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Morphometric Characterization of *Trypanosoma* spp. and blood parameters in *Pterygoplichthys pardalis* (Pisces: Loricariidae) from the Brazilian Amazon

LUCICLARA F. DE SOUSA, DARLISON C. DE SOUZA, TÁSSIO A. COÊLHO, MARCOS TAVARES-DIAS & LINCOLN L. CORRÊA

Abstract: The present study describes *Trypanosoma* spp. infection and blood parameters in *Pterygoplichthys pardalis* from the Tapajós River basin in eastern Amazon (Brazil). Of 32 fish examined, 40.6% were infected by *Trypanosoma* spp., while a total of 112 trypomastigotes were found. Two *Trypanosoma* morphotypes were characterized and compared with species described in literature infecting other Loricariidae, and a similarity of 94% was found with one species described for another host. The plasma glucose and aspartate aminotransferase levels, hematocrit, number of total erythrocytes, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) in the infected and uninfected fish were similar, but hemoglobin was lower in fish infected with *Trypanosoma* spp. Hemoglobin levels declined with the abundance of the hemoparasites, but the condition factor was similar among fish infected and uninfected by *Trypanosoma* spp. This is the first study on the hemoparasitism by *Trypanosoma* spp. and blood parameters in *P. pardalis*.

Key words: Hemoparasites, infection, freshwater fish, protozoan.

INTRODUCTION

Loricariidae is the largest Neotropical fish family, representing 25% of Siluriformes diversity, with around 800 known species (Nelson 2016). These Siluriformes are characterized by a body covered by bony plates and a single pair of barbells (Armbruster 2004), and by their omnivorous habits, as they feed on algae, debris and microorganisms from the sediment of lakes and rivers. The fish have sedentary behavior and inhabit the bottom of water bodies (Baumgartner et al. 2012, Froese & Pauly 2019).

The Loricariidae *Pterygoplichthys pardalis* Castelnau, 1855 is widely distributed in South America, mainly in the Amazon River system (Nelson 2016, Cardoso et al. 2017, Froese & Pauly 2019). This fish is consumed by Amazonian riverine populations (Moroni et al. 2015, Cardoso et al. 2017), and as it is used as an ornamental fish due to its exotic appearance, thus can now also be found in other regions of the world due transportation (Cardoso et al. 2017). However, there are no studies of infection by hemoparasites of the *Trypanosoma* genus and their effects on the physiology of this fish.

Fish can be infected by hemoparasites of the *Trypanosoma* genus. These are among the most significant Euglenozoa, with approximately 30 species registered in around 13 genera of Loricariidae from the Amazon, Mogi-Guaçú, Piracicaba, Guaíba, Pardo, Tocantins, Paracatu,

Paraná, Tietê, Jamanxizinho and Ribeira do Iguape Rivers (Eiras et al. 2010, 2012, Corrêa et al. 2016). *Trypanosoma* spp. are heterozygous parasites that spend one phase of their lives in the bloodstream of different species of aquatic vertebrates (fish, amphibians and reptiles) and another stage in the intestines of leeches, which infect fish populations on several continents (Corrêa et al. 2016, Molina et al. 2016, Lapirova & Zabotkina 2018, McAllister et al. 2019). The trypomastigote forms of these parasites have a more or less elongated morphology, notably in the nucleus, kinetoplast, undulating membrane and flagellum (Eiras et al. 2010). This shape appears to be based on the level of digestion of the blood ingested, with the trypomastigote appearing only when the blood is fully digested. This characteristic is also influenced by the temperature and amount of blood ingested (Molina et al. 2016).

Trypanosoma spp. can cause numerous clinical manifestations in fish populations, with anorexia, dorsal depigmentation, anemia and splenomegaly, manifestations that can lead to the death of the hosts. Some species of these hemoparasites can produce hemolytic factors and reduce the oxygen carrying capacity of the blood, causing erythropenia and anemia in hosts (Ahmed et al. 2011, Gupta & Gupta 2012, Maqbool & Ahmed 2016, Lapirova & Zabotkina 2018, McAllister et al. 2019). However, few blood manifestations have been identified in species of Loricariidae (Fujimoto et al. 2013, Corrêa et al. 2016). Additionally, it is suggested that fish that recover from infection by these hemoparasites can become immune against possible reinfection (Molina et al. 2016). The aim of the present study was to describe infection by *Trypanosoma* spp. and blood parameters in *P. pardalis* from the eastern Amazon, northern Brazil.

