Haematological response of snow barbell, *Schizothorax plagiostomus* Heckel, naturally infected with a new *Trypanosoma* species

Aamir Maqbool · Imtiaz Ahmed

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Abstract The present study deals with the description of a new piscine trypanosome species found infecting the fresh water fish *Schizothorax plagiostomus* Heckel from river Jhelum, Srinagar, J&K, India and evaluating the haematological parameters of the infected fish. Haematological examination of *S. plagiostomus* revealed 61.1 % infection with an intensity of 1–9 trypanosomes/100 RBC's. Small ($26.9 \pm 1.39 \mu\text{m}$) and large ($47.17 \pm 3.50 \mu\text{m}$) forms of the trypanosome were observed in light microscopy investigations, revealing the dimorphic nature of the species. The trypanosome species was found to be distinct from the other related dimorphic species in morphometric dimensions including cell length, cell breadth, kinetoplast index, flagellar index, and cytological peculiarities, respectively. The detailed descriptions of the two morphological forms found in the blood of *S. plagiostomus* are provided. Based on the geographical location, morphometrics, cytological peculiarities, host status and comparative study, the new species is named *Trypanosoma kashmirensis* n. sp. The parasitic infestation caused a significant decrease ($p < 0.05$) in red blood cell counts, haematocrit and haemoglobin concentrations while, the leucocyte (WBC) count, mean cellular volume and mean cellular haemoglobin showed a significant increase ($p < 0.05$) in the infected fish as compared to the non-infected. The above alterations of the haematological parameters could be used as an important tool for the indication of *Trypanosoma* infection in the fish.

Keywords *Schizothorax plagiostomus* · *Trypanosoma* · Morphometric dimensions · Haematological parameters

Introduction

Fish is an excellent and one of the primary source of protein for humans in many parts of the world. Fish is more affordable and accessible animal source food and it is estimated that around 60 % of people in many developing countries depend on fish, for over 30 % of their animal protein supplies (FAO 2005). Consumption will increase in future years as the demand will further be compounded by the growth in population and this additional supply will have to come from aquaculture (FAO 2012). Development of aquaculture during the last decade has resulted in much greater attention being paid to problems posed by parasites and their importance for fishery leading to constraints in the productivity of aquaculture (Kennedy 1994). Besides direct losses caused by mortality, parasites may have considerable impact on growth and behavior of fish, their resistance to other stress factors, susceptibility to predation, etc. and their presence may also reduce marketability of fish (Crowden and Boom 1980; Brassard et al. 1982; Lom and Dyková 1992; Williams and Jones 1994; Kumaraguru et al. 1995; Woo 1995).

Trypanosoma probably is the best known extra erythrocytic protozoan in fish (Clauss et al. 2008) and undoubtedly represents one of the most important groups of pathogens which negatively affects the health state of cultured and feral fish. *Trypanosoma* are single celled flagellated protozoans that are generally found in the blood of vertebrates and can cause trypanosomiasis of fish, including fresh water fish (Smit et al. 2004; Davies et al. 2005; Padua et al. 2011). Piscine trypanosomes are always

A. Maqbool (✉) · I. Ahmed
Fish Nutrition Research Laboratory, Department of Zoology,
University of Kashmir, Hazratbal, Srinagar 190006, J&K, India
e-mail: aamir.aquaculture@yahoo.co.in

I. Ahmed
e-mail: imtiazamu1@yahoo.com

transmitted by leeches (Woo 2006) and can be detected in the peripheral blood of various freshwater and marine fish species (Bruno et al. 2006). Hoare and Wallace (1966) proposed the use of terms amastigote, promastigote, opisthomastigote, epimastigote, trypomastigote and choanomastigote for the various stages in the life cycle of the parasite based on the position of the flagellum and is widely accepted. However, trypomastigotes are the predominant stages reported from fish blood. Approximately 200 piscine trypanosomes have been reported from all over the world (Gupta and Gupta 2012) and host specificity of the vast majority of species is not known (Woo 1995; Gupta and Gupta 2012).

The study of the physiological and haematological characteristics of cultured fish species is an important tool for the development of aquaculture system, particularly in regard to the use in detection of healthy fish from diseased or stressed animal (Rainza-paiva et al. 2000; O'Neal and Weirich 2001). Haematological studies are an important tool for diagnosis and prognosis of morbid conditions, including the deleterious effects of infection (Tavares-Dias and Moraes 2003). Biological monitoring techniques involving hematological variables have become attractive and useful for monitoring environmental quality, water pollution and the health condition of aquatic organisms (Kori-Siakpere and Ubogu 2008; Olufayo 2009; Qiang et al. 2013). The evaluation of haematological parameters could be useful for the diagnosis of fish pathologies and physiological status (Stoskopf 1993). Therefore, the variations of haematological parameters associated with various parasite infections could be established into a database, which could be used as biomarkers in disease diagnosis and in guiding the implementation of treatment or preventive measures. The studies related to haematological alterations in *Trypanosoma* infected fish are scanty (Joshi and Dabral 1981; Khan 1985; Islam and Woo 1991; Gupta and Gupta 2010) and further research is required involving diverse hosts, so that the haematological variations associated are elucidated and used as an efficient *Trypanosoma* infection indicator.

