

Short note

Morphometric and morphological characterization of *Trypanosoma* sp. (Kinetoplastea: Trypanosomatidae) hemoparasite of Crested Eagle, *Morphnus guianensis* in the Brazilian Amazon

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ABSTRACT. *Morphnus guianensis* is a species belonging to the Accipitridae family classified as almost threatened by the International Union for Conservation of Nature (IUCN). Trypanosomes are flagellated protozoa that carry out their life cycle in the circulatory system of vertebrate hosts and within the digestive tract of invertebrate hosts. This study recorded *Trypanosoma* sp. parasitizing *M. guianensis* in the Brazilian Amazon, providing data related to the morphology and morphometry of the trypomastigote forms of peripheral blood of this bird. The mean values of the measured morphological regions were used to compare morphometric similarity with morphotypes described for *Trypanosoma* sp. through the Bray-Curtis method and, after statistical analysis, the dendrogram was generated from the morphological similarities with a similarity greater than 95% with *T. paddae* described for different hosts in different geographical regions.

Keywords: morphology, hemoparasite, neglected taxon, birds

Introduction

Crested Eagle (*Morphnus guianensis*) is a species of eagle belonging to the Accipitridae family classified as almost threatened by the International Union for Conservation of Nature [1] and as vulnerable by the National List of Endangered Brazilian Fauna Species [2,3]. It mainly inhabits well-preserved tropical and subtropical forests, occurring in great part of the neotropics, from Mexico to Argentina [3–5] It feeds mainly on arboreal mammals such as primates, marsupials,

birds and reptiles [6,7].

Trypanosomes are flagellated protozoa that carry out their life cycle in the circulatory system of vertebrate hosts and in the digestive tract of invertebrate hosts, and transmission to birds can occur through a variety of vectors such as: mites, louse flies, biting midges, culicid and simuliid mosquitoes [8–10]. The pathological changes observed in birds are very similar to the pathological manifestation in mammals. An experiment demonstrated that birds infected with *Trypanosoma*, present alterations in the cardiac tissue and in the

spleen, resulting in focal myocarditis [8].

Although the presence of *T. avium* has been described for some species of birds of prey [11], trypanosomes are traditionally hematozoa, but compilations of parasite surveys in New World birds by blood smears show that trypanosomes are relatively rare in peripheral blood. For example, the analysis of blood smears from a number of passerine species resulted in a prevalence of only 18% [12]. Due to low prevalence, trypomastigote forms were described by the culture method of [13].

The presence of *Trypanosoma* has not been reported or described so far in trypomastigote forms parasitizing *M. guianensis*. Thus, this manuscript aims to record, in an unprecedented way, *Trypanosoma* sp. parasitizing *M. guianensis* in the Brazilian Amazon, providing data related to the morphology and morphometry of the trypomastigote forms.

Materials and Methods

Host characterization. A young specimen of *M. guianensis* (Fig. 1) was sent to the Zoo of the Universidade da Amazônia (ZOOUNAMA), located in Santarém, western Pará, Brazil (Fig. 2). The individual was rescued by the Fire Department



Figure 1. Juvenile individual of *Morphnus guianensis* infected with *Trypanosoma* sp. (scale bar: 5 cm) (Photograph E.V.L)

on the Bela Vista suburb periphery of the urban area of the municipality of Santarém on December 9th, 2015 (2°29'S, 54°43'W). This specimen was in the quarantine sector of ZOOUNAMA for five days before the blood collection.

Hematological collection and procedures. During a routine evaluation in the Quarantine Sector of ZOOUNAMA, on December 14th, a blood sample was collected by puncture of the alar vein (brachial/ulnar) using a needle and syringe

