Infection by Trypanosoma spp. in Platydoras armatulus (Siluriformes, Doradidae), in Southwestern Amazon, Brazil

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Research Article

Keywords: Acre, Fishes, Parasitology, Hemoparasite

Posted Date: March 31st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-355103/v1

Abstract

Trypanosoma is a hemoflagellate capable of infecting a wide variety of invertebrates and vertebrates, such as Neotropical freshwater fish. Thus, the present study described and morphologically compared *Trypanosoma* sp., found in *Platydoras armatulus*, Valenciennes, 1840, in southwestern Amazon. A sampling of fish specimens was carried out in a river located in Guajará, Amazonas, Brazil. Fish blood samples were collected through a cardiac puncture. Thus, smears were made for quantification, morphometric measurements, and morphotyping of trypanosomes found. Prevalence, mean abundance, and intensity of parasitism were estimated in the specimens of parasitized fish. Five fish specimens were collected, showing a 100% prevalence of parasites in the host. We found two *Trypanosoma* morphotypes, A and B, in which A had the highest infection intensity in host specimens. Thus, the present study showed the first report of *Trypanosoma* spp. in *P. armatulus*, besides the probability of two parasitic morphospecies in the blood of these fish specimens.

Introduction

Blood parasites of the genus *Trypanosoma* occur in diverse invertebrates (Meneguetti et al. 2014) and vertebrates (Molyneux 1983), such as Neotropical freshwater fish. Most of these hemoparasites are heterogeneous, i.e., they involve two hosts to complete their life cycle (Molina et al. 2016). Trypanosomes in the blood of infected fish undergo many morphological transformations (amastigote, spheromastigote, epimastigote, and trypomastigote) until they are consumed by leech species, which ingest the infected blood. These flagellates then begin to divide in the leech stomach (Lom and Dykova 1992; Eiras 1994, Eiras et al. 2008; Corrêa et al. 2016).

Trypanosoma infection can cause anemia in fish (Khan 1985) and is directly related to parasitemia (Woo 2006, Ahmed et al. 2011). Besides, some studies have reported several changes in vital organs of infected fish, and in some cases, anorexia associated with high infection by trypanosomes (Dyková and Lom 1979; Islam and Woo 1991). However, although some *Trypanosoma* species cause mortality and morbidity of vertebrate hosts (Ardelli and Woo 1998), studies have recorded persistence of infection with host survival, which may indicate parasite-host coevolution, with a delicate balance between the mechanisms of parasite evasion and fish immune system (Overath et al. 1999; Wiegertjes and Forlenza 2010). Thus, studies with fish trypanosomes are important not only for knowing potential ichthyofauna pathogens but helping understand these parasite adaptations to survive in different hosts and different geographical regions (Kelly et al. 2014), as found in mammalian trypanosomes (Echodu et al. 2015, Greif et al. 2015).

In Brazil, there are approximately 60 *Trypanosoma* species described infecting diverse freshwater and saltwater fish families (Eiras et al. 2012). For the family Doradidae, *Trypanosoma* spp. has been found in *Pterodoras granulosus* (Albuquerque et al. 1996), *Trachydoras paraguayensis* (Eiras 1991), *Rhinodoras dorbignyi* (Fonseca e Vaz 1928), *Franciscodoras marmoratus* (Fonseca 1935), and *Corydoras* sp. (Eiras et al. 2012). However, there was no study of *Trypanosoma* hemoparasitizing *Platydoras armatulus*

Valenciennes, 1840. Thus, the present study aims to report, for the first time, the occurrence of *Trypanosoma* spp. in *P. armatuluse* and morphologically compare two morphotypes of these hemoparasites found in this fish species.

Material And Methods

Study area

The fish were collected (authorization from the Brazilian Institute of Environment and Renewable Natural Resources No. 59642-2/2019) in the Ipixuna River (7°17'13"S 72°36'49" W), a tributary of the Juruá micro-basin, located in the municipality of Guajará, State of Amazonas, Brazil (Fig. 1).