Lingard (1904) recorded the first trypanosome from *Barbus carniticus* from Poona and Nandi et al. (2002) provided a bibliographic record of more than fifty trypanosome species from Indian fishes. Since then, many *Trypanosoma* species have been recorded from different Indian fishes including *T. piscidium* from *Colisa fasciata* (Gupta et al. 2003), *T. sauli* from *Channa punctatus* (Gupta et al. 2006), *T. heteropneusti* from *Heteropneustes fossilis* (Gupta et al. 2006), *T. danilewskyi* from *Clarias batrachus* (Saha et al. 2007), *T. mukasai* from *Triplophysa marmorata*, *Carassius carassius* and *Schizothorax curvifrons* (Shahi et al. 2013). However, there is a dearth of information and a lack of morphometric characterization on schizothoracine species with only a single report of

trypanosome infection from *S. curvifrons* Heckel (Shahi et al. 2013). Snow barbell, *S. plagiostomus* is one of the economically important coldwater fish species of Himalayan and sub-Himalayan region including Kashmir. It is locally known as 'khont' and is found inhabiting the snow and stream fed waters of the Kashmir valley. The available literature shows no previous records of *Trypanosoma* spp. infecting *S. plagiostomus*. Therefore, the present study is the first case report on *Trypanosoma* infection in *S. plagiostomus* in the natural environment, provides a morphological characterization of the trypanosome and evaluates the haematological response of the infected fish.

Materials and methods

Host collection

S. plagiostomus Heckel (340 ± 25.4 g; 32 ± 2.8 cm) were captured with the help of local fishermen in June 2012 from river Jhelum, located in Srinagar, Jammu and Kashmir, India ($34^{\circ}5'N$ – $74^{\circ}47'E$). Three sampling sites (S_1 , S_2 and S_3) were selected in river Jhelum (Fig. 1). The live fish were transported in plastic containers filled with water and brought to the wet-lab. These fishes were first given a prophylactic dip in $KMnO_4$ (5 mg L^{-1}) to rule out any possible microbial infection and randomly stocked in indoor circular plastic fish tanks (water volume = 600 L) fitted with a continuous water flow-through system for a fortnight. During this period, the fish were fed to satiation a soybean based formulated diet twice a day at 08:00 and 18:00 h. Fish showing any external signs of disease or parasitized with helminth species were excluded from the study.

Haematological analysis and parasite processing

During blood sampling, fishes were carefully netted minimizing the stress and placed in a water containing (half filled) fish trough. They were positioned ventral side up (with gill region submerged) so that the blood could be easily drawn from the caudal vein, with the whole sampling event usually taking <2 min. All blood samples (1 ml from each fish) were collected from the caudal vein using a chilled needle with a 2 ml heparinized syringe and transferred to anticoagulant coated (Na-Heparin) blood collection vials which were kept in an ice-bath until further analysis. A drop of blood was placed on a glass microscope slide, covered with a glass cover-slip and screened for blood parasites using a light microscope at 400× magnification. Low intensity infection detection was done by using microhaematocrit method (Woo 1969). To determine the parasitaemia, number of parasites per 100 RBC's were

Fig. 1 Map showing the sampling sites (S_1 , S_2 and S_3). Inset: map of India showing the location of Srinagar

Fig. 2 Photomicrographs showing giemsa stained pleomorphic forms of *Trypanosoma* from *S. plagiostomus*. **a–b** small forms, **c–d** large forms. **F** free flagellum, **K** kinetoplast, **N** nucleus, **UM** undulating membrane, Arrows indicate the end of the free flagellum. Scale bar = 10 μ m

calculated in the blood smears (Gupta et al. 2006). Using grease-free slides, thin layer blood smears were made and left to air-dry. Dried smears were fixed in 100 % methanol

for 5–8 min and stained for 30 min with phosphate-buffered Giemsa (pH 6.8–7.2). All hematological analyses were carried out within 2 h after each extraction.

Haemoglobin (Hb)

Haemoglobin (Hb) content of the blood was estimated by the method of Drabkin (1946). 20 µl of blood was mixed with 5 ml of Drabkin solution (Loba Chemie, Mumbai, India) and left to stand for at least 15 min. Haemoglobin concentration was determined spectrophotometrically by measuring the absorbance at 540 nm and compared to that of Hb standard (Ranbaxy, New Delhi, India). Prior to reading the absorbance, Hb test samples were centrifuged to remove dispersed nuclear material.

Haematocrit (Hct)

Haematocrit (Hct) was determined on the basis of sedimentation of blood. Heparinized blood (50 µl) was taken in a micro-haematocrit capillary (Na-heparinized) and spun in a micro-haematocrit centrifuge (REMI RM-12C, Mumbai, India) at 12,000 g for 5 min to obtain Hct value. The Hct value was measured using a Hct reader and reported as percentage (Zaragoza et al. 2008).

