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Morphological and hematological studies of *Trypanosoma* spp. infecting ornamental armored catfish from Guamá River-PA, Brazil

RODRIGO Y. FUJIMOTO[1], MIKAELLE S. NEVES[2], RUDÃ F.B. SANTOS[2], NATALINO C. SOUZA[2], MÁRCIA V.S. DO COUTO[2], JOSIANE N.S. LOPES[2], DANIEL G. DINIZ[3] and JORGE C. EIRAS[4,5]

[1]Embrapa Tabuleiros Costeiros, Avenida Beira Mar, 3250, Caixa Postal 44, 49025-040 Aracaju, SE, Brasil
[2]Universidade Federal do Pará, Instituto de Estudos Costeiros,
Rua Leandro Ribeiro, s/n, Aldeia, 68370-000 Bragança, PA, Brasil
[3]Universidade Federal do Pará, Instituto de Ciências Biológicas,
Laboratório de Investigações em Neurodegeneração e Infecção, Hospital Universitário João de Barros Barreto,
Rua dos Mundurucus, 4487, Guamá, 66073-005 Belém, PA, Brasil
[4]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto,
Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal
[5]CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental/CIMAR,
Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

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ABSTRACT

A total of 281 specimens of freshwater armored ornamental fish species (*Leporacanthicus galaxias*, *Lasiancistrus saetiger*, *Cochliodon* sp., *Hypostomus* sp., *Pseudacanthicus spinosus*, *Ancistrus* sp. and *Rineloricaria* cf. *lanceolata*) were captured at the hydrological basin of Guamá River, Pará, Brazil. The infection by *Trypanosoma* spp. was inspected. The morphological and morphometric characterization of the parasites and the hematological parameters were determined. *Leporacanthicus galaxias* and *Pseudacanthicus spinosus* presented 100% infection prevalence, and the other species showed a variable prevalence of infection. The parasites showed clearly different morphotypes and dimensions, and probably belong to different species. The hematological response to the infection varied with the host. *Cochliodon* sp. showed no differences between infected and not infected fish. In other species several modifications on some hematological parameters were found, but apparently without causing disease. It is emphasized the possibility of introduction of the parasites in new environments due to the artificial movements of these ornamental fish.

Key words: freshwater fish, hematological parameters, infection, *Trypanosoma* spp.

INTRODUCTION

The northeast of Pará State, in Brazil, especially the hydrographic basin of Guamá River, is an important area for the fishery of ornamental freshwater fish. This is due to the facility of reaching the fishing grounds and to the proximity of Belém, from where the fish are exported to other countries. According to Torres (2007), the capture of ornamental armored fish represents up to 55% of the monthly income to fishermen. Most of these fish are exported, but a sanitary inspection of the specimens is not performed. Therefore, the dissemination risk of parasites and diseases is high.

Correspondence to: Daniel Guerreiro Diniz
E-mail: danielguerreirodiniz@gmail.com

There are some observations on the parasites of ornamental fish in Brazil, mostly about ectoparasites (Garcia et al. 2009, Piazza et al. 2006, Prang 2007, Tavares-Dias et al. 2010), and some studies deal with the infection by *Trypanosoma* spp. These parasites may not harmful to the fish hosts (Untergasser 1989), or cause anemia, damage of the hematopoietic tissues and, finally, the death of the fish (Noga 1996). Massive infections in some hosts (over 10^5 specimens /mm^3), like *Cyprinus carpio* and *Carassius auratus*, may cause anemia, anorexia and ascites (Paperna 1996).

According to Eiras et al. (2010, 2012), there are at least 62 nominal species of trypanosomes infecting freshwater fish in Brazil, a number of them parasitizing armored fish like *Hypostomus* spp. and *Pterodoras* spp. (D'Agosto et al. 1985, Lopes et al. 1991, Bara et al. 1985, Fróes et al. 1979). It must be stressed out that a number of these parasites were identified only with basis on morphology and morphometry, and in most of the times assuming specificity in parasitization.

In this paper we report the infection of 7 different species of ornamental armored freshwater fish from the Guamá River by *Trypanosoma* spp. The morphology and morphometry of each form is described, and the prevalence, abundance and mean intensity of infection is reported. Furthermore, the hematological characteristics of the hosts are referred to.