MATERIALS AND METHODS

Fish and collection

A total of 32 specimens of *P. pardalis* (Figure 1) were captured in Igarapé dos Reis and Enseada Grande in the region of the mouth of the Tapajós River, in the state of Pará, Brazil (Figure 2), with the aid of gill nets of varying mesh sizes, for hemoparasite analysis. A blood sample was

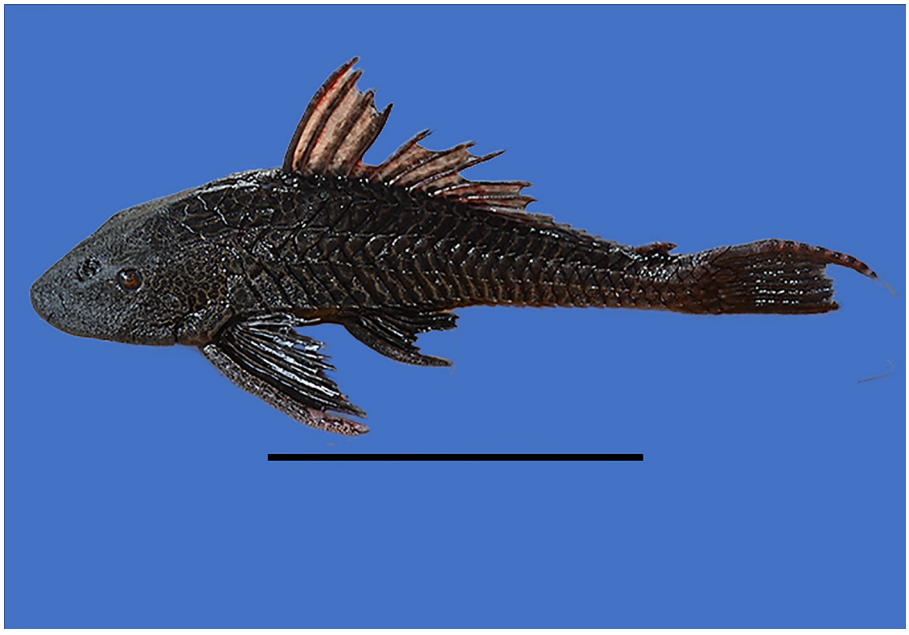

Figure 1. *Pterygoplichthys pardalis* from the Brazilian Amazon. (Scale bar = 10 cm).

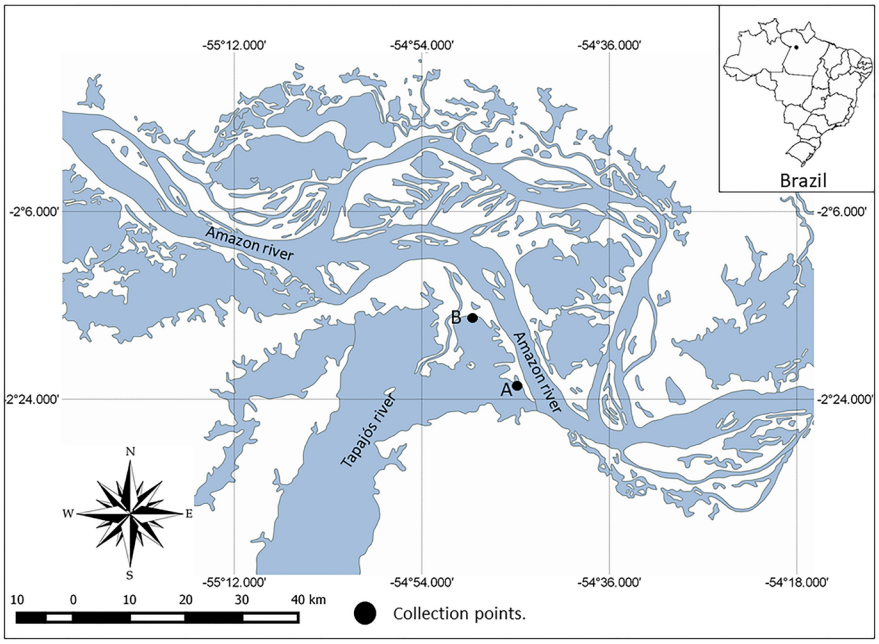

Figure 2. Collection locations of *Pterygoplichthys pardalis* from the mouth of the Tapajós River, in eastern Amazon (Brazil).

