.424	1	36.179
<i>T. mega</i>	75.807	78.512	80.01	61.064	59.786	81.494	36.179	1

Present study*.

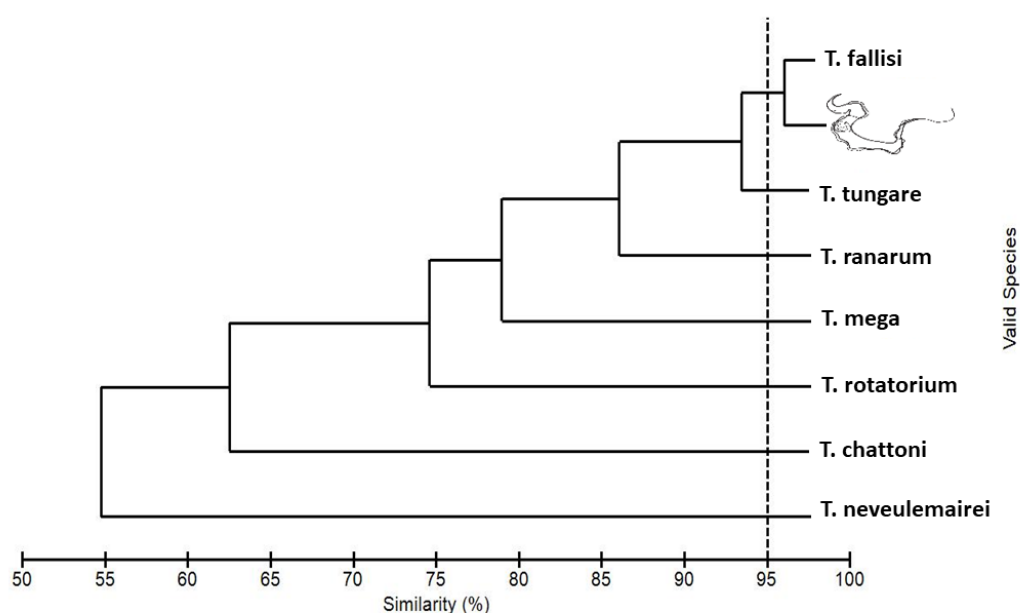


Figure 2. Dendrogram of morphometric similarity between valid species of trypanosomes for anurans and morphotype identified in *Rhinella major* collected in Curuá-Una, Santarém, Pará (Brazil).

region of the body with absent cariosoma. In the posterior region, two to three vacuoles are observed, being predominantly located near the nucleus. The kinetoplast presents a shape varying from oval to circular, displaced to the margin of the cytoplasmic membrane, having a distance between the posterior extremity of easy visualization, as well as the undulating membrane.

Morphometric analyzes of *Trypanosoma* sp. showed that in *R. major* (Figure 3) was infected by a morphotype of these hemoparasites (Figure 4), and the measurements are presented in Table II.

DISCUSSION

The present study identified low prevalence rates, mean intensity and mean abundance of *Trypanosoma* sp. in *R. major*. Indeed, these parasites appear to be found at a low frequency in these host. Leal et al. (2009) found a prevalence of 20% in 40 anurans captured in the Brazilian states of Mato Grosso do Sul and São Paulo.

Besides, Ferreira et al. (2007) reported prevalence rates of 36% of infection in 75 anurans collected in the Amazon biome and a study performed by Desser (2001) in Costa Rica, trypanosomes occur in 12% of anurans from seven species.

However, Spodareva et al. (2018) found in two lakes in Kiev, Ukraine region, a high prevalence of isolated amphibian trypanosomes. Their work used fewer individuals, when compared to our study, totalling eight trypanosomes isolates of 11 individuals in an Oxbow lake (prevalence of 73%) and three of 12 individuals in an acidic peatbog (prevalence of 25%).

In a pathological study of blood parasites, using 140 specimens of the species *Hoplobatrachus rugulosus* (Rice Field Frogs) in Thailand, revealed prevalence of *Trypanosoma rotatorium*-like (11.4%), *Trypanosoma chattoni* (22.2%) and other hemoparasites were detected: *Hepatozoon* sp. (5.7%) and *Lankesterella minima* (7.1%). Also, histopathological study was conducted in liver, kidney, lungs and spleen, but no evidence of pathological lesions associated with trypanosomes was found, only for *Hepatozoon* sp. and *Lankesterella*

minima (Sailasuta et al. 2011). Parasitism by trypanosomes in amphibians is not uncommon and is mostly reported as nonpathogenic (Wright 2006, Densmore & Green 2007).

In our study, no physical changes were found in hosts corroborating with Bardsley & Harmsen (1973) which describe pathogenicity caused by *Trypanosoma* spp. in anurans is an extremely difficult phenomenon to establish, as it does not result in changes in the physical development of the host.

Trypanosomes of anurans are traditionally described based on their host and geographical origin, but this approach is weakened when the extreme polymorphism and plasticity of trypomastigote forms is considered (Ferreira et al. 2007). Recently, Rodrigues et al. (2019) described *Trypanosoma* sp. parasitizing two amphibian species from Brazilian midwest using morphology and morphometry. The morphological and morphometric study of trypanosomes continues to be used for the description and validation of new hosts and records of new distributions (Miyata & Yong 1990, Desser 2001, Lemos et al. 2008, Leal et al. 2009).