MATERIALS AND METHODS

A total of 281 armored ornamental specimens were captured at Guamá River, including 41 *Leporacanthicus galaxias* (Isbrüker and Nijssen 1989) (common name: acari pinima), 48 *Lasiancistrus saetiger* (Armbruster 2005) (acari canoa), 35 *Cochliodon* sp. (acari pleco), 10 *Pseudacanthicus spinosus* (Castelnau 1855) (acari assacu), 57 *Rineloricaia cf. lanceolata* (acari loricaria), 42 *Hypostomus* sp. (acari picoto), and 48 *Ancistrus* sp. (acari ancistrus).

Immediately after capture, a blood sample was taken from the caudal vein with syringes coated with 10% EDTA. After blood sampling the fish were measured (total and standard length) and inspected for ectoparasites, and integument or gill lesions. The following blood parameters were determined: glucose (mg/dL) using the automatic meter Prestige IQ 50, hematocrit (Ht, at 13,000 rpm during 3 minutes), total plasma protein (g/dL) using a Quimis refractometer, total hemoglobin (HB, g/dL) using a Celmi 500 and Celmi 550 meter, and number of erythrocytes per mm^3 counted in Neubauer chamber.

Blood smears were air dried and stained with May Grunwald Giemsa modified by Rosenfeld (1947). The smears were used for leukocyte differential counting. The determination of mean corpuscular volume (MCV = Ht / Er x 10, fentoliter), mean corpuscular hemoglobin (MCH = Hb / Er x 10, picograms), and mean corpuscular hemoglobin concentration (MCHC= Hb / Ht x 100, g/dL) were recorded according to Vallada (1999).

Blood smears and hematocrit were also used for determining the presence of trypanosomes. In the positive samples the mean intensity of infection was determined indirectly relating the number of parasites with the amount of 1,000 erythrocytes (modified from Ranzani-Paiva 1995 method of differential counting of white blood cells). The parasites were photographed using a digital camera, and the photographs were used latter (employing the software Motic Images Advanced 3.0) to determine the cell characteristics: total length (TL), maximum width of the body (W), nucleus length (NL) and width (NW), distance between the middle of nucleus and anterior (DNA) and posterior (DNP) extremities, length of the free flagellum (FF) and number of folds of the undulating membrane (UM). With the data obtained, the nuclear index (IN) according to D'Agosto and Serra-Freire (1993) and Gu et al. (2006) was calculated.

All the hematological results, as well as the morphometric ones were submitted to analysis of variance (BioEstt 4.0 programme). For F significant values, it was employed the Tukey test (5% of probability) to compare the means values. For the analysis of the variance of folds of the undulating membrane, it was employed the Kruskal-Wallis method for non-parametric data. It was also used the normality test with basis in the deviations values to verify the existence of outliers which were eliminated.

RESULTS

The species *Pseudacanthius* spinosus and *Leporacanthicus galaxias* presented 100% of prevalence of infection, the other hosts showed a prevalence value varying between 22.6% (*Cochliodon* sp.) and 58.3% (*Lasanciastrus* sp.) (Table I). Therefore, most of the fishes were infected, and a high proportion of them were also infected by unidentified leeches. However, some of the specimens infected with leeches were not parasitized by trypanosomes.

Hypostomus sp. presented the highest intensity of infection (1.3 parasites by 1,000 erythrocytes), and *Rineloricaria cf. lanceolata* showed the lowest one (0.5). The other species presented intermediate values (Table I). *Pseudacanthicus spinosus* had the highest abundance level, and *Rineloricaria cf. lanceolata* the smallest one, while the other species presented intermediate values (Table I).

The morphology (Fig. 1) of the parasites varied. In general, they had a rounded anterior extremity and a tapered posterior one. In some cases both the extremities were slightly tapered. The nuclei were most of the times oval-shaped, sometimes almost circular, in some cells occupying all the cell width. The kinetoplast was mostly rounded, in most of cases having a sub-terminal location. Usually the small part of the cell located before the kinetoplast was difficult to observe clearly due to poor staining of this part of the body.

