Infection with *Trypanosoma* spp. in *Platydoras armatulus* (Siluriformes, Doradidae), in southwestern Amazon, Brazil

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Abstract *Trypanosoma* is a hemoflagellate capable of infecting a wide variety of invertebrates and vertebrates, such as Neotropical freshwater fish. The present study described and morphologically compared *Trypanosoma* spp., found in *Platydoras armatulus*, Valenciennes, 1840, in southwestern Amazon. Fish specimens were sampled in Ipixuna and Juruá rivers located in the states of Amazonas and Acre, Brazil. Fish blood samples were taken by cardiac puncture, and smears were prepared for quantification, morphometric measurements, and morphotyping (characterization of the trypanosomes according to their morphological variations) of trypanosomes found. Prevalence, mean abundance, and intensity of parasitism were estimated in the parasitized fish specimens. 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 presented the first report of *Trypanosoma* parasitizing *P. armatulus*, with different morphological variations.

Keywords Neotropical · Hemoparasitism · Morphotype · Distribution

Introduction

Blood parasites of the genus *Trypanosoma* occur in diverse invertebrates (Meneguetti et al. 2014) and vertebrates (Molyneux 1983), such as Neotropical freshwater fish (Rodrigues et al. 2019; Souza and Corrêa 2019; Sousa et al. 2020). Most of these hemoparasites are heteroxenous, i.e., they require two hosts to complete their life cycle (Molina et al. 2016; Bernotiene et al. 2020; Smit et al. 2020). Trypanosomes in blood of infected fish undergo morphological alterations until they are consumed by leech species, which ingest the infected blood (Lemos et al. 2015; Smit et al. 2020). These flagellates then begin to divide in the leech stomach (Lom and Dykova 1992; Eiras et al. 2008).

*Trypanosoma* infection can cause anemia in fish (Khan 1985) and is directly related to parasitemia (Woo 2006; Ahmed et al. 2011). In addition, some studies have reported several changes in vital organs of infected fish, and in some cases, anorexia associated with high infection with trypanosomes (Dyková and Lom 1979; Islam and Woo 1991). However, although some *Trypanosoma* species cause mortality and morbidity of vertebrate hosts (Ardelli and Woo 2001), 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 on fish trypanosomes are important not only for knowing potential ichthyofauna pathogens but also for helping understand these parasite adaptations to survive in different hosts and different geographical regions (Kelly et al. 2018), as found in mammalian trypanosomes (Echodu et al. 2015).

For the family Doradidae, Trypanosoma species has been found in Pierodoras granulosus (Albuquerque et al. 1996), Trachyodoras paraguayensis (Eiras et al. 1990), Rhinodoras dorbigiyni (Fonseca and Vaz 1928), Franciscodoras marmoratus (Fonseca 1935), and Corydoras sp. (Eiras et al. 2012). However, there was no study of Trypanosoma parasite Platydoras armatulus Valenciennes, 1840. Thus, the present study aimed to report, for the first time, the occurrence of Trypanosoma in P. armatulus and morphologically compare two morphotypes of these hemiparasites found in this fish species.

Material and methods

Study area

Fish were collected (authorization from the Brazilian Institute of the Environment and Renewable Natural Resources 59,642–2/2019) in the Ipixuna River (7°17′13″S 72°36′49″W) located in the municipality of Guajará, state of Amazonas and Jurua River (7°40′34.1″S 72°39′39.5″W) located in the municipality of Cruzeiro do Sul, state of Acre, 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 knots. We used two nets, each with 12 mm mesh, 2 m high, and 12 m open. Nets were launched ten times at each sampling site. We also used beach tows of 9 m long and 2 m and 40 m high, with a 13 mm mesh. Fish collected were sent to the Aquatic Ecology Laboratory of the Federal University of Acre-UFAC, where they were identified, measured, and weighed. The collected specimens were placed in an aquarium with 3L water and anesthetized with 250 mg L⁻¹ menthol dissolved in water, according to Façanha and Gomes (2005), the time needed to reach the total loss of balance and the reduction of opercular beats are smaller from concentrations above 150 mg L⁻¹.

