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First report of trypanosomiasis in farmed largemouth bass (*Micropterus salmoides*) from China: pathological evaluation and taxonomic status

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Abstract

The aim of this study was to evaluate the effect of trypanosomes on cultured largemouth bass (*Micropterus salmoides*) and describe the taxonomic identification of the parasite. The effects of the parasite on *M. salmoides* were examined based on clinical symptoms, hemograms, histopathology, and serum biochemistry. Diseased fish showed typical clinical symptoms of trypanosomiasis, which included lethargy, anorexia, and histopathological lesions in the liver, head kidney, and spleen. The serum of diseased fish had significantly lower concentrations of glucose, triglyceride, and low-density lipoprotein, and significantly higher alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) activities. The morphology of the trypanosomes was also analyzed using light microscopy, and their 18S rDNA sequence was analyzed to establish genetic relationships with other known strains. We found that the trypomastigote form of the trypanosomes from *M. salmoides* was similar to those isolated from *Pelteobagrus fulvidraco*. The trypanosomes had a slender and narrow body with a relatively long free flagellum, not well-developed undulating membrane, and an oval kinetoplast located near the subterminal posterior end of the body. The 18S rDNA sequences of the trypanosome from *M. salmoides* had the highest similarity (99.8%) with that of *P. fulvidraco*, suggesting they are identical species. Based on the differences in morphological characteristics and 18S rDNA sequence compared to trypanosomes isolated from other freshwater fish, it is considered as a new species and we propose the name *Trypanosoma micropteri* n. sp.

Keywords *Trypanosoma* · Hemoparasite · Largemouth bass · Biochemical serum parameters · 18S rDNA

Introduction

The largemouth bass *Micropterus salmoides* (Lacépède 1802) is native to North America and has become an important freshwater economic fish in China, generating an annual yield up to 350, 000 t (Coyle et al. 2000; Bai et al. 2008; Li et al. 2018). Foshan City, Guangdong Province, is an area that produces over 60% of the total largemouth bass output in China (Chen et al. 2015). Unfortunately, outbreaks of diseases in farms have become more frequent and damaging to the aquaculture of largemouth bass (Ma et al. 2013). In May 2018, some largemouth bass farms in Foshan experienced persistent mortalities, which was indicative of a disease. Trypomastigotes were observed in the blood smears of the dying fish, and diseased fish showed reduced feeding and lethargy. Based on these observations, trypanosomiasis was suspected to be the cause of the death.

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Trypanosomiasis is a disease caused by *Trypanosoma* (Euglenozoa: Kinetoplastea) protozoans, and this disease affects all classes of vertebrates across the world (Woo 1998; Gibson et al. 2005). In aquaculture settings, more than 200 trypanosome species have been recorded in both marine and freshwater fish (Gupta and Gupta 2012; Eiras et al. 2012; Lemos et al. 2015). Many studies have demonstrated that bloodsucking aquatic leeches transmit trypanosomiasis to the fish (Jones and Woo 1991; Su et al. 2014; Hayes et al. 2014; Corrêa et al. 2016). Trypanosomes primarily parasitize the blood and depend on the energy resources of the host (Corrêa et al. 2016). These hemoparasites can proliferate in the blood of the host by binary fission, and their proliferation impairs the physiological functions of the blood (Maqbool and Ahmed 2016). Although most fish infected with trypanosomes remain asymptomatic, high levels of parasites in the blood can lead to symptoms such as lethargy, anemia, leukocytosis, hypoglycemia, and splenomegaly (Islam and Woo 1991; Fujimoto et al. 2013; Fink et al. 2015).

Fish trypanosomes have been mainly described in wild populations, and how hemoparasites affect commercial farms is poorly understood (Jesus et al. 2018). To our knowledge, this is the first report of trypanosomiasis in cultured largemouth bass in China. Hence, more information on trypanosomiasis outbreaks in largemouth bass intensive farming is required to reduce economic damage caused by this disease. Here, we identified the clinical symptoms and pathological changes of infected largemouth bass and compared the morphological and molecular characteristics of the trypanosome with other known trypanosomes.

Materials and methods

Investigation of diseased fish

In early May 2018, a disease with persistent mortality occurred in the largemouth bass population in Foshan City (Fig. S1), Guangdong Province, located in Southern China. At the time of the outbreak, the water temperature was 25~27 °C. The mortality of fish in three ponds (10,000 m^3/pond and 45,000~50,000 fish/pond) was recorded by the farmers, and the symptoms of fish were investigated by observing swimming patterns, body surface, and visceral organs of the fish.