The undulating membrane was well defined, developing all over the body length, or about half of the length. In some cases it was observed only near the extremity, presenting only two folds in the specimens with smaller values of body width. The undulating membrane was especially evident in *Cochliodon* sp., presenting in this host more folds. The cytoplasm varied from basophilic to eosinophilic. The free flagellum was sometimes hard to distinguish because it was not so intensely stained, and its length varied between short and long.

There was a great morphometrical variation in the several characteristics as it can be seen in Table II, and several features presented a great variation depending from the host species.

The hematological study showed the infection caused varied effects on the hosts. Interestingly, in *Cochliodon* sp. no hematological alterations were found between infected and not infected specimens. In *Ancistrus* sp. and *Hypostomus* sp. the repercussions were minimal – the first specimens showed only a pronounced increase of the concentration of mean corpuscular hemoglobin, and the second revealed increase in the percentage of lymphocytes. In *Rineloricaria cf. lanceolata* the infection caused decrease of mean corpuscular hemoglobin concentration and glucose, and modifications in the white blood cells (decrease of lymphocytes and neutrophils and increase in monocytes). Finally, *Lasiancistrus saetiger* showed increase in the hematocrit and erythrocytes, and decrease of total plasma proteins and of mean corpuscular hemoglobin.

DISCUSSION

The first conclusion to be drawn from our results is that the prevalence of the infection varied considerably with the host species, in two of them (*L. galaxias* and *P. spinosus*) reaching a prevalence of 100%. It is known that these parasites are transmitted by the bite of leeches. Therefore, the facility of infection by leeches promotes the parasitization by trypanosomes, and the behavior of the fish may contribute to a higher or lesser probability of leech infection. The armored fish have a benthic behavior that facilitates the infection by leeches, and high values of infection by trypanosomes are not uncommon. D'Agosto and Serra-Freire (1990) reported 100% of prevalence for *Trypanosoma chagasi* and *T. guaiabensis* infecting the armored *Hypostomus punctatus* from lake Açú at Rio de Janeiro. Other reports on infections in several species of armored fish showed a high variability on the prevalence and intensity of infection values (Fróes et al. 1978, 1979, Lopes et al. 1989, Ribeiro et al. 1989, Eiras et al. 1989, 1990). Considering these facts, and the fact that apparently the probability of leech infection in the fishes from our sample was the same for all the host species, it is possible to conclud that the resistance of the fish to the infection varies with the fish species.

There are in Brazil at least 62 species of trypanosomes described from freshwater fish, and at least 28 from those were described from armored fish (Eiras et al. 2010). Most of the descriptions were done assuming a strict specificity of infection, and a form observed in a new host was considered a new species (Thatcher 2006). Today it is recognized that strict specificity may be an exception but not a role, and it is urgent to review the Brazilian fish trypanosomes as it was done with trypanosomes from Africa performed by Baker (1960), resulting in a substantial reduction of the number of blood flagellate species. Besides, one confusing factor is the variability in length during infection and the existence of pleomorphic species (Gibson et al. 2005).

Our data do not allow the identification of the parasite species and, for the reasons described above, a comparison with the Brazilian species of trypanosomes would be useless. The identification based solely on morphological features is usually not possible, and the absence of specific infections,

TABLE I
Total weight (W), standard (SL) and total length (TL) of fish, prevalence of infection (P), mean intensity of infection (MI), abundance of infection (AB), and hematological parameters in infected and uninfected fish. Figures highlighted in ictalicized bold indicate significant differences between infected and uninfected fish; Ns: not significant.