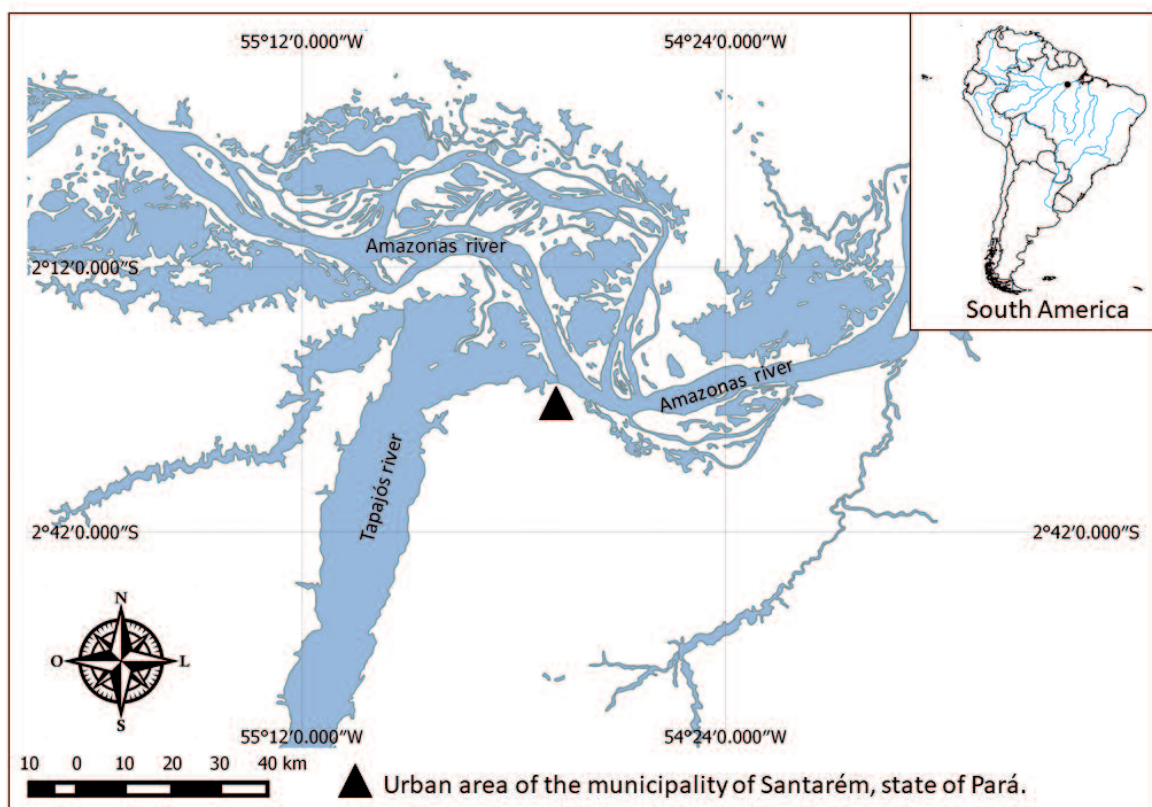


Figure 2. Rescue location of the Crested Eagle in Santarém city state of Pará, Brazilian Amazon

Table 1. Morphometric parameters (μm) of trypomastigotes of *Trypanosoma* sp. from *Morphnus guianensis* and *Trypanosoma avium*

Measuremc	<i>Trypanosoma</i> sp.*	<i>T. avium</i> [16] (A)	<i>T. avium</i> [16] (B)	<i>T. avium</i> [17]
TL	44.4	56.0	51.1	43.5
BL	39.2	52.1	43.7	40.6
PK	3.5	–	–	10.7
PN	18.2	24.9	20.0	19.5
NA	20.9	–	–	–
KN	13.8	–	–	–
FF	6.1	6.2	7.4	7.0
BW	3.6	4.6	4.6	5.2

Explanations: * present study; TL: total length; BL: length of the body without the flagellum along the midline; PK: distance from the posterior end to the kinetoplast; PN: distance from the posterior region to the center of the nucleus; NA: distance from the center of the nucleus to the anterior end; KN: distance from the kinetoplast to the center of the nucleus; FF: free flagellum; BW: body width at the center of the nucleus.

containing EDTA anticoagulant. Blood smears were made in duplicates and sent to the Universidade Federal do Oeste do Pará-UFOPA. The slides were fixed with methanol and stained with a solution of GIEMSA in buffered water at a 1:10 dilution and analyzed under an optical microscope with a 1000 \times magnification in search of trypomastigote forms. The shapes were quantified and measured with the aid of a Zeiss Axioplan optical microscope with an Axiocam ERc 5s camera.

Morphometric characterization of the trypomastigote form of *Trypanosoma* sp. To determine the morphometric characteristics, the software Zen Blue edition 2 was used. The morphometric measurements of the trypomastigotes were adapted from [14,15] being measured the.