Thirty diseased fish, which were identified by their slow swimming and lethargy, and 30 asymptomatic fish (300~400 g) were randomly selected to check for the presence of blood parasites. Sampled fish were anesthetized using tricaine methanesulphonate (MS-222) (Sigma-Aldrich, Steinheim, Germany), and whole blood was collected from the caudal vein using 1-mL syringes. A drop of blood was used for making blood smears, which were stained using Wright–Giemsa stain (Yuanmu, Shanghai, China) following the manufacturer's protocol. Smears were observed under light microscopy (Nikon 80i, Japan) at × 400 magnification. The blood smears were also used to determine the ratio of parasites to red blood cells. Meanwhile, five diseased fish and five asymptomatic fish were investigated using parasitology and bacteriology methods. Gills and body surface were examined under the microscope for the presence of parasites. Spleen and liver were cultured on brain–heart infusion (BHI) agar plates and incubated at 28 °C for 48 h to isolate the bacteria.

Serum biochemical and histopathology analysis

Five diseased fish and five control fish (asymptomatic with no trypanosomes in the blood) were used for subsequent experiments. Fish were anesthetized using MS-222, and whole blood was collected from the caudal vein using 5-mL heparinized syringes and centrifuged at 4000 g for 10 min to collect serum for biochemical analyses. The concentrations of glucose, cholesterol, triglyceride, albumin, total protein, high-density lipoprotein, and low-density lipoprotein, and the activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were determined using the automated Hitachi Automatic Biochemical Analyzer 7180 (Hitachi, Yokohama, Japan) system with commercial kits (BioSino Bio-Technology & Science Inc., China).

The fragments of gills, liver, heart, spleen, and kidney were collected from five diseased and five control fish, and samples were fixed in 10% neutral buffered formalin. After 48 h of fixation, the fragments were dehydrated in an ethanol series and embedded in paraffin wax, and 4 μm of transversal sections were prepared. Sections were stained with hematoxylin and eosin (H&E) and observed under light microscopy (Nikon 80i, Japan).

Morphological examination

Morphological analyses of the trypanosomes were conducted by taking microscope images of well-stained trypanosomes at × 1000 magnification (Leica DM5000B, Germany). Following the morphometric values and standards described by Gu et al. (2006, 2007b), the following 13 morphological parameters were measured: body length (BL), total length with flagellum (L), posterior end to mid-nucleus (PN), posterior end to kinetoplast (PK), kinetoplast to mid-nucleus (KN), free flagellum length (FF), mid-nucleus to anterior end (NA), parasite maximum body width (BW), nucleus width (NW), nucleus length (NL), the nuclear index (NI = PN/NA position of nucleus in the body), the kinetoplast index (KI = PN/KN position of kinetoplast in the body), and flagellar index (FI = FF/BL position of free flagellum in the body). Incomplete and distorted trypanosomes were removed, and 100 trypanosomes were randomly selected for morphological analysis using ImageJ 1.51J8 (Wayne Rasband National Institutes of Health, USA).

Phylogenetic analysis of 18S rDNA

Parasite DNA was extracted from the blood of diseased fish using the blood DNA kit (TaKaRa, Dalian, China) following the manufacturer's instructions and stored at − 20 °C. The 18S rDNA sequences of trypanosomes were amplified using a forward primer S-762 (5′-GACTTTTGCTTCCTCTATTG-3′) and a reverse primer S-763 (5′-TATGCTTGTTTCAA GGAC-3′) designed by Maslov et al. (1996). The predicted PCR product was approximately 2200 bp. PCR reactions were conducted using the Ex Taq polymerase (TaKaRa, Dalian, China) according to the manufacturer's protocol. PCR cycling parameters were as follows: 1 cycle at 94 °C for 5 min and then (94 °C, 30 s; 55 °C, 30 s; 72 °C, 2 min) × 35 cycles, followed by 1 cycle at 72 °C for 10 min. The PCR products were cloned into the pMD18-T plasmid (TaKaRa, Dalian, China) and transformed into DH5α competent cells (TaKaRa, Dalian, China). Positive clones were identified using colony PCR and sequenced by the Beijing Genomics Institute, China.

Nucleotide similarities of the 18S rDNA sequences were obtained to examine the relationship of the trypanosomes isolated from the diseased fish to known trypanosome species using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analyses were performed using 26 trypanosomes species (Fig. 3), and *Trypanosoma* spp. from mammalian and bird species were used as an outgroup. The 18S rDNA sequences were aligned using Clustal X (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/) and concatenated into single alignments for phylogenetic analyses. Maximum likelihood (ML) was used for phylogenetic construction by MEGA 7.0 (Arizona State University, USA) under default settings. The consensus tree was obtained after bootstrap analysis with 1000 replications.

Statistical analysis

Serum biochemical parameters of diseased fish were compared to control fish using a Student's *t* test. The data are shown as mean ± SEM, and $P < 0.05$ was considered significant.