Sampling

Fish sampling was performed using 80 m long and three m high gillnets, with mesh sizes of one and a half cm, two and a half cm, three and a half cm, and five and a half cm between opposite nodes. We used two nets, each with 12 mm mesh, two meters high, and 12 meters open. The nets were launched ten times at each collection point. We also used beach trawls of nine meters long and two meters and forty centimeters high, with a 13 mm mesh. The fish collected were sent to the Aquatic Ecology Laboratory of the Federal University of Acre-UFAC, where they were identified, measured, and weighed.

The specimens collected were anesthetized using menthol in a similar way to benzocaine. Then, to assess the presence of hemoparasites, blood samples were collected by cardiac puncture, using a hypodermic syringe containing an anticoagulant (5% EDTA). Thus, duplicate blood smears were made per fish sample. The blood smears were stained using Quick Panoptic/LABORCLIN® and examined by optical microscopy with 400 and 1000x magnification, at the Microscopy Laboratory I, at the Federal University of Acre (UFAC), Campus Cruzeiro do Sul, Acre, Brazil.

The description of the trypanosomes was made with 14 specimens of Morphotype A and 11 specimens of Morphotype B. For the morphometric evaluation, the parasites were photographed using a Leica DM 500 optical microscope, with an ICC50 HD coupled camera. The photos were used to determine the morphometric characteristics of the trypanosomatids, using the ImageJ software. Cytomorphometric measurements of the trypanosomatids were performed according to Borges (2016) (Fig. 2).

Data analysis

Prevalence, mean abundance and mean intensity were calculated according to Bush et al. (1997). We used the direct method, adapted from De Carli (2001), to estimate the infection intensity (expressed in parasites/mL). We recorded and calculated all parasites found in 100 microscopic fields, with 1000x magnification. It is estimated that 100 microscopic fields are equivalent to 0.2 µL of blood. Thus, the intensity of infection = (number of parasites × 5) × 1,000 = (parasites/mL) (De Souza and Corrêa 2019).

The morphometric variables of *Trypanosoma* spp. underwent homoscedasticity (Levene) and normality (Shapiro-Wilk) tests. Thus, we used the Student's T-Test ($p < 0.05$) for the parametric data to verify a difference in the morphometric parameters between the morphotypes of *Trypanosoma* spp. The analyses were performed using the R software version 3.6.1.

Results

We collected five *P. armatulus* individuals (length $\bar{x}$ =9.3 ± 1.25; weight $\bar{x}$ =4.15 ± 10.4), all infected by *Trypanosoma* spp. Morphotype A (n = 14) was identified infecting three individuals of *P. armatulus* and Morphotype B (n = 11) only two, with the intensity of *Trypanosoma* infection in the specimens averaging 25.10^3 parasites/mL of blood.

The prevalence of Morphotype A in the specimens was 60%, with a mean abundance of 2.8, and a mean infection intensity of 4.6. Morphotype B had a prevalence of 40%, with a mean abundance of 2.2, and a mean infection intensity of 5.5 in fish specimens.

The morphometric parameters of Morphotypes A (Fig. 3A) and B (Fig. 3B) of *Trypanosoma* spp. trypomastigotes are available in Table 1.

Table 1
Morphometric parameters of Morphotypes A and B, expressed as mean and standard deviation (µm).