Then, to assess the presence of hemiparasites, blood samples were collected by cardiac puncture, using a hypodermic syringe containing anticoagulant (5% EDTA). Thus, duplicate blood smears were prepared per fish sample. Blood smears were stained using Quick Panoptic/LABORCLIN® and examined by optical microscopy at 400 and 1,000 × magnification, at the Microscopy Laboratory I, at the Federal University of Acre (UFAC), Campus Cruzeiro do Sul, state of Acre, Brazil.

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

Nuclear index NI = PN/NA (position of nucleus in the body) and kinetoplasmatic index KI = PN/KN (position of kinetoplast in the body) were calculated in the present study according to the terminology of morphometric values and standards commonly adopted by Hoare (1972) and Smit et al. (2004).

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, at 1,000 × magnification. It is estimated that 100 microscopic fields are equivalent to 0.2 μL blood. Thus, the intensity of infection = (number of parasites × 5 (number of hosts)) × 1,000 = (parasites/mL) (De Souza and Corrêa 2019).

Thus, we used the Student’s T-Test (p < 0.05) for parametric data to check for differences in morphometric parameters between the morphotypes of Trypanosoma. The analyses were performed using the R 3.6.1 software.

Results

We collected five individuals of P. armatulus (length x = 9.3 cm ± 1.25; weight x = 4.15 g ± 10.4), all infected with Trypanosoma Morphotypes A and B. Trypanosoma spp. were observed in different hosts, in which three individuals infected with P. armatulus presented an average abundance of 5 ± 2.4 hemiparasites in 1 mL blood, with a total of 14 Trypanosoma spp. belonging to morphotype A, with an average infection intensity of 25.10³ parasites/mL blood. However, only two hosts showed an average abundance of 4 ± 0.5 Trypanosoma of morphotype B, with infection intensity of 2.10³ parasites/
mL blood. The prevalence of *Trypanosoma* of morphotype A in *P. armatulus* was 60% and morphotype B, 40%.

The morphometric parameters of morphotypes A (Fig. 3A) and B (Fig. 3B) of *Trypanosoma* species trypomastigotes are listed in Table 1. The morphometric measurements with statistical differences (p < 0.05) between the two morphotypes were the total length (μm) (t = 4.12; p = 0.001), flagellum size (μm) (t = 4.48; p = 0.001), body length (μm) (t = 4.30; p = 0.001), nucleus length (μm) (t = 3.56; p = 0.01) and distance from the posterior end to the kinetoplast center (PK); Distance from the 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 (NP).

**Morphotype A (Fig. 3A)**

Morphotype A showed both ends of the body tapered, mainly the anterior towards the flagellum, with cytoplasm having vacuole. The free flagellum was relatively short, and the plasmatic membrane was well defined and wavy, extending to the tapered part anterior to the flagellum. The
Fig. 3 Morphotypes of the trypomastigote forms of *Trypanosoma* spp. found parasitizing *P. armatulus* in the Jurumá River basin system. A—Morphotype A; B—Morphotype B (scale = 10 μm)

Table 1 Morphometric parameters (μm) of morphotypes A and B, expressed as mean and standard deviation (μm). Total body length (TL); Body length (BL); Nucleus length (NL); Kinetoplast length (KL); Free flagellum length (F); Body width (BW); Nucleus width (NW); Kinetoplast width (WK); Distance from the posterior end to the kinetoplast center (PK); Distance from the 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)

| Measurements | Morphotype A       | Morphotype B       | t   | p     |
|--------------|--------------------|--------------------|-----|-------|
| TL           | 68.10 ± 5.3        | 54.12 ± 3.72       | 4.12| 0.001**|
| BL           | 48.39 ± 6.2        | 35.67 ± 3.55       | 4.30| 0.001**|
| NL           | 2.84 ± 0.5         | 2.93 ± 0.67        | 3.56| 0.01*  |
| KL           | 1.01 ± 0.25        | 0.90 ± 0.23        | 0.73| 0.47   |
| F            | 20.03 ± 2.68       | 18.55 ± 2.19       | 4.48| 0.001**|
| BW           | 2.79 ± 0.73        | 2.03 ± 0.42        | 0.42| 0.099  |
| NW           | 1.86 ± 0.50        | 1.39 ± 0.35        | 2.01| 0.01*  |
| WK           | 0.88 ± 0.16        | 0.70 ± 0.16        | 1.05| 0.13   |
| PK           | 1.90 ± 1.3         | 0.95 ± 0.38        | 0.36| 0.32   |
| NK           | 26.99 ± 9.05       | 20.89 ± 5.80       | 3.15| 0.01*  |
| NA           | 19.55 ± 5.10       | 16.93 ± 4.43       | 1.12| 0.22   |
| PN           | 28.82 ± 9.33       | 21.88 ± 6.03       | 0.69| 0.25   |