Using Bray-Curtis similarity indices, the present study corroborates the morphometric characterization and descriptions of the trypanosomes, providing statistical support for the morphological determination of the *Trypanosoma* sp. species, presenting high degrees of similarity with the species type, described by Martin & Desser (1990). Using molecular tools have been able to describe new species of *Trypanosoma* parasitizing amphibians species of genus *Leptodactylus* from Caatinga and Cerrado biomes and three species of alligators from the Amazon and Pantanal, all in Brazil (Fermino et al. 2015, Attias et al. 2016). Besides, were able to identify the leech of the genus *Haementheria* as *Trypanosoma* vector

for alligators, being found in the oral cavity of the animals (Fermino et al. 2015). However, more studies using a broad sample of *Trypanosoma* species and characters are necessary, since the morphometric and morphological similarity presented in the present study does not match the molecular phylogenies presented by Martin et al. (2002), Ferreira et al. (2008) and Spodareva et al. (2018). The present study, records *R. major* parasitized by trypanosomes, which represents a new vertebrate host for *Trypanosoma* sp. in Brazil.

Hematophagous diptera can act as vectors of different lineages of *Trypanosoma* spp., especially for Amazon terrestrial anurans (Ferreira et al. 2008). *Rhinella major* is a species with predominantly terrestrial habits in the adult phase and uses holes in the ground to shelter, which could increase the chances of contact with hematophagous dipteran. There are evidence that some species of *Corethrella* may transmit *Trypanosoma* by feeding on calling frogs and this association is likely an ancient one (Johnson et al. 1993, Borkent 2008). Nevertheless, the mechanism of transmission of these hemoflagellates in the Amazon region is relatively unknown.

More comprehensive studies on the physiological aspects of host response to trypanosomatid infection are needed, as well as studies to improve and extend the taxonomy of this group using molecular tools. It is important to know the diversity of hosts, since their performance can be affected by parasitism.

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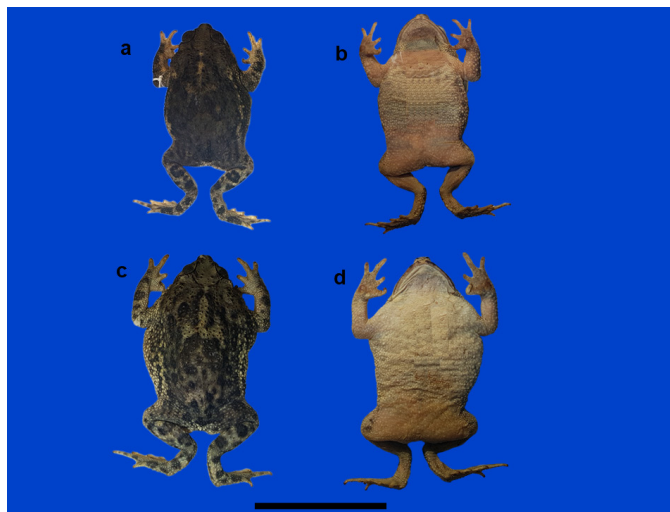


Figure 3. *Rhinella major* collected on the right bank of the Curuá-Una River. a – Male in dorsal position; b – Male in ventral position; c – Female in dorsal position; d – Female in ventral position (bar 5 cm).



Figure 4. Trypomastigote forms of *Trypanosoma* sp. observed in the blood circulation of *Rhinella major* (bar 10 μ m).

Table II. Morphometric measurements of the Trypomastigote shape (n=10) of *Trypanosoma* spp. in *Rhinella major* from the Curuá-Una Hydroelectric Power Plant, Santarém, western Pará in the Eastern Amazon (Brazil).

Parameters	Mean \pm Deviation	Minimum/Maximum
Total body length with flagellum	65.97 \pm 6.32	53.55-76.47
Body length along the midline	51.97 \pm 5.44	43.97-60.83
Distance from the center of the nucleus to the posterior extremity	21.76 \pm 3.21	15.04-26.53
Distance from the center of the nucleus to the anterior extremity	30.22 \pm 3.68	22.86-34.58
Length of the free flagellum	12.60 \pm 6.42	13.58-17.92
Nucleus Length	3.46 \pm 0.39	2.61-3.95
Nucleus width in center portion	2.09 \pm 0.90	1.34-4.64
Body width in the center of the nucleus	2.84 \pm 0.94	2.11-5.45
Distance from the center of the kinetoplast to the center of the nucleus	15.27 \pm 2.62	9.20-18.77
Distance from the center of the kinetoplast to the posterior extremity	6.14 \pm 2.55	0-10.13
Kinetoplast length	0.80 \pm 0.21	0.46-1.15
Kinetoplast Width	0.84 \pm 0.12	0.62-1.05

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The authors declare that there is no conflict of interests regarding the publication of the manuscript. The authors state that MSc. Tássio A. Coelho and MSc. Darlison C. de Souza designed the study, collected research data, analyzed the data, wrote, and revised the article. Dr. Lincoln L. Corrêa and Dr. Ricardo A. Kawashita-Ribeiro designed the study, analyzed the data and revised the article. All authors read and approved the final manuscript.