immediately collected from each fish, and the fish were then euthanized by the spinal cord transection method. The blood was examined for Trypanosomatidae and the identification procedures were performed in the Universidade Federal do Oeste do Pará (UFOPA), Santarém, PA, Brazil.

The weight (g) and standard length (cm) of each fish were used to calculate the relative condition factor (Kn) of the infected and uninfected fish (Le Cren 1951), which were compared using the Mann-Whitney test (Zar 2010).

The study was approved by the Animal Experimentation Ethics Committee of the Universidade Federal do Oeste do Pará (CEUA N° 1020180045). It was also submitted to the genetic heritage and associated traditional knowledge national management system, as summarized below, and was registered with SisGen, in compliance with the provisions of Law 13,123/2015 and its regulations.

Blood collection and analysis of blood parameters

The blood was collected by cardiac puncture using syringes containing EDTA (10%). This was used to determine the hematocrit using the microhematocrit method, Red Blood Cells count in Neubauer chamber, and hemoglobin concentration by the cyanmethemoglobin method. These data were used to calculate the Wintrobe hematological indices: mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) (Ranzani-Paiva et al. 2013). The remaining blood was centrifuged at 75G for five minutes to obtain plasma and determine the glucose and Aspartate Amino Transferase (AST) levels, using kits from Labest (MG, Brazil) and a spectrophotometer.

Method for detection of hemoparasites and *Trypanosoma* spp morphotypes

The blood was used to determine Trypanosomatidae morphotypes and hemoparasites counts. Samples of blood were homogenized before made the blood smears

to quantify trypomastigotes. Blood smears of each fish were confectioned in duplicates and panchromatic-stained using Fast Panoptic (Laborclin®, Brazil). Blood smears were examined with the aid of an optical microscope with 100x magnification and 100 fields were counted. The parasites were photographed using an optical microscope (Zeiss Axioplan) with an Axiocam ERc 5s camera at the Microscopy and Sample Laboratory of the Universidade Federal do Oeste do Pará (UFOPA). In order to determine the morphometric characteristics of Trypanosomatidae, the Zen Blue edition 2 software package was used, and the following measurements were carried out following the recommendations of Borges et al. (2016): total body length with flagellum (TL), body length along the midline (BL), body width at the center of the nucleus (BW), free flagellum length (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 end (KP) (Figure 3).

Data analysis

The ecological terms (prevalence and mean abundance) used were those proposed by Bush et al. (1997). Bray-Curtis dissimilarity analysis was used to verify possible morphological compatibilities between the valid *Trypanosoma* species described in literature, for hosts of the Brazilian Amazon, using Past. 3.0 software (Hammer et al. 2001).

All data were previously evaluated based on the assumptions of normality and homoscedasticity using Shapiro-Wilk and Bartlett, respectively. The blood parameters of the infected and uninfected fish were compared using the Mann-Whitney (*U*) test, for comparison between medians. The Spearman correlation coefficient (*rs*) was used to verify possible correlations of parasite abundance with hemoglobin levels, as well as with the condition factor. All analyzes were performed using a confidence interval of 95% (Zar 2010).