Morphometric parameters	Morphotype A	Morphotype B	T	p
Total body length (CT)	55.6 ± 1.5	59.8 ± 0.4	4.12	**0.004****
Body length (CC)	37.0 ± 1.3	39.1 ± 1.1	2.30	**0.04***
Nucleus length (CN)	2.8 ± 0.2	3.0 ± 0.3	1.01	0.35
Kinetoplast length (CK) (CK)	0.9 ± 0.1	1.1 ± 0.1	0.73	0.48
Free flagellum length (F)	18.5 ± 0.9	20.4 ± 1.1	2.48	**0.04***
Body width (LC)	2.1 ± 0.1	2.2 ± 0.1	0.42	0.68
Nucleus width (LN)	1.5 ± 0.1	3.4 ± 0.1	2.01	0.06
Kinetoplast width (LK)	0.7 ± 0.07	0.7 ± 0.07	1.05	0.32
Distance from the posterior end to the kinetoplast center (PK)	1.1 ± 0.1	1.25 ± 0.3	0.36	0.56
Distance from the kinetoplast center to the nucleus center (NK)	22.1 ± 1.2	23.0 ± 1.6	0.59	0.53
Distance from the anterior end to the nucleus center (NA)	13.7 ± 1.5	14.8 ± 1.1	1.12	0.29
Distance from the posterior end to the nucleus center (PN)	23.3 ± 2.2	24.0 ± 1.2	0.69	0.53
*p > 0.05; ** p > 0.01				

The morphometric measurements with statistical differences ($p < 0.05$) between the two morphotypes were the total length ($t = 4.12$; $p = 0.004$), flagellum size ($t = 2.48$; $p = 0.04$), and body length ($t = 2.30$; $p = 0.04$).

Trypanosoma spp. (Morphotype A).

Their body was attenuated, slender, forming undulations in the anterior part and another close to the nucleus. This morphotype showed 11 cytoplasmic vacuoles, six in the anterior part, five in the posterior part to the nucleus, and one close to the kinetoplast. The kinetoplast and the nucleus had an ovoid shape, and the *Trypanosoma* spp. plasma membrane was quite wavy and defined, medium flagellum, and with few flexions.

Trypanosoma spp. (Morfotipo B).

These specimens had a slender body, with undulations, one more accentuated in the nucleus region, and four cytoplasmic vacuoles, two in the anterior and posterior part to the nucleus. These specimens had ovoid nucleus and kinetoplast, a plasma membrane wavy in the anterior and posterior part of the body. The flagellum was short with a ripple.

Discussion

The present study showed the first report of *Trypanosoma* spp. in *P. armatulus*, increasing the number of hosts for these hemoparasites. In the Amazon region, most host fish reported are from the family Loricariidae (Fujimoto et al. 2013; Corrêa et al. 2016; Souza and Corrêa; 2019). For the family Doradidae, the only species with these hemoparasites was *Pterodoras granulosus*, in the Tocantins River (Lopes et al. 1991). Thus, there may be more Doradidae species infected by these parasites in the region. The presence of this organism in *P. armatulus* possibly indicates the occurrence of an intermediate host, being leeches in these systems. Although we did not find leeches in fish, these infections by *Trypanosoma* spp. suggest the existence of these hosts in this natural environment (Lemos et al. 2015; Molina et al. 2016; Woo and Black 1984).

The prevalence of parasites in the blood of hosts was 100%. This fact may be associated with this fish species behavior since they are usually found in rivers (Britski et al. 1981) and feed on organisms hidden in these environments (Sterba 1963). In this sense, there is a chance that these organisms become susceptible to attack by leeches contaminated by *Trypanosoma* spp. that live in these environments of margin and aquatic vegetation (Woo, 2006; Ahmed et al. 2011). In this way, identifying new hosts of *Trypanosoma* spp. is vital since it can assist in resolving common problems in the study of pathogens caused by these parasites, in addition to contributing to the phylogeny of the trypanosomatid group (Lom 1979).

The present study showed two trypomastigote morphotypes of *Trypanosoma* spp. in *P. armatulus*, with a higher prevalence of Morphotype A. However, we did not find both morphotypes were in the same individual. Some authors consider that the same fish species may be infected by more than one *Trypanosoma* species (Fróes et al. 1979; Ribeiro et al. 1990; Lemos et al. 2015). Thus, there is no specificity of this hemoparasite with a host. However, *Trypanosoma* spp. showed high pleomorphism (Eiras et al. 2012). Therefore, it is impossible to determine that Morphotypes A and B found here represent different species since the measurements with significant differences may suffer variations during the parasite development in the host. According to Lom (1979), pleomorphism can be expressed in aspects such as changes in the body's total length and width (thin or wide shapes) and the free flagellum length. The number of undulations in the undulating membrane, the presence and number of cytoplasmic granules, and the distance from the posterior kinetoplast are also aspects expressed by pleomorphism. It was mainly these morphological characteristics that varied in Morphotypes A and B.