Results

Gross observation

In early May 2018, cultured largemouth bass in Southern China showed high rates of morbidity in some farms. The water temperature of the farms with disease fish was 25–27 °C. The morbidity continued for more than a month before this investigation, and 0.2~0.5% of the fish that were being cultured at the time were dying each day. Diseased fish swam along the sides of the pond and gasped at the surface of water, and showed reduced feeding and lethargy. The primarily morphological difference between diseased fish and control fish was exophthalmos (Fig. 1b), or the bulging of the eye. Trypomastigotes were observed in the blood smears of all diseased fish and 6.67% of the asymptomatic fish. The asymptomatic fish had fewer parasites in the blood compared to diseased fish, and the ratios of parasite to red blood cells were 0.17 ± 0.02 and 0.69 ± 0.25 (Fig. S2), respectively. In addition, no other parasites were found on the fish and bacterial growth was not observed on BHI agar plates inoculated from the liver and spleen of fish from the diseased farm.

Serum biochemistry and histopathology

Trypanosomes significantly changed glucose, triglyceride, low-density lipoprotein concentration and alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) activities in the serum of diseased fish (Table 1). Glucose (0.05 ± 0.03 nmol/L), triglyceride (1.87 ± 1.25 nmol/L), and low-density lipoprotein (1.14 ± 0.50 nmol/L) concentrations were significantly lower ($P < 0.05$) in diseased fish compared to healthy fish collected from the same farm (control) (5.85 ± 1.37, 5.40 ± 1.70 and 2.70 ± 0.50 nmol/L, respectively). ALT (90.00 ± 37.55 U/L), AST (207.25 ± 56.77 U/L), and LDH (1439.00 ± 454.43 U/L) activities were significantly higher ($P < 0.05$) in diseased fish compared to control fish (17.20 ± 6.12, 51.40 ± 17.11 and 253.00 ± 108.56 U/L, respectively). However, we did not observe differences ($P > 0.05$) in cholesterol, low-density lipoprotein, albumin, globulin, and total protein concentration and alkaline phosphatase (ALP) activity between diseased and control fish.

Histopathological examination revealed dramatic changes in the liver, head kidney, and spleen. Liver tissue sections from the diseased fish showed disorganized hepatocytes, steatosis, and foci necrosis (Fig. 2b). There were obvious signs of lymphocytic reduction, renal congestion, and destruction of red blood cells in the head kidney (Fig. 2d). Lymphocytic reduction and severe hemorrhages were observed in the spleen (Fig. 2f). No obvious pathological changes were found in the heart and gill (data not shown). In addition, a large number of trypomastigotes were found in the gill (Fig. 3a), liver (Fig. 3b), head kidney (Fig. 3c), and blood (Fig. 3d).

Taxonomic summary

Phylum: Sarcomastigophora Honigberg and Balamuth 1963
Class: Kinetoplastea (Honigberg, 1963) Vickerman, 1976
Order: Trypanosomatida (Kent, 1880) Hollande, 1952
Family: Trypanosomatidae (Doflein, 1901) Grobben, 1905
Trypanosoma micropteri Jiang et al. n. sp

Fig. 1 Clinical signs of diseased fish. **a** Eye of uninfected fish. **b** Exophthalmos of diseased fish. **c** Liver and spleen of uninfected fish. **d** Liver appeared pale and spleen showed swollen of disease fish

Type material: hapantotype: culture 2018T001. Paratypes: cultures 2018T002 and 2018T003; their respective collection sites were State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong Province, China.

Type host: *Micropterus salmoides* (Teleostei, Centrarchidae). Additional host: *Pelteobagrus fulvidraco* (Siluriformes, Bagridae).

Locality: town of Juijiang, Foshan city, Guangdong Province, China (22° 55′ N, 113° 10′E). Niushan Lake, Wuhan City, Hubei Province, China (30° 19′ N, 114° 31′ E)

Site of infection: peripheral blood

Etymology: the specific name is derived from the generic name of the host species, *M. salmoides*. The species was first discovered in *P. fulvidraco* by Gu 2007, and it was not described as a new species. The species name of *P. fulvidraco*

Table 1 Hematological parameters in the healthy and diseased largemouth bass

Biochemical parameters	Control fish	Diseased fish
Blood glucose (nmol/L)	5.85 ± 1.37	0.05 ± 0.03*
Cholesterol (nmol/L)	15.28 ± 1.53	13.14 ± 3.56
Triglyceride (nmol/L)	5.40 ± 1.70	1.87 ± 1.25*
High-density lipoprotein (nmol/L)	2.19 ± 0.14	1.97 ± 0.05
Low-density lipoprotein (nmol/L)	2.70 ± 0.50	1.14 ± 0.50*
Albumin (g/L)	10.26 ± 0.80	11.16 ± 1.96
Globulin (g/L)	36.40 ± 0.76	40.46 ± 5.62
Total protein (g/L)	47.74 ± 2.88	51.72 ± 7.46
Alanine transaminase ALT (U/L)	17.20 ± 6.12	90.00 ± 37.55#
Aspartate transaminase AST (U/L)	51.40 ± 17.11	207.25 ± 56.77#
Alkaline phosphatase ALP (U/L)	76.60 ± 10.04	71.00 ± 12.13
Lactate dehydrogenase LDH (U/L)	253.00 ± 108.56	1439.00 ± 454.43#