	Cochliodon sp.		*Ancistrus sp.*		*Lasiancistrus saetiger.*	
	Not infected	Infected	Not infected	Infected	Not infected	Infected
W	22.0±5.73ns	21.8±12.03ns	39.2±13.98ns	26.6±5.46ns	28.4±15.68ns	32.7±15.23ns
SL	9.2±1.90ns	9.8±1.77ns	11.2±1.43ns	11.4±0.30ns	10.1±1.76ns	9.8±1.99ns
TL	12.2±1.02ns	12.9±1.99ns	13.9±2.07ns	14.1±0.81ns	11.4±2.30ns	11.4±3.50ns
P	_	26.66	_	20	_	58.3
MI	_	1	_	1	_	1
AB	_	0.26	_	0.2	_	0.58
GLUC	92.4±10.80ns	42.7±11.74ns	72.4±28.72ns	95±13.85ns	55.2±18.37ns	44.1±8.75ns
HT	20.6±10.76ns	21.8±15.56ns	20.4±10.46ns	14.3±8.96ns	***17.8±7.49 b***	***28.0±11.69 a***
TPP	7.8±2.45ns	8.4±3.16ns	4.6±1.69ns	3.0±1.17ns	***8.9±2.30 a***	***6.5±2.53b***
HB	6.8±4.20ns	5.2.54ns	8.9±5.54ns	9.3±5.95ns	9.23±4.52ns	9.7±4.97ns
ER	0.3±0.46ns	0.2±0.18ns	0.6±16.99ns	0.6±0.26ns	***0.3±0.32 b***	***1.3±0.06 a***
MCV	1097.9±286.90ns	568.9±250.54ns	373.7±156.62ns	289.2±264.15ns	845.8±786.60ns	230.9±99.29ns
MCH	270.8±175.63ns	211.9±108.40ns	170.5±107.62ns	162.3±87.09ns	1106.4±1917.10ns	71.6±37.18ns
CMCH	38.6±29.07ns	35.7±2.94ns	***41.3±17.67 b***	***155.4±216.18 a***	***63.4±39.44 a***	***29.4±10.97 b***
LYM	56.1±20.65ns	47.2±20.12ns	75.6±19.56ns	81.3±2.51ns	94.4±13.23	64.4±15.33
NEU	39.5±21.16ns	48.2±21.29ns	19.9±16.89ns	11.3±1.52ns	4.0±9.6	28.0±13.8
MON	4.2±1.48ns	4.5±2.51ns	3.2±3.24ns	7.3±3.21ns	1.2±0.8	7.5±4.4

TABLE I (continuation)

	Leporacanthicus galaxias	*Pseudacanthicus spinosus*	*Hypostomus sp.*		*Rineloricaria cf. lanceolata*	
	Infected	Infected	Not infected	Infected	Not infected	Infected
W	28.4±14.6	25.2±27.9	21.4±8.76ns	23.9±6.05ns	19.4±6.88ns	13.0±5.38ns
SL	10.1±2.0	9.5±2.7	8.7±1.33ns	9.1±0.96ns	15.3±2.10ns	15.5±2.53ns
TL	12.9±2.6	12.6±2.7	11.0±1.53ns	11.±1.51ns	18.0±2.80ns	17±4.69ns
P	100	100	_	20	_	46.6
MI	1.1	1.2	_	1.3	_	0.5
AB	1.1	1.2	_	0.2	_	0.2
GLIC	60.2±27.8	27.6±21.6	62.3±15.29ns	48.6±8.5ns	***102.4±30.04a***	***59.5±22.12b***
HT	31.6±13.2	10.1±5.9	16.9±7.01ns	21±7.54ns	16.0±6.76ns	17.5±3.93ns
TPP	8.6±3.4	6.3±2.6	8.2±2.18ns	10.3±0.91ns	9.0±2.00ns	9.0±1.85ns
HB	10.9±3.8	5.7±3.2	9.9±5.09ns	9.7±0.92ns	9.5±4.73ns	4.9±2.86ns
ER	0.4±0.3	0.2±0.2	0.6±0.30ns	0.4±0.20ns	0.6±0.34ns	0.2±0.18ns
MCV	843.7±661.4	1887.0±180.6	693.7±740.41ns	490.9±167.64ns	557.4±848.57ns	1070.4±1073.02ns
MCH	353.6±348.3	1541.2±145.1	342.1±354.24ns	246.2±108.77ns	199.5±1066.66ns	216.5±110.35ns
CMCH	37.4±17.7	62.7±17.2	58.8±24.71ns	50.4±18.28ns	***58.3±47.05a***	***26.4±11.84b***
LYM	27.3±7.8	62.8±8.7	***29.4±5.85a***	***14.66±9.23b***	***80.2±7.71a***	***64.7±9.46b***
NEU	68.9±8.5	28.8±6.9	67.5±5.61ns	76±15.09ns	***14.6±7.16b***	***25±5.29a***
MON	3.7±1.9	8.4±2.6	3±1.75ns	9.3±7.57ns	***4.3±3.25b***	***10.2±4.27a***

Abbreviations: GLUC, glucose (mg/dl), HT, hematocrit, TPP, total plasma proteins g/dl, HB, hemoglobin g/dl, ER, number of erythrocytes (number of cells x $10^6/mm^3$); MCV, mean corpuscular volume (fentoliter); MCH, median corpuscular hemoglobin (pg); CMCH, concentration of the median corpuscular hemoglobin in g/dl; LYM, total number of lymphocytes; NEUT, neutrophils (%); MON, monocytes (%).