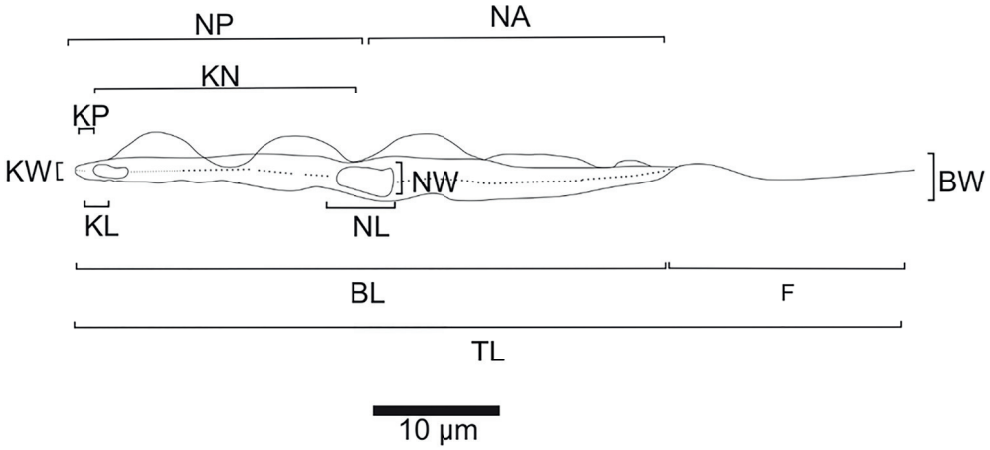

Figure 3. Measurements of *Trypanosoma* spp. in *Pterygoplichthys pardalis* from the Tapajós River, in eastern Amazon (Brazil). Total body length with flagellum (TL), body length along the midline (BL), body width at the center of the nucleus (BW), free flagellum length (F), nucleus length (NL), nucleus 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 end (KP).

RESULTS

The specimens of *P. pardalis* examined had a mean weight of 359.7 ± 98.6 g and a mean standard length of 33.4 ± 2.6 cm.

Of the 32 fish analyzed, 13 (40.6%) were infected by *Trypanosoma* spp. and a total of 112 trypomastigotes were found. Morphometric analyzes of *Trypanosoma* spp. showed that the *P. pardalis* were infected by two morphotypes of these hemoparasites (Figure 4a-d), and the measurements are shown in Table I.

For the morphological survey, the *Trypanosoma* morphotypes were described by analyzing 20 specimens (Figure 5). The trypomastigotes exhibited a flagellate body with attenuation in the anterior and posterior directions. This was more evident in the anterior region where flagella with one or two flexions are located, comprising on average a quarter of the total size of the parasite. Cytoplasm with the presence of one or two vacuoles in the anterior and posterior regions and an overall shape varying between a letter C and a letter S were observed. The morphology of the nucleus is between circular and ovoid, situated in the median region of the cell body, without karyosome, but with a narrow and discrete undulating membrane.

When compared morphologically with species of *Trypanosoma* from Loricariidae from the Amazon region using the Bray-Curtis dissimilarity index (Table II), the trypomastigote forms of *P. pardalis* revealed a similarity of 94% with the *Trypanosoma* morphotype IV described by Lemos et al. (2015), grouping in an isolated class (Figure 6), providing evidence for a positive morphological identification.

The relative condition factors of the *P. pardalis* infected (Kn = 1.00, p = 0.04) and uninfected (Kn = 1.00, p = 0.04) by *Trypanosoma* spp. were similar (U = 117.0, p = 0.803). No significant correlation (rs = -0.074, p= 0.687) between the Kn of the hosts and the abundance of *Trypanosoma* spp. was observed.

The weight and length, aspartate amino transferase and glucose levels, number of erythrocytes, MCV and CHCM were similar among the fish that were infected and uninfected by *Trypanosoma* spp., while the hemoglobin levels were lower in the infected fish (Table III). There was a significant negative correlation (rs = -0.358, p = 0.044) between the abundance of *Trypanosoma* spp. and hemoglobin levels.