Data shown as mean ± SEM; $N = 5$

*Significance decrease ($P < 0.05$) in the diseased fish

Significance increased ($P < 0.05$) in the diseased fish

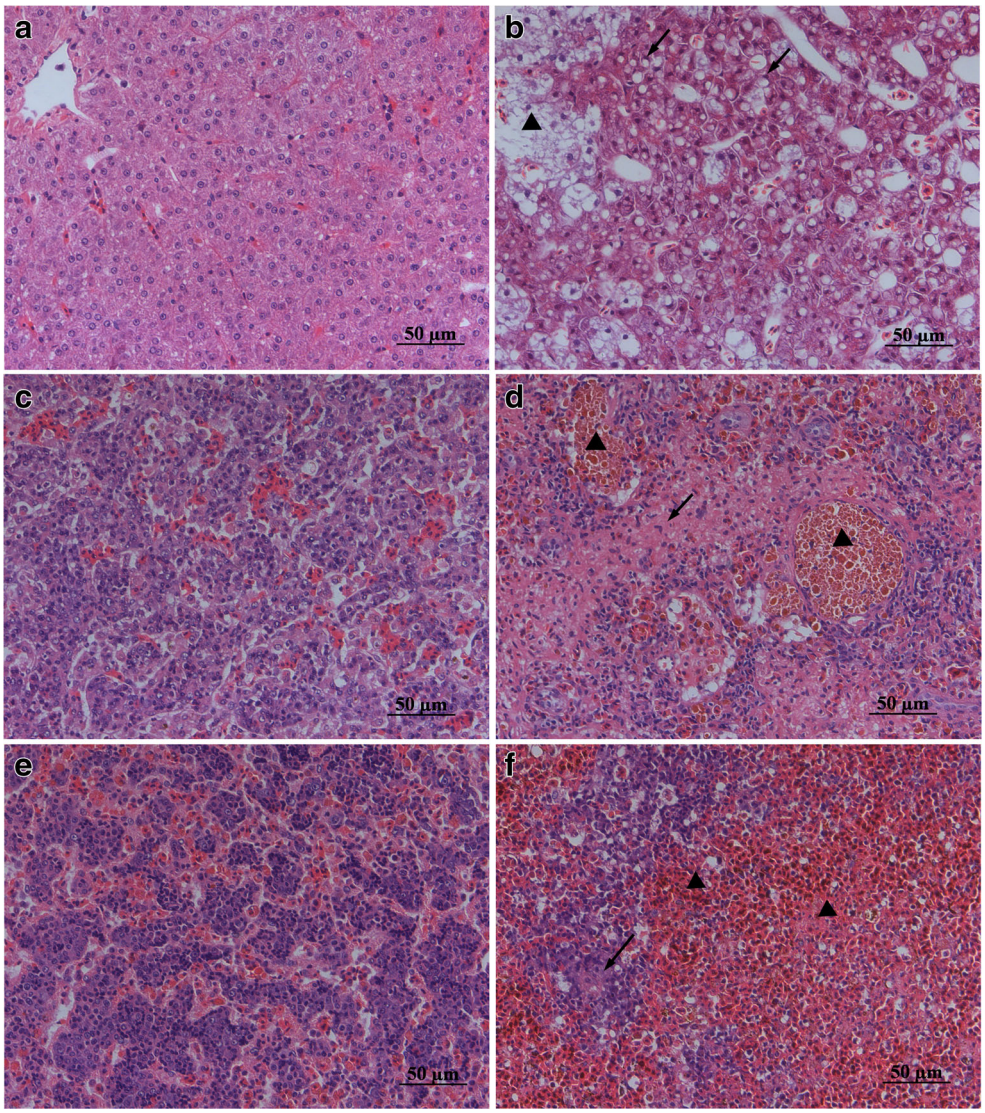

Fig. 2 Pathological changes in infected largemouth bass. **a**, **c**, **e** Normal liver, head kidney, and spleen, respectively. **b** Liver showed hepatocyte steatosis (arrow) and foci necrosis (triangle). **d** Head kidney showed lymphocytic reduction (arrow), renal congestion, and red blood cells were destroyed (arrow). **f** Lymphocytic reduction (arrow) and severe hemorrhage (triangle) were observed in spleen

has been used to describe another species, *Trypanosoma fulvidraco* (Gu et al. 2007). The species was named *T. micropteri* n. sp. to distinguish these two species.