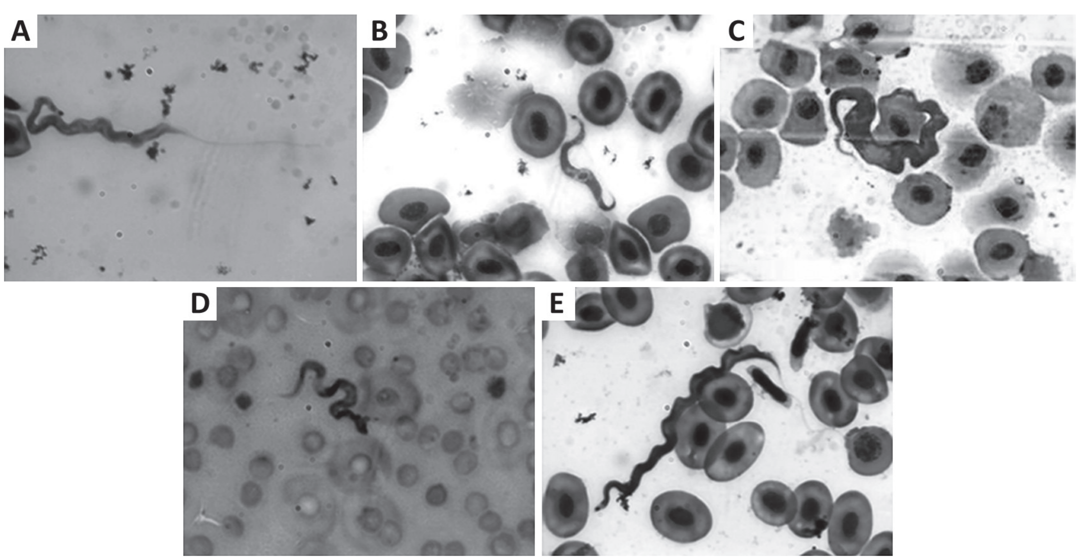

Figure 1 - Types of Trypanosomes found in some host species (**A**, *Cochliodon* sp.; **B**, *Lasiancistrus saetiger*; **C**, *Leporacanthicus galaxia*; **D**, *Pseudacanthicus spinosus*; **E**, *Cochliodon* sp.). Note the very different morphotypes especially concerning the width of the body and the length of free flagellum. Magnification: 1,000.

TABLE II
Morphometric characteristics (average plus standard deviation, figures in micrometers) of *Trypanosoma* spp. infecting armored fish.

	Leporacanthicus galaxias	*Cochliodon sp.*	*Pseudacanthicus spinosus*	*Hypostomus sp.*	*Lasiancistrus saetiger*	*Ancistrus sp.*	*Rineloricaria cf. lanceolata*
TL	52.1±3.6 a	58.0±9.4 a	38.1±4.3 ab	47.4±13.0 abc	44.9±5.7 abc	38.1±7.7 c	47.5±4.3 c
W	5.2±1.1 a	3.9±0.6 b	3.4±0.3 bc	4.9±1.3 bcd	5.3±0.7 bcd	3.5±0.9 d	3.8±1.1 d
NL	5.9±1.1 a	6.0±1.4 a	4.8±0.9 a	3.9±0.7 ab	5.4±1.1 ab	4.2±0.8 bd	5.4±0.9 d
NW	4.6±1.2 a	3.5±0.6 b	3.2b±0.4 bc	4.4±1.4 bc	5.0±0.7 bc	3.3±0.9 c	3.7±1.0 c
DNA	26.1±3.2 a	32.0±2.5 ab	21.2ab±4.6 ab	27.1±11.3 ab	25.8±5.0 ab	23.2±2.6 b	22.4±3.5 b
DNP	24.8±4.7 a	26.3±5.4 a	15.5b±4.0 ab	20.3±4.3 ab	19.3±5.4 abc	15.1±5.7 c	24.6±2.8 c
UM	7.9±1.9 ab	8.7±1.5 a	3.5cd±0.9 abc	6±1.4 abcd	5.7±2.4 abcde	3.6±1.3 e	2.8±1.1 e
FF	5.6±1.4 a	20.9±13.0 ab	4.0±1.5 bc	6.9±2.1 bc	2.3±0.6 bc	13.5±9.2 c	2.1±0.8 c
NI	0.9±0.1 a	0.8±0.1 b	0.6±0.2 b	0.8±0.3 b	0.7±0.2 b	0.7±0.3 b	1.1±0.1 b