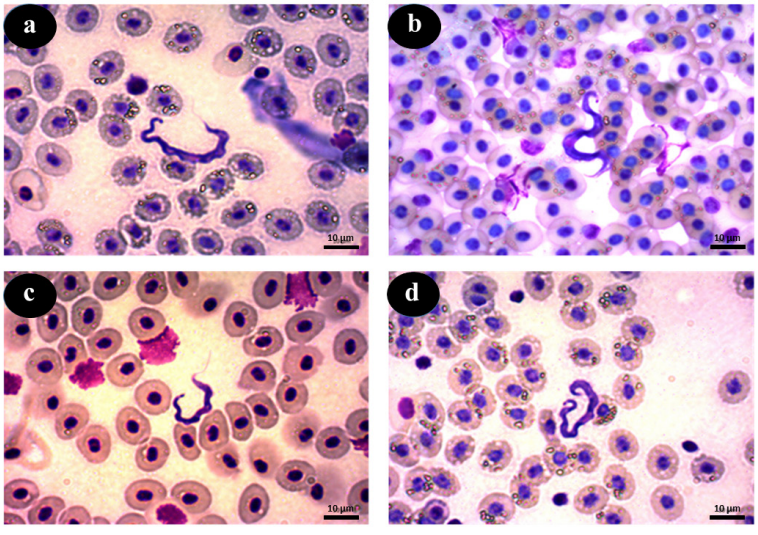

Figure 4. a-d Trypomastigote shapes, characterizing parasitemia by *Trypanosoma* spp. in the blood of *Pterygoplichthys pardalis* from the mouth of the Tapajós River, in eastern Amazon (Brazil). Panoptic Staining. Scale = 10 µm.

Table I. Morphometric measurements of the trypomastigote shape of *Trypanosoma* spp. in *Pterygoplichthys pardalis* from the Tapajós River in eastern Amazon (Brazil).

Parameters	Mean ± Deviation	Minimum/Maximum
Total body length with flagellum	47.1 ± 8.9	32.3-73.7
Body length along the midline	39.6 ± 5.9	32.3-53.2
Distance from the center of the nucleus to the posterior extremity	21.4 ± 3.0	18.4-27.1
Distance from the center of the nucleus to the anterior extremity	19.6 ± 3.9	12.8-26.1
Length of the free flagellum	9.6 ± 7.2	0-28.7
Nucleus Length	3.0 ± 0.9	1.5-5.6
Nucleus width in center portion	1.6 ± 0.6	1.5-3.9
Body width in the center of the nucleus	2.4 ± 0.7	1.5-3.9
Distance from the center of the kinetoplast to the center of the nucleus	21.2 ± 3.0	14.7-26.8
Distance from the center of the kinetoplast to the posterior extremity	0.2 ± 0.1	0-0.4
Kinetoplast length	1.0 ± 0.2	0.7-1.4
Kinetoplast Width	0.7 ± 0.1	0.5-1.2

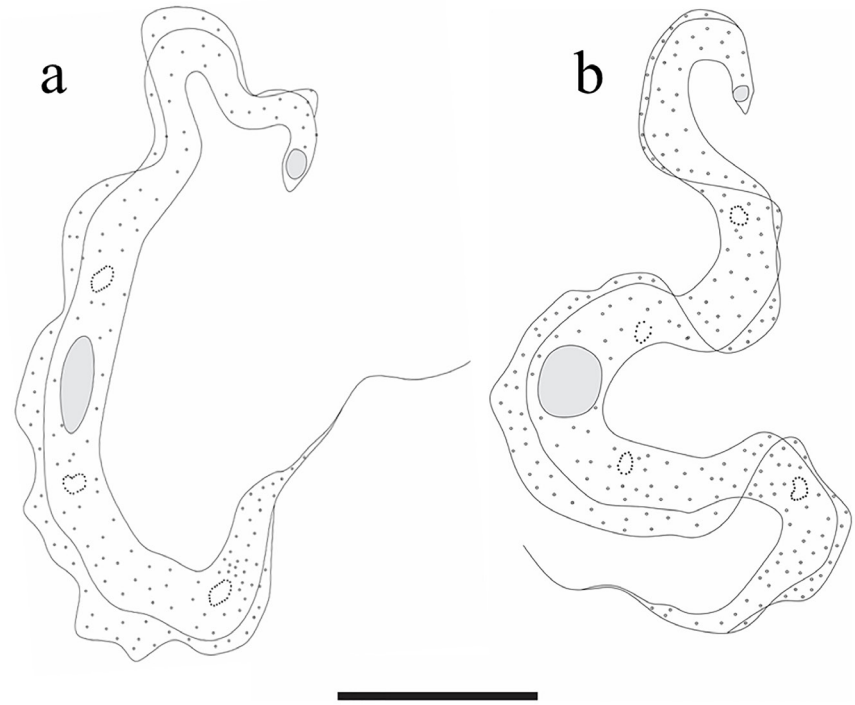

Figure 5. Shapes of *Trypanosoma* sp. in *Pterygoplichthys pardalis* from the mouth of the Tapajós River, in the Eastern Amazon (Brazil). *Trypanosoma* sp. in C-shape (a) and *Trypanosoma* sp. in S-shape (b). Scale bar: 10 µm.