We found, for the first time, *Trypanosoma* spp. infecting *P. armatulus*, increasing the number of hosts for these trypanosomatids. This study also suggests that different forms of these hemoparasites can occur

in the same fish species. We emphasize that the material collected in this study is stored, and future molecular analyses will be carried out, including the genetic material sequencing, which will confirm whether these differences are pleomorphism or are related to different *Trypanosoma* species.

Declarations

Author Contributions Statement

For the preparation of this short communication, for analysis and illustrations of the following work were done by Gabriele Oliveira Texeira and Henrique Paulo Silva de Melo for intellectual part and corrections Sérgio Luis Prolo, Ricardo Massato Takemoto, Luís Marcelo Aranha Camargo e Dionatas Ulisses Meneguetti

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Figures

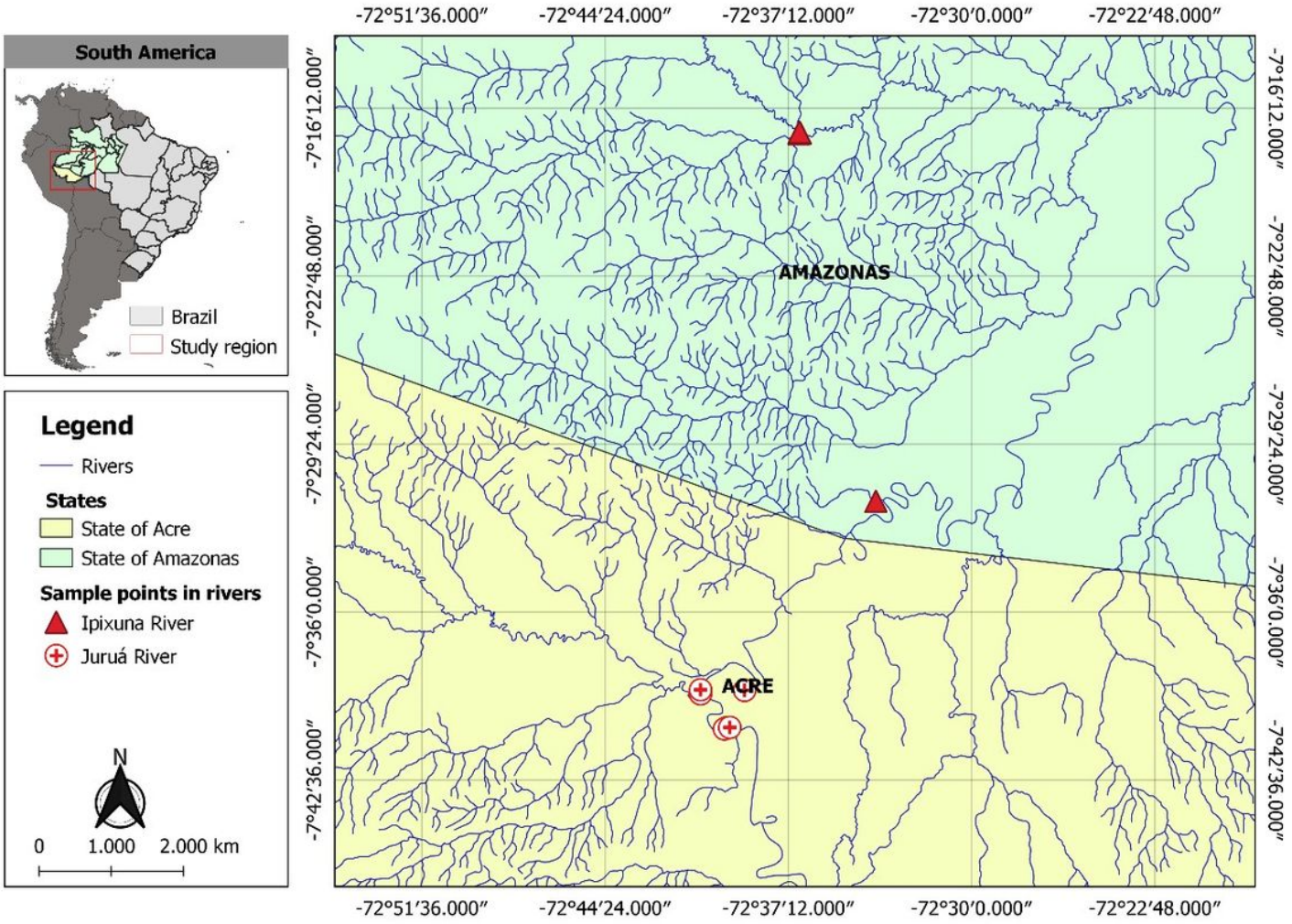

Figure 1

Location and georeferencing of river where P. armatulus samples were collected. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

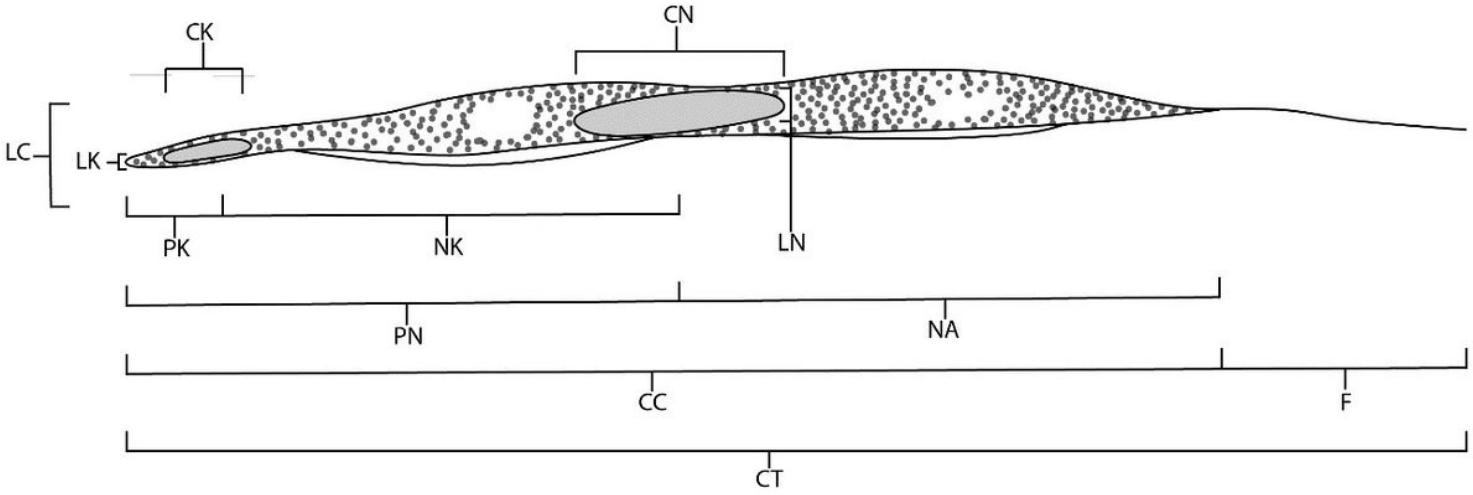

Figure 2

Scheme of the morphometric measurements of trypanosomatids found in P. armatulus. Total body length (CT); Body length (CC); Nucleus length (CN); Kinetoplast length (CK); Free flagellum length (F); Body width (LC); Nucleus width (LN); Kinetoplast width (LK); Distance from the posterior end to the kinetoplast center (PK); Distance from kinetoplast center to the nucleus center (NK); Distance from the anterior end to the nucleus center (NA); Distance from the posterior end to the nucleus center (PN).