*p > 0.05; **p > 0.01

nucleus had an oval shape and was located in a posterior position, close to the cell center (NI = 1.44 ± 0.11), with the sickle-shaped chromatin in all individuals. The kinetoplast had a circular shape and was located close to the posterior end in most individuals observed (KI = 1.02 ± 0.01).

Morphotype B (Fig. 3B)

Morphotype B, on the other hand, showed undulations in the anterior part of the body and near the nucleus. This morphotype had 11 cytoplasmic vacuoles, with six in the anterior and five in the posterior part of the nucleus, in which one was close to the kinetoplast. The nucleus had an ovoid and elongated shape, located in a posterior position close to the cell center (NI = 1.28 ± 0.12). The kinetoplast had an oval shape and was located close to the posterior...
extremity in most individuals observed \((K_{I} = 1.05 \pm 0.04)\). The plasmatic membrane of \textit{Trypanosoma} sp. was quite wavy and defined, with a medium and flexed flagellum.

**Discussion**

The present study is the first report of \textit{Trypanosoma} specie in \textit{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; Souza and Corrêa; 2019). For the family Doradidae, reported are from the family Loricariidae (Fujimoto et al. 2013; Souza and Corrêa; 2019). For the family Doradidae, the only species with these hemoparasites was \textit{Pterodoras granulosus}, in the Tocantins River (Lopes et al. 1991). Thus, there may be more Doradidae species infected with these parasites in the region. The presence of this organism in \textit{P. armatulus} possibly indicates the occurrence of an invertebrate vector, such as leeches. Although we did not find leeches in fish, these infections with \textit{Trypanosoma} species suggest the existence of these hosts in this natural environment (Lemos et al. 2015; Molina et al. 2016; Woo and Black 1984).

The present study showed the presence of \textit{Trypanosoma} in blood of all hosts analyzed, indicating a high prevalence of these parasites in this fish species. Although leeches were not found parasitizing these fish, the present study suggests that these organisms were susceptible to attack by leeches contaminated with \textit{Trypanosoma}. This because \textit{P. armatulus} has the habit of foraging in search of small organisms, such as macroinvertebrates, in the aquatic vegetation present on the banks of rivers (Woo 1963). Thus, as these riverbank environments and aquatic vegetation are habitats for leech species (Woo 2006; Ahmed et al. 2011), it may have facilitated predation on these fish. And the fact that we do not find leeches in this species may be related to the behavior of these organisms, which can attach to a host and feed until engorgement, reaching up to eleven times their body weight, and then detach (Mann 1962).

The present study registered two trypomastigote morphotypes of \textit{Trypanosoma} in \textit{P. armatulus}, with a higher prevalence of morphotype A. However, we did not find both morphotypes in the same individual. Some authors consider that the same fish species can be infected with more than one \textit{Trypanosoma} species (Ribeiro et al. 1990; Lemos et al. 2015). Nevertheless, it is impossible to determine that morphotypes A and B found here represent different species, since measurements with significant differences such as total body length, flagellum length and characteristics, such as changes in total body width (thin or wide) and the distance from the posterior kinetoplast, found in the present study, may vary in individuals of the same species, thus they are known as aspects expressed by pleomorphism, according to Lom (1979). In this sense, only molecular analysis could identify the species of these hemoparasites.

We found, for the first time, \textit{Trypanosoma} infecting \textit{P. armatulus}, increasing the number of hosts for these trypanosomatids, which is fundamental, as it can help solve common problems in the study of diseases caused by these parasites (Lom 1979). This study also suggests that different morphological forms of this hemoparasite can occur in the same fish species. However, molecular analyses are required, including the sequencing of the genetic material, to confirm whether the morphological differences are a pleomorphism or are related to different \textit{Trypanosoma} species.

**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 Luís Prolo, Ricardo Massato Takemoto, Luís Marcelo Aranha Camargo e Dionatas Ulisses Meneguetis.}

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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