Description: the trypanosomes only exist as trypomastigotes in the blood of largemouth bass (Fig. 3d). The body is slender and narrow, with a tapered anterior end and a sharpened posterior end; the length of the body are 17.0–26.6 μm, mean 21.5 μm with width 1.1–2.2 μm, mean 1.6 μm; undulating membrane not well developed; cytoplasm hyaline and sometimes deeply stained granules. Free flagellum relatively long, ranging from 8.5–22.3 μm with mean flagellum length of 15.7 μm. The nucleus is elongated oval, always parallel to the long axis, and located in the anterior part of the body. The nucleus is 1.9–3.3 μm long with mean of 2.6 μm, and 0.7–1.8 μm wide and mean 1.2 μm, and NI = 0.7–3.8 and mean = 1.7. The kinetoplast is oval, located near the subterminal posterior end of the body, and the KI = 1.1–1.2, mean = 1.12. The 18S rDNA gene of *T. micropteri* n. sp. is 2160 bp (GenBank no. MH635421).

Molecular characterization

The variation in 18S rDNA sequences extracted from different infected largemouth bass individuals was 0.005~0.02% (N = 6). Previously isolated sequences from *T. micropteri* n. sp. and T. sp. ex *P. fulvidraco* were nearly identical (99.8%), with the exception of one variable site and three gaps between them. In addition, the 18S rDNA sequences of *T. micropteri* n. sp. were also highly similar (99.1%) to *Trypanosoma* sp. Marv, a trypanosome from carp also grown experimentally in goldfish (Gibson et al. 2005), with only two variable sites and 13 gaps between these variable sites. The sequences that were

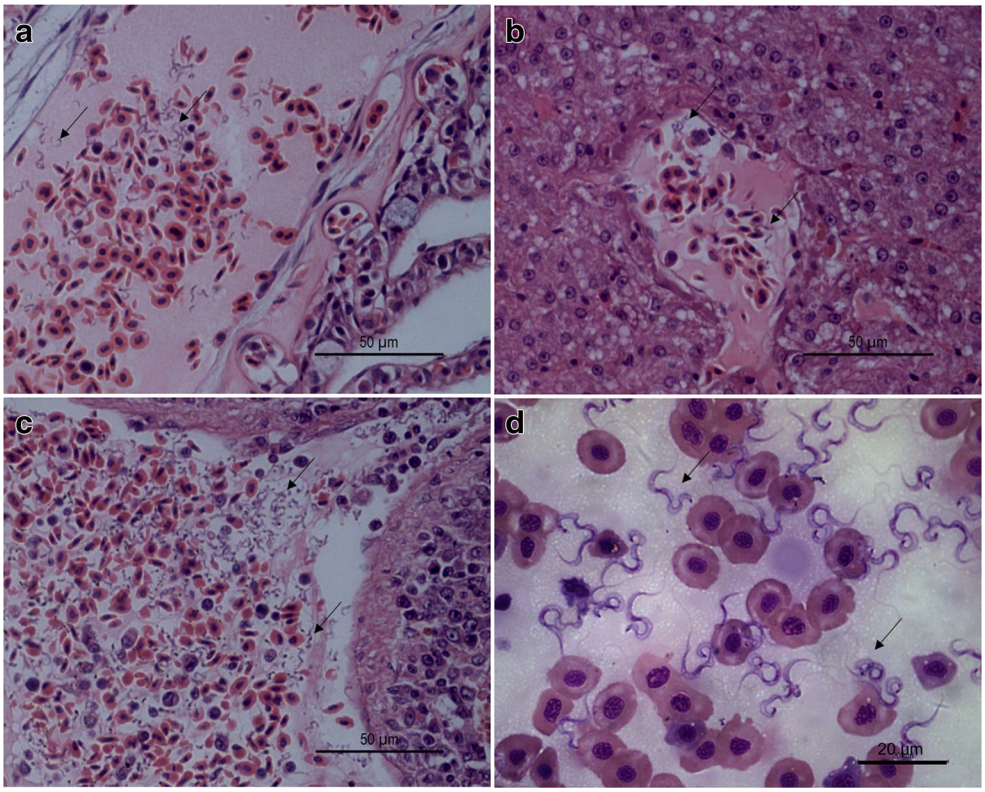

Fig. 3 *Trypanosoma micropteri* n. sp. trypomastigotes in gill (**a**), liver (**b**), head kidney (**c**), and blood (**d**). **a**–**c** Histological sections stained with H&E. **d** Wright–Giemsa stained peripheral blood smear

generated in this study were 91.0–98.6% similar to *Trypanosoma* species that infect other freshwater fish, and 89.0–96.2% similar to *Trypanosoma* species that infect marine fish (Table S1). The phylogenetic tree of the *Trypanosoma* spp. was generally consistent with the topology of previously published phylogenies, and the 18S rDNA maximum likelihood tree strongly supported the existence of an aquatic clade (Fig. 4). The fish trypanosome isolates from freshwater and marine fish formed two clades, and these two clades were separated from the clades that consisted of *Trypanosoma* species that infect other animals. *T. micropteri* n. sp. clustered into the freshwater fish subclade along with *T.* sp. ex *P. fulvidraco*, *T.* sp. Marv, and *Trypanosoma.* sp. ex *Carassius carassius*, and these sequences had 98.6%, 99.1%, and 99.8% sequence similarity, respectively.