The mean values of the measured morphological regions were used to compare morphometric similarity with described morphotypes of *Trypanosoma avium*, using the Bray-Curtis method with the aid of the Past 3.0 statistical program.

Compliance with ethical standards. The collection of peripheral blood from *M. guianensis* was authorized by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA)/Chico Mendes Institute for Biodiversity Conservation (ICMBio) (n^o 46202-3/2017). The blood collection procedures were approved by the Animal Research Ethics Committee of the Universidade Federal do Oeste do Pará (Nucleus 06001/2015-CEUA/UFOPA).

After identification, representative specimens of

the parasites were deposited in the Zoological Collection of the Zoological Museum of UNICAMP, under the number ZUEC SAR 03.

Results

Taxonomic summary

Host: *Morphnus guianensis* (Daudin, 1800)

Family: Accipitridae

Type locality: Bela Vista suburb, Santarém municipality, Pará state, Brazil

Habitat: bloodstream of *M. guianensis*

Deposited material: ZUEC SAR 03

New host

Species: *Morphnus guianensis* (Daudin, 1800)

Geographic distribution: Central America parts of Guatemala and Belize, Panama, South America

Collection location: Bela Vista suburb, Santarém municipality, west of the state of Pará, Brazil. Coordinates (2^o29'S, 54^o43'W).

During the search in two smears, seven trypomastigotes of *Trypanosoma* sp. were found (Fig. 3), showing elongated C-shaped body profile with attenuation in the anterior and posterior extremities, with a prevalence of two to three vacuoles in the anterior region and one to two in the posterior region. Thin and discrete undulating membrane with a predominantly ovoid-shaped nucleus slightly displaced towards the posterior region. The flagellum is short on average, less than one fifth of the total body length. Small kinetoplast with a circular-oval shape, displaced to the lateral

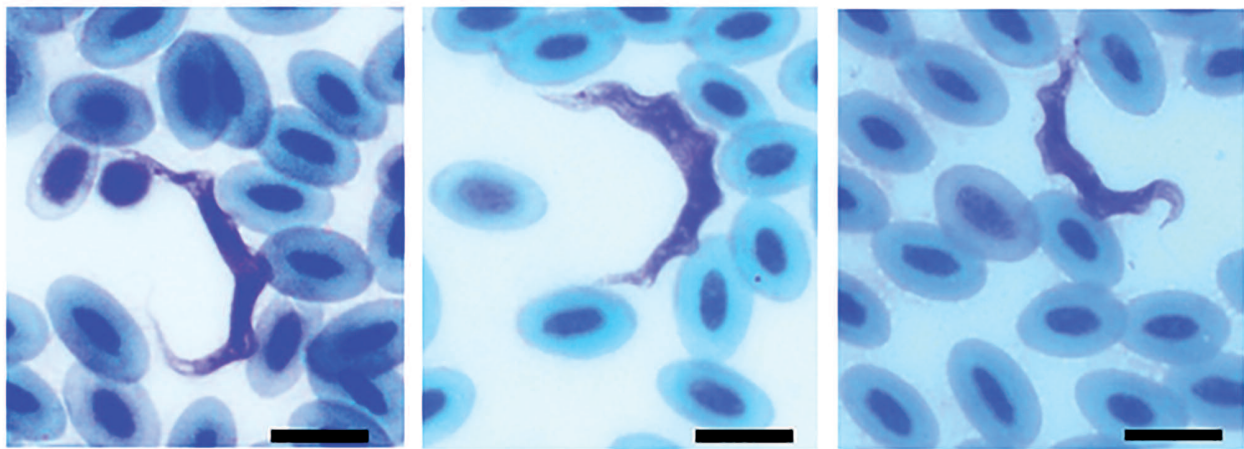


Figure 3. Bloodstream trypomastigotes of *Trypanosoma* sp. observed in a Giemsa-stained blood smear from *Morphnus guianensis* (Crested Eagle) from Santarém municipality, PA, Brazil (Scale bar: 10 μ m)

end of the cytoplasm, the region between the kinetoplast and the posterior end of easy visualization (Fig. 4).

After the morphological features were described, the measurement of the morphometric values of the trypomastigote forms was performed, considering the average values of the measured regions (Table 1) and comparing the similarities by the Bray-Curtis method with the studies of [11,16–20].