Table II. Matrix of morphological similarity of *Trypanosoma* spp. from *Pterygoplichthys pardalis* from the Tapajós River in eastern Amazon (Brazil) and valid species for other Loricariidae described in literature.

	Borges et al. (2016)	**Corrêa et al. (2016) Morphotype I**	**Corrêa et al. (2016) Morphotype II**	**Corrêa et al. (2016) Morphotype III**	**Fujimoto et al. (2013)**	**Lemos et al. (2015) Morphotype I**	**Lemos et al. (2015) Morphotype II**	**Lemos et al. (2015) Morphotype III**	**Lemos et al. (2015) Morphotype IV**	**Present study**
Borges et al. (2016)	1	94.00	76.00	90.82	96.68	84.72	86.98	95.58	99.10	95.60
Corrêa et al. (2016) Morphotype I	95.60	1	78.17	84.84	93.23	83.83	90.72	89.67	96.14	95.99
Corrêa et al. (2016) Morphotype II	95.99	99.10	1	87.09	81.73	82.25	93.99	89.13	89.08	95.52
Corrêa et al. (2016) Morphotype III	95.52	96.14	95.58	1	83.95	86.77	86.06	91.68	90.02	90.03
Fujimoto et al. (2013)	90.03	89.08	89.67	86.98	1	92.67	89.59	83.25	92.25	87.62
Lemos et al. (2015) Morphotype I	87.62	90.02	89.13	90.72	84.72	1	91.78	92.44	84.13	90.79
Lemos et al. (2015) Morphotype II	90.79	92.25	91.68	93.99	83.83	96.68	1	93.99	91.55	81.77
Lemos et al. (2015) Morphotype III	81.77	84.13	83.25	86.06	82.25	93.23	90.82	1	93.10	93.73
Lemos et al. (2015) Morphotype IV	93.73	91.55	92.44	89.59	86.77	81.73	84.84	76.00	1	95.41
Present study	95.41	93.10	93.99	91.78	92.67	83.95	87.09	78.17	94.00	1