Discussion

At least 200 trypanosome species that can infect freshwater and marine fish have been described to date, and only certain trypanosomes appear to be harmful against certain fish species (Lom and Dykova 1992; Woo 1998; Eiras et al. 2012;). Some trypanosomes have a considerably negative effect on fish, causing lethargy, anemia, splenomegaly, hematopoeitic damage, and weight loss (Khan 1985; Ahmed et al. 2011; Lemos et al. 2015). Prior investigations showed that mortality ranges from 0 to 65% in freshwater fish that are infected with *Trypanosoma* spp. (Woo and Black 1984; Corrêa et al. 2016; Rodrigues et al. 2017). In this study, we found that *T. micropteri* n. sp. mainly caused lethargy and pathological alteration in the gills, spleen, and liver of largemouth bass. The mortality of largemouth bass caused by *T. micropteri* n. sp. was 0.2 to 0.5% per day. The numbers of trypanosome in diseased fish were significantly higher than infected fish that did not have symptoms. These results suggest that the trypanosome can severely impair the physiological functions of largemouth bass when it reaches high numbers in the fish. Prior studies have noted that emaciation is one of the main symptoms of trypanosomiasis in fish (Khan 1985; Molina et al. 2016). Contrary to these studies, body weight was similar in diseased and healthy largemouth bass. A possible explanation may be that the time from illness to death is too short for there to be a significant drop in weight in largemouth bass. In addition, all diseased fish had trypomastigotes in the blood, whereas trypanosome levels were low (6.67%) in the asymptomatic fish. These results suggest that *T. micropteri* n. sp. is not spreading quickly in largemouth bass. We did not find leeches on the analyzed fish, which is consistent with previously studies focusing on freshwater fish in China (Li and Wang 1996; Gu et al., 2007). However, it is a consensus that leeches transmit these parasites (Hayes et al. 2014; Corrêa et al. 2016). We speculate that there are few leeches in these ponds, and they may only attach to the fish when feeding, making it difficult to detect their presence. This may be the main reason why this disease appears to spread slowly in