Abbreviations: TL, total length; W, width; NL, nucleus length; NW, nucleus width; DNA, distance from the nucleus till the anterior extremity; DNP, distance from the nucleus till the posterior extremity; UM, number of folds of the undulating membrane; FF, length of the free flagellum; NI, nuclear index. Values followed by different letters in the same line indicate significant differences (5% probability in Tukey test).

at least in most of the cases, do not allow a positive identification without the aid of molecular tools, and characterization of the development of the parasite within the vector, which were not considered in the present research. However, it is highly probable that we face different species due to the so pronounced differences in morphology and morphometry of the parasites, as depicted in Figure 1 and Table II. It is the authors' intent to pursue this study in the future in order to elucidate this question.

According to the hematological data obtained, it seems that some host species (*Lasiancistrus saetiger* and *Rineloricaria cf. lanceolata*) were more affected than others (*Ancistrus* sp. and *Hypostomus* sp.), while *Cochliodon* sp. apparently had the hematological parameters not altered by the infection. Therefore, it can be concluded that some hosts adapted better than others to the infection.

Some results of other authors for different freshwater hosts species show results sometimes similar to ours: anemia in *Carassius auratus* infected with *Trypanosoma danilewskyi* (Dyková and Lom 1979), in *Barilius blendelisis* parasitized by *Trypanosoma* sp. (Rauthan et al. 1995), and in *Cyprinus carpio* infected with *T. borreli* (Clauss et al. 2008). Other authors reported minimal changes in blood parameters for different species of parasites and hosts, and Aguilar et al. (2005) consider that *T. granulosum* has only minor effects in the host *Anguilla anguilla*. According to Lom (1979), "it seems, on the evidence obtained from observations of natural infections, that species of this genus live in a more or less balanced state with their host".

In summary, we conclude that the armored ornamental freshwater fish species studied are highly infected by trypanosomes (representing most probably different species) and react differently to the infection as showed by the hematological observations.

Furthermore, it is important to emphasize the risk of dissemination of these parasites, due to the artificial movements of the hosts, in spite of the need of a vector to transmit the parasites to uninfected fish. This problem is especially important because the infection is not detectable by visual inspection of the fish.

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RESUMO

Um total de 281 espécimes de peixes ornamentais de água doce – das espécies *Leporacanthicus galaxias*, *Lasiancistrus saetiger*, *Cochliodon* sp., *Hypostomus* sp., *Pseudacanthicus spinosus*, *Ancistrus* sp. e *Rineloricaria* cf. *lanceolata* – foram capturados na bacia hidrográfica do rio Guamá, Pará, Brasil. A infecção por *Trypanosoma* spp. foi inspecionada. A caracterização morfológica e morfométrica dos parasitas e os parâmetros hematológicos foram determinados. Todas as espécies foram infectadas e todos os espécimes de *Leporacanthicus galaxias* e *Pseudacanthicus spinosus* estavam parasitados. As outras espécies mostraram uma prevalência variável da infecção. Os parasitas mostraram claramente morfotipos e dimensões diferentes, e provavelmente, pertencem a espécies diferentes. A resposta hematológica à infecção variou de acordo com o hospedeiro. Em *Cochliodon* sp. não houve diferença entre peixes infectados e não infectados. Em outras espécies diversas modificações em alguns parâmetros hematológicos foram encontrados, mas aparentemente sem causar doença. Ressalta-se a possibilidade de introdução de parasitas em novos ambientes devido aos movimentos artificiais destes peixes ornamentais.

Palavras-chave: peixes de água doce, parâmetros hematológicos, infecção, *Trypanosoma* spp.

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