After the statistical analysis, a dendrogram (Fig. 5) was generated from the morphological similarities with a similarity of 96.03% according to the contingency values (Table 2) with the *T. padadae* morphotype described by [18] parasitizing *Corvus brachyrhynchos brachyrhynchos*.

Discussion

According to [11], a large part of the studies of avian trypanosomes are based on observations of trypomastigotes by light microscopy as they are conducted in remote areas, making it possible to perform more specific analyzes, such as: biochemical, immunological and in vitro cultivation, in only a few records. Despite the limitations in the identification of trypomastigote forms based solely on light microscopy analysis, its use as a low-cost technique has been helping to determine the distribution pattern of the main *Trypanosoma* spp. trypomastigote morphotypes in wild animals, particularly in tropical areas [19]. In the present study, the observations were made during a routine clinical examination of the host located in an area with scarce resources for carrying out more robust analyzes such as DNA sequencing.

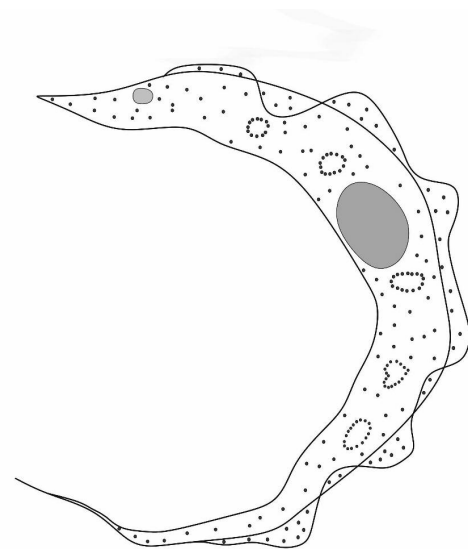


Figure 4. Diagrammatic illustration of *Trypanosoma* sp. morphotype trypomastigote as seen in a stained preparation made from the blood of *Morphnus guianensis* (Crested Eagle) based on light microscopy observation

We are aware that, with molecular analysis, we could characterize and possibly identify new species of *Trypanosoma* in peripheral blood from birds.

According to [11,21] despite the importance of taxonomy and systematics for understanding the biological cycle in the natural environment, these are still fields with limited development concerning avian trypanosomes. The negligence in the study of these hemoparasites in avian hosts is more evident when compared to the numbers of studies in mammalian hosts. In the case of this specimen, it cannot be said whether the infection occurred in free life or in captivity, as the blood sample was

Table 2. Contingency values for morphometric similarity of different *T. avium* and *Trypanosoma* species records from the Bray-Curtis matrix

	<i>Trypanosoma</i> sp.*	<i>T. avium</i> (A) [16]	<i>T. avium</i> (B) [16]	<i>T. avium</i> [17]	<i>T. anguiformis</i> [11]	<i>T. polygranularis</i> [11]	<i>T. irwini</i> [19]	<i>T. naviformis</i> [20]	<i>T. paddae</i> [18]	<i>T. antarioensis</i> [18]
<i>Trypanosoma</i> sp.	1	76.93	78.66	81.54	92.38	89.88	86.31	82.64	96.03	83.82
<i>T. avium</i> (A) [16]	76.93	1	96.43	88.23	72.03	70.24	64.91	63.53	75.52	68.25
<i>T. avium</i> (B) [16]	78.66	96.43	1	91.10	73.79	72.88	67.51	65.24	77.23	71.03
<i>T. avium</i> [17]	81.54	88.23	91.10	1	76.06	74.25	71.37	67.59	83.80	71.95
<i>T. anguiformis</i> [11]	92.38	72.03	73.79	76.06	1	93.25	86.22	88.00	91.65	88.75
<i>T. polygranularis</i> [11]	89.88	70.24	72.88	74.25	93.25	1	87.37	89.14	89.53	93.06
<i>T. irwini</i> [19]	86.31	64.91	67.51	71.37	86.22	87.37	1	78.84	86.71	84.57
<i>T. naviformis</i> [20]	82.64	63.53	65.24	67.59	88.00	89.14	78.84	1	82.74	87.82
<i>T. paddae</i> [18]	96.03	75.52	77.23	83.80	91.65	89.53	86.71	82.74	1	84.13
<i>T. antarioensis</i> [18]	83.82	68.25	71.03	71.95	88.75	93.06	84.57	87.82	84.13	1

* Present study

collected during quarantine, five days after the bird's arrival at the Zoo.