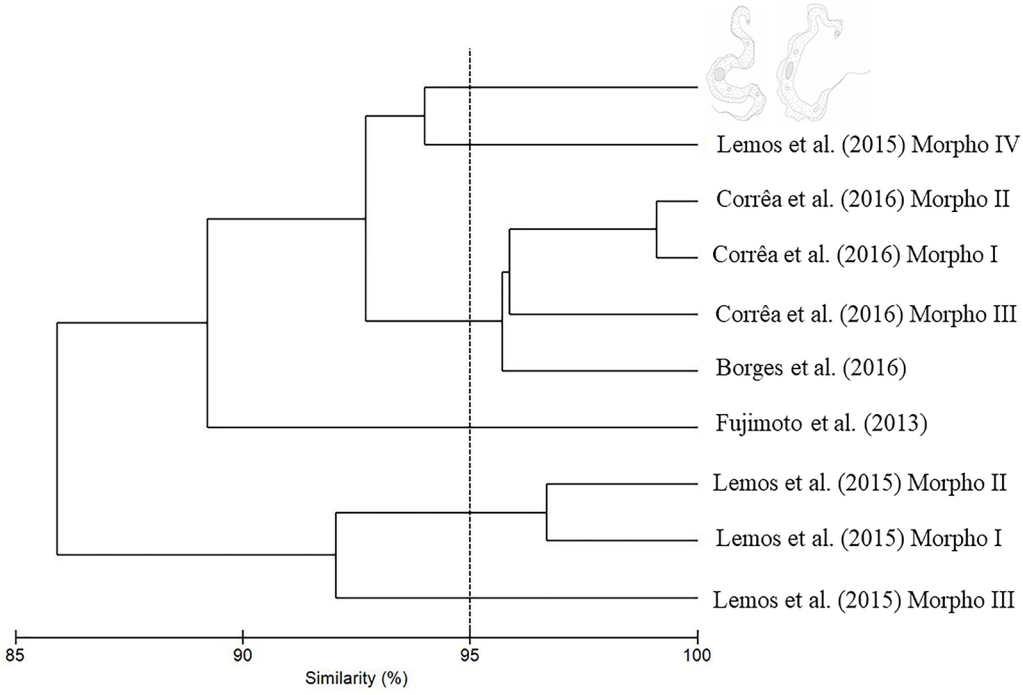

Figure 6. **Morphological dissimilarity dendrograms (Bray-Curtis) of *Trypanosoma* spp. in *Pterygoplichthys pardalis* from the mouth of the Tapajós River and other species of Loricariidae in literature.**

Table III. Body and blood parameters of *Pterygoplichthys pardalis* from the Tapajós River in eastern Amazon (Brazil).

Parameters	Non-parasitized fish (n = 19)	Parasitized fish (n = 13)	U	*p*
Weight (g)	340.6 ± 110.5[a]	312.3 ± 125.6[a]	111.0	0.631
Length (cm)	32.9 ± 2.9[a]	32.1 ± 3.9[a]	117.5	0.818
Aspartate aminotransferase (U/L)	99.8 ± 68.8[a]	80.0 ± 51.2[a]	81.0	0.418
Glucose (mg/dL)	104.1 ± 59.0[a]	101.5 ± 53.0[a]	91.0	0.719
Red blood cells ($x10^6 \mu L^{-1}$)	2.04 ± 1.33[a]	1.80 ± 0.42[a]	15.0	0.391
Hematocrit (%)	41.5 ± 9.3[a]	44.8 ± 9.2[a]	83.0	0.209
Hemoglobin ($g\ dL^{-1}$)	14.8± 6.5[a]	10.9 ± 4.5[b]	62.0	0.018
MCV (fL)	247.3 ± 110.2[a]	239.7 ± 37.1[a]	21.0	1.00
MCHC (g/dL)	27.6 ± 12.8[a]	34.5 ± 12.9[a]	72.0	0.088
White blood cells (μL^{-1})	30,710 ± 14,3[a]	36,726 ±16,2[a]	16.0	0.418
Thrombocytes (μL^{-1})	33,595 ± 16,8[a]	21,300 ± 12,8[a]	15.0	0.398

MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin Concentration; U: Mann-Whitney; Different letters (a) and (b) on the same line indicate differences between parasitized and non-parasitized fish.

DISCUSSION

The limitations of morphological identification did not allow the *Trypanosoma* species in *P. pardalis* to be determined, as these hemoparasites have a plasticity that generally requires the use of molecular tools for precise identification (Fujimoto et al. 2013). However, analysis of the dissimilarity of *Trypanosoma* spp. in *P. pardalis* and other species of Loricariidae from the Amazon and Mato Grosso Pantanal (wetland) basins revealed the existence of morphologically distinct species, as well as one species that was similar. In *P. pardalis*, both *Trypanosoma* morphotypes exhibited elevated similarity (94%) with the morphotype IV described by Lemos et al. (2015) for *Hypostomus affinis* and *Hypostomus luetkeni*, therefore indicating the occurrence of the same morphotype for these hosts.

Pterygoplichthys pardalis lotic environments may favor infection by *Trypanosoma* transmission vectors to maintain the biological cycle of these hemoparasites. Hirudinea vectors form part of the maintenance of the biological cycle of *Trypanossoma* spp. (Molina et al. 2016, Corrêa et al. 2016, McAllister et al. 2019), and during the fish collection phase of the present study the presence of leeches was observed in several specimens of *P. pardalis*. Similarly, other studies (Corrêa et al. 2016, Lapirova & Zabotkina 2018) have also observed an increase in infection by leeches in fish infected by *Trypanossoma* spp. In fish, pathogenicity can therefore also be caused by leeches, and can be more than a simple hemorrhage at the feeding site of these parasites. In severe cases of infection, leeches can cause anemia and even the death of hosts (Molina et al. 2016). However, in *P. pardalis*, parasitism did not influence the condition factor of fish infected by *Trypanosoma* spp. Similar results has been described for *Abramis brama* infected by *Trypanosoma* sp. (Lapirova & Zabotkina 2018).