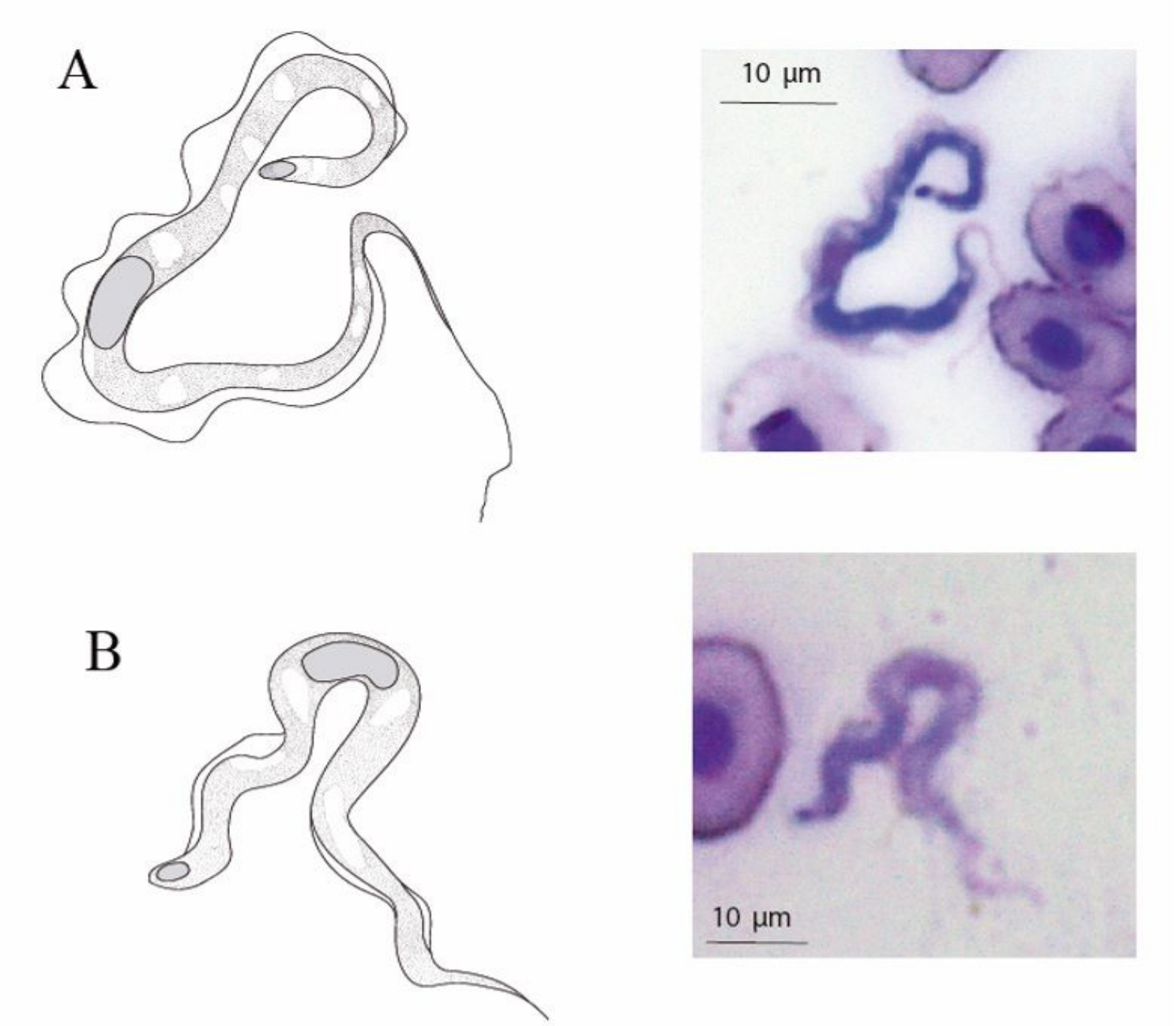

Figure 3

Morphotypes of the trypomastigote forms of Trypanosoma spp. found parasitizing P. armatulus in the Juruá River basin system. A - Morphotype A; B - Morphotype B (scale = 10 µm).