The main differences between the trypomastigote form recorded in this study and the morphometric patterns presented by [11] for *T. polygranulares* and *T. anguiformes*, by [22] for *T. antarioensis*, by [19] for *T. irwini*, by [23] for *T. maviformis*, by [16] and [20] for *T. avium* are associated with the shape and size of the cell body and flagellum, as well as a structural organization of cytoplasmic organelles.

Despite the existing morphometric similarities (>95%) with the morphotype recorded by [18] parasitizing *C. brachyrhynchos brachyrhynchos* in Ontario, Canada, these similarities are not enough for us to conclude that it is the same species of *Trypanosoma*, since the plasticity of these organisms makes identification at the species level unfeasible based solely on morphological and morphometric characteristics, requiring the application of molecular tools. However, it is important to note that there is no overlap in the geographic distribution of both host species and that both species diverge considerably in their preferred

habitats [24,25].

It may be understandable due to the lack of evidence regarding the economic importance of these organisms related to risks to human health. However, understanding the real diversity of these organisms, as well as the implications of the parasite-host relationship, are still little explored fields in the Brazilian Amazon region, which harbor the highest bird diversity of the planet, and there is a need for taxonomic and more robust systematic tests related to these hemoparasites.

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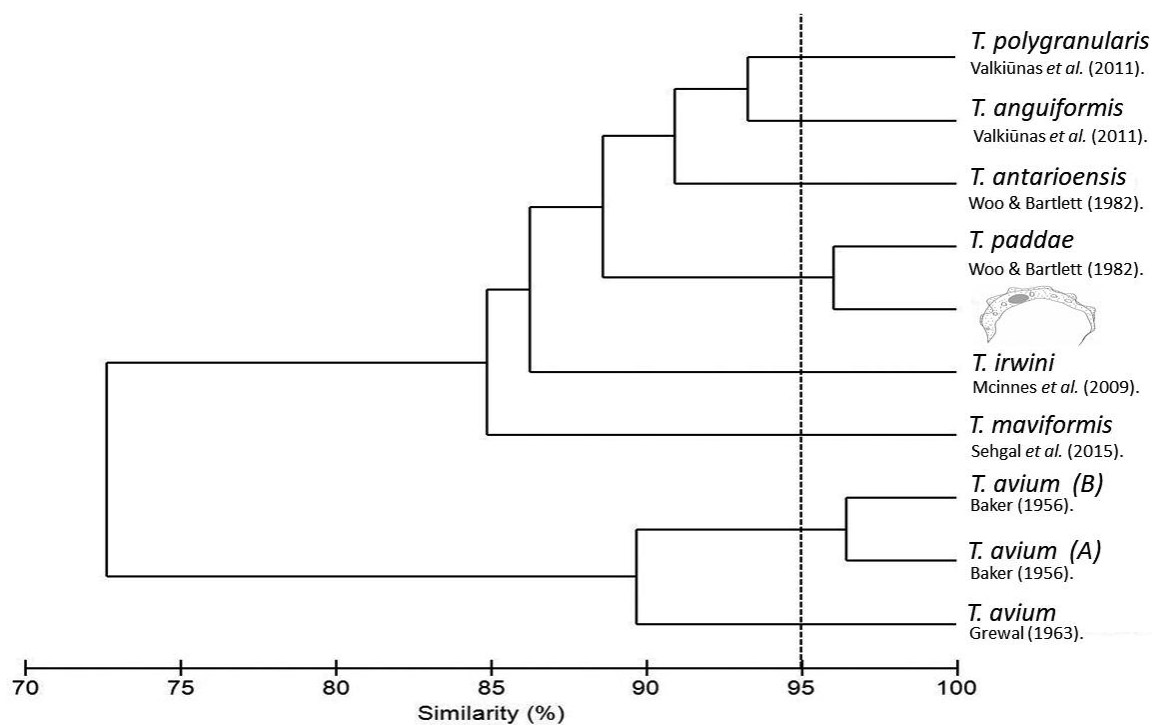


Figure 5. Dendrogram of Bray-Curtis similarity of the morphological features of the morphotypes of the *Trypanosoma avium* species complex

– Finance Code 001.

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