The prevalence of *Trypanosoma* spp. in *P. pardalis* was 40.6%. Similar results have been described for *Hypostomus strigaticeps* (52.0%), *Hypostomus regani* (44.0%) and *Hypostomus albopunctatus* (46.6%) from the Mogi-Guaçu River, São Paulo (Molina et al. 2016). However, higher level of parasitism (90-100%) was described for *H. affinis* and *H. luetkeni* of the Pomba River, in Minas Gerais (Lemos et al. 2015). Parasitaemia can also vary in loricariid hosts (Fujimoto et al. 2013, Molina et al. 2016), and such differences may be related to host behavior, abundance of vectors in the environment and/or the diagnostic method used in the study (Borges et al. 2016).

Blood parameters can be important indicators for monitoring fish health in response to infections (Ranzani-Paiva et al. 2013). In fish, anemia due to erythropenia is one of the most prominent disorders of the manifestations caused by *Trypanosoma* spp. (McAllister et al. 2019). However, such infections have a complex etiology (McAllister et al. 2019). Some *Trypanosoma* spp. can cause erythropenia and anemia in the host fish (Ahmed et al. 2011, Gupta & Gupta 2012, Maqbool & Ahmed 2016, Lapirova & Zabotkina 2018, McAllister et al. 2019), due to the production of hemolytic agents. Recent studies suggested that the synergistic activity of the proinflammatory cytokines is needed to maintain prolonged anemia, while some *Trypanosoma* species can directly or indirectly suppress erythropoiesis in hosts (McAllister et al. 2019), leading to an anemic process. In *P. pardalis* infected by *Trypanosoma* spp., however, there was a reduction only in hemoglobin levels, which increased with the abundance of these hemoparasites. Lapirova & Zabotkina (2018) also reported a reduction in hemoglobin levels in *A. brama*, as well as an increase in

immature erythrocytes in compensation for this reduction. In *Schizothorax plagiostomus*, a moderate infection by *Trypanosoma* spp. caused normocytic-normochromic anemia due to the reduction in the number of Red Blood Cells, hematocrit and hemoglobin (Maqbool & Ahmed 2016).

Trypanosoma spp. from *P. pardalis* exhibited close similarity with another species infecting Loricariidae described in literature. A moderate level of infection caused a reduction in hemoglobin concentration, without altering the body condition of the hosts. This was the first study on *Trypanosoma* and blood parameters for *P. pardalis*.

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LUCICLARA F. DE SOUSA[1]
https://orcid.org/0000-0003-3036-3307

DARLISON C. DE SOUZA[2]
https://orcid.org/0000-0002-7610-9665

TÁSSIO A. COÊLHO[2]
https://orcid.org/0000-0002-0264-4526

MARCOS TAVARES-DIAS[3]
https://orcid.org/0000-0002-8376-1846

LINCOLN L. CORRÊA[1]
https://orcid.org/0000-0002-6453-4824

[1]Universidade Federal do Oeste do Pará/UFOPA, Instituto de Ciências e Tecnologia das Águas/ICTA, Av. Mendonça Furtado, 2946, Fátima, 68040-470 Santarém, PA, Brazil

[2]Programa de Pós-Graduação em Biodiversidade(PPGBEES), Instituto de Ciências e Tecnologia das Águas/ICTA, Av. Mendonça Furtado, 2946, Fátima, 68040-470 Santarém, PA, Brazil

[3]Embrapa Amapá, Rodovia Juscelino Kubitschek, Km 5, N° 2600, Universidade, 68903-419 Macapá, AP, Brazil

Correspondence to: **Lincoln Lima Corrêa**
E-mail: lincorre@gmail.com

Author contributions

Each author made a relevant contribution to the preparation of this manuscript as follows:
Luciclara F. de Sousa: collaboration in data sampling, methodology and discussion of results.
MSc. Darlison C. de Souza sampling, analysis and interpretation of data. MSc. Tássio A. Coêlho statistical analysis and its interpretation. Dr. Marcos Tavares-Dias: Collaboration and review of related procedures. Dr. Lincoln L. Corrêa: Main responsible for the final text and collaboration and review of related procedures.