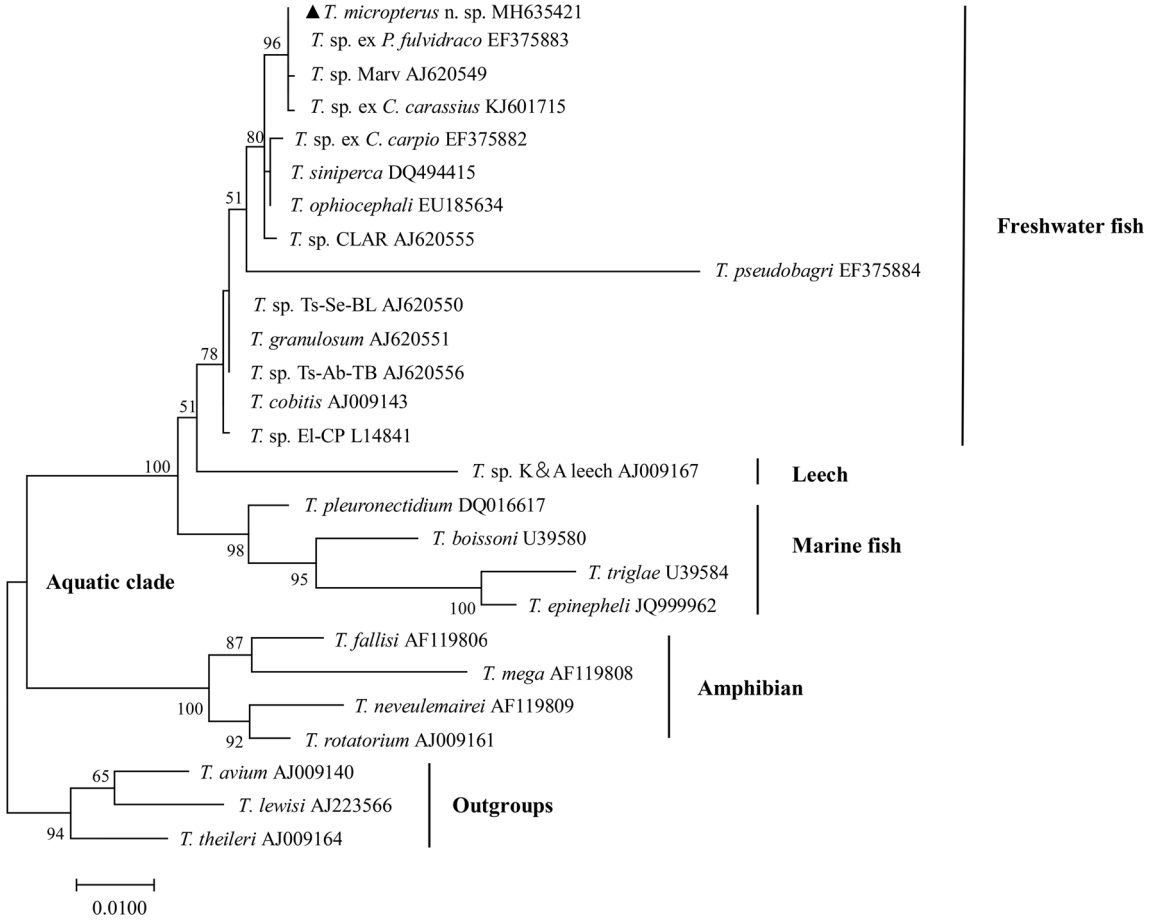

Fig. 4 Maximum likelihood (ML) phylogenetic tree constructed based on the 18S rDNA gene sequences in different trypanosome species using MEGA 7. A bootstrap analysis is performed using 1000 replicates to test the relative support for particular clades. GenBank accession numbers of each gene follow the name of each species

largemouth bass aquaculture. Therefore, it is urgent to clarify how *Trypanosoma* spp. is transmitted in Chinese freshwater fish.

Biochemical profiles are often used as a tool to assess the health of the farmed fish. Biochemical constituents of the fish blood, proteins, nutritional, metabolic parameters, and internal organs can easily be assessed (Newman et al. 1997; Jamalzadeh et al. 2009). Trypanosomes mainly depend on the energy resources of the host fish, which can alter the biochemical composition of the infected fish (Woo 1998; Gupta and Gupta 2012). In general, glucose is a constant source of energy for all cells in the body and is maintained at adequate levels in the plasma (Percin and Konyalioglu 2008). We found significantly lower glucose levels ($P < 0.05$) in diseased fish (0.05 ± 0.03 nmol/L) compared to control fish (5.85 ± 1.37 nmol/L). The fall of glucose content in the serum suggests that *T. micropteri* n. sp. utilized the energy resources of the infected largemouth bass, which may underlie the symptoms of lethargy observed in diseased fish. Triglyceride and low-density lipoprotein concentrations were significantly lower ($P < 0.05$) in diseased fish compared to control fish. Changes in the triglyceride and low-density lipoprotein concentrations may be caused by liver damage and lack of nutrition (Torre et al. 2000). ALT, AST, and LDH activities were significantly higher ($P < 0.05$) in diseased fish, and this may be caused by impaired functions of the liver and spleen, which can further impair metabolism (Vaglio and Landriscina 1999). Based on these findings, we can infer that *T. micropteri* n. sp. parasitized in the blood vascular system to deplete circulating nutrition and severely impair the physiological functions of the host fish.

In China, more than 30 species of trypanosomes have been recorded in freshwater fish (Gu et al. 2007b). Modern taxonomic identification relies on both morphological and genetic characteristics of the species (Gibson et al. 2005; Gu et al., 2007), and genetic analyses focus primarily on 18S rDNA for trypanosomes (Davies et al. 2005; Gu et al., 2007; Su et al. 2014). Incorporating 18S rDNA sequences greatly improves the accuracy of species identification, as well as allowing for inferring the phylogenetic relationships within other trypanosomes (Davies et al. 2005; Gu et al., 2007). Following the current standards, the morphometric characteristics and phylogenetic relationship of *T. micropteri* n. sp. are listed in Table 2 and Fig. 4. There was only one

Table 2 Comparison the dimensions in *Trypanosoma micropteri* n. sp. from largemouth bass with other *Trypanosoma* spp. (high similarity in the 18S rDNA sequences)

Characters	*T. micropteri* n. sp.	*T.* sp. ex *P. fulvidraco*	*T.* sp. ex *C. carassius*	*T.* sp. ex *C. carpio*
PK (μm)	1.2 ± 0.2(0.8–1.9)	0.9 ± 0.1 (0.7–1.1)	1.3 ± 0.1 (1.1–1.5)	1.3 ± 0.2 (1.1–1.6)
KN (μm)	12.0 ± 1.7(8.0–16.1)	10.9 ± 2.3 (6.0–14.3)	12.9 ± 0.3 (7.5–15.4)	18.9 ± 2.3 (14.0–22.8)
PN (μm)	13.2 ± 1.7(9.0–17.2)	12.7 ± 2.4 (7.3–16.2)	–	21.5 ± 2.4 (15.5–23.5)
NA (μm)	8.4 ± 1.9 (3.9–13.0)	9.7 ± 1.3 (8.0–11.2)	13.5 ± 0.8 (11.9–15.0)	8.1 ± 0.9 (5.6–10.0)
BL (μm)	21.5 ± 2.2 (17.0–26.6)	22.4 ± 3.2 (15.5–26.4)	27.6 ± 1.3 (21.7–30.5)	29.7 ± 2.5 (24.5–32.5)
FF (μm)	15.7 ± 2.2 (8.5–22.3)	15.3 ± 0.9 (12.5–15.8)	8.4 ± 0.7 (7.7–22.0)	17.0 ± 2.1 (15.0–22.4)
L (μm)	37.2 ± 3.4(26.2–46.7)	37.7 ± 3.9 (28.7–42.0)	–	46.6 ± 3.5 (40.3–52.7)
NL (μm)	2.6 ± 0.3 (1.9–3.3)	2.4 ± 0.2 (2.1–2.8)	–	2.8 ± 0.3 (2.5–3.8)
NW (μm)	1.8 ± 0.2 (0.7–0.9)	1.1 ± 0.1 (0.9–1.2)	–	1.5 ± 0.3 (1.1–2.3)
BW (μm)	1.6 ± 0.2 (1.1–2.2)	1.2 ± 0.1 (1.1–1.3)	1.6 ± 0.1 (1.4–1.7)	1.4 ± 0.3 (1.3–2.1)
NI	1.7 ± 0.6 (0.7–3.8)	1.3 ± 0.2 (0.8–1.9)	–	2.8 ± 0.5 (2.0–4.3)
KI	1.1 ± 0.02 (1.1–1.2)	1.2 ± 0.05 (1.1–1.3)	–	1.2 ± 0.06 (1.1–1.4)
FI[a]	2.4 ± 0.2 (1.9–3.1)	1.5 ± 0.2 (1.0–1.7)	–	1.8 ± 0.2 (1.4–2.2)
Host species	*Micropterus salmoides*	*Pseudobagras fulvidraco*	*Carassius carassius*	*Cyprinus carpio*
References	The present study	Gu et al. 2007	Grybchuk et al. 2014	Gu et al. 2007

trypomastigote morphotype in the blood of the largemouth bass, and it exhibited similar morphological characteristics as *T.* sp. ex *P. fulvidraco* isolated from *P. fulvidraco* in China, which was previously described by Gu et al. (2007a). *T. micropteri* n. sp. is about 37.2 μm in length, which is close to the size of *T.* sp. ex *P. fulvidraco* (mean 37.7 μm). The values for PN, BL, FF, NL, and KI were consistent, and there were overlaps in the ranges of other morphological standards (Table 2). Moreover, according to the 18S rDNA analysis, sequences from largemouth bass and *P. fulvidraco* trypanosomes examined in this study were nearly identical (sequence similarity 99.8%) and clustered together in a clade. These differences may be caused by intra-population variability, where there was 0.005~0.02% variation in the sequence of isolates of this new species from different largemouth bass individuals. These results suggest that *T. micropteri* n. sp. and *T.* sp. ex *P. fulvidraco* are likely the same species. In addition, *T.* sp. Marv and *T.* sp. ex *C. carassius* are also clustered together with *T. micropteri* n. sp. in the phylogenetic analysis, and share high sequence similarity. However, the description of *T.* sp. Marv lacks (Gibson et al. 2005) morphological data and *T.* sp. ex *C. carassius* (Grybchuk et al. 2014) has longer body length (27.6 vs 21.5 μm), shorter free flagellum length (8.4 vs 15.7 μm), and longer NA distance (13.5 vs 8.4 μm). Moreover, the strains of *T.* sp. Marv and *T.* sp. ex *C. carassius* were isolated from Europe (Gibson et al. 2005; Grybchuk et al. 2014). Therefore, we are not able to identify whether *T.* sp. Marv, *T.* sp. ex *C. carassius*, and *T. micropteri* n. sp. are the same species based on the current data.

In summary, we demonstrate the presence of *T. micropteri* n. sp. in cultured largemouth bass in China. The macroscopic findings were similar with the common characteristics of *T. micropteri* n. sp. in infected fish, such as *Hypostomus luetkeni* (Lemos et al. 2015) and *Oreochromis niloticus* (Jesus et al. 2018). An evaluation of the histopathology and serum biochemical parameters revealed that *T. micropteri* n. sp. severely impaired the physiological functions of the largemouth bass and caused high mortality in infected fish. Based on the morphological and phylogenetic analyses, *T. micropteri* n. sp. appeared to be closely related to *T.* sp. ex *P. fulvidraco* (Gu et al., 2007). Future research is needed to confirm the vectors of *T. micropteri* n. sp. in largemouth bass aquaculture in order to prevent the spread of this disease.

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Compliance with ethical standards

All protocols were approved by China Institute of Veterinary Drug Control and the Animal Experimentation Ethics Committee at Sun Yat-sen University in Guangzhou, China.

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