Total erythrocyte count (RBC) and total leucocyte count (WBC)

For RBC and WBC counts, blood sample (20 µl) was taken with a micro pipette (Finpipette, Thermo Scientific, Finland) and diluted with (Natt and Herrick 1952) diluent (1:200). The diluted sample was placed in a Neubauer improved haemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany) and blood cells were counted using a light microscope.

MCV, mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis (1991).

$$\text{MCV (fl)} = \text{Hct/RBC} \times 10$$

$$\text{MCH (pg)} = \text{Hb/RBC} \times 10$$

$$\text{MCHC (g/dl)} = \text{Hb/Hct} \times 100$$

Parasite morphometry

The Giemsa stained smears were examined under a compound microscope (Magnus MLX-Tr, New Delhi, India) fitted with a digital camera and digital photomicrographs of trypanosomes were obtained using 100x oil immersion lens. Morphometric analysis was done with image analysis software ImageJ (National Institute of Health), calibrated to a stage micrometer. 90 randomly selected parasite specimens were used for morphometric measurements and 15 morphometric parameters including cell body length (CL), total length including flagellum (TL), posterior end

to kinetoplast (PK), posterior end to mid-nucleus (PN), kinetoplast to mid-nucleus (KN), mid-nucleus to anterior end (NA), free flagellum length (FL), breadth of cell body (CB), nucleus length (NL), nucleus breadth (NB), kinetoplast length (KL), kinetoplast breadth (KB), nuclear index $\text{NI} = \text{PN/NA}$ (position of nucleus in body), kinetoplasmic index $\text{KI} = \text{PN/KN}$ (position of kinetoplast in body), and flagellar index $\text{FI} = \text{CL/FL}$ were calculated in the present study as according to the terminology of morphometric values and standards commonly adopted by Hoare (1972) and Smit et al. (2004).

Water quality analysis

The physico-chemical parameters of water (temperature, dissolved oxygen, free carbon dioxide, total alkalinity and pH) in the fish tanks were recorded daily, following the standard methods (APHA 1992). The water sample for analysis was collected early in the morning (07:00 h) before the feeding was done. Water temperature ($22.6 \pm 1.92^\circ\text{C}$) was recorded using a mercury thermometer, dissolved oxygen ($6.8 \pm 0.86 \text{ mg L}^{-1}$) was estimated by Winkler's iodometric test, free carbon dioxide ($7.8 \pm 2.35 \text{ mg L}^{-1}$), total alkalinity ($85 \pm 3.39 \text{ mg L}^{-1}$) by titrimetric methods, respectively while, pH (7.3 ± 0.66) was measured using a digital pH meter (pHep-HI 98107, USA).

Statistical analysis

Haematological indicators were summarized as mean $\pm$ standard deviation (SD). Statistical significance was determined by student's *t* test, and a *p* value < 0.05 was considered significant. Statistical analysis of data were carried out with SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

Results

Examination of fresh blood samples from *S. plagiostomus* specimens revealed the presence of parasites seen actively wriggling about, pushing the blood cells randomly during their course of movement. Out of 90 specimens, 55 (61.1 %) were found to be infected. Parasitaemia, was moderate (6–9 trypanosomes/100 RBC's) in about 7.2 % of infected fish and the remaining had low infection level (1–5 trypanosomes/100 RBC's). Pleomorphism was observed with the presence of two distinguishable forms: 'small' and 'large'. Table 1 summarizes the morphometric parameters of the present *Trypanosoma* species with other related species.

Table 1 Morphometric dimensions (in μm) of *Trypanosoma kashmirensis* n. sp from *Schizothorax plagiostomus* compared with other related dimorphic species

		TL	CL	CB	NL	NB	KL	KB	
<i>T. aligaricus</i> (Gupta and Jairajpuri 1982)	Small	Mean (range)	34.37 (34.25–35.5)	1.65 (1.5–1.8)	3.00 (2.5–3.5)	1.12 (1.0–1.2)	10.5 (0.5–1.6)	0.45 (0.4–0.5)	
	Large	Mean (range)	54.68 (53.25–55.3)	2.45 (2.1–2.6)	3.73 (3.6–3.8)	2.02 (1.8–2.2)	0.51 (0.4–0.6)	0.25 (0.21–0.3)	
	Small	Mean (range)	35.8 (30.0–39.8)	3.0 (2.6–4.2)	1.9 (1.3–3.2)	1.5 (1.0–2.5)	0.9 (0.6–1.2)	0.65 (0.4–0.9)	
	Long	Mean (range)	43.6 (38.5–48.0)	4.6 (4.0–5.2)	3.1 (2.2–4.4)	1.4 (0.9–2.2)	1.0 (0.6–1.3)	0.4 (0.2–0.9)	
<i>T. saulii</i> (Gupta et al. 2006)	Minuta	Mean (range)	24.0 (18.0–33.0)	1.9 (1.0–2.5)	3.2 (1.0–6.0)	1.6 (1.0–3.0)	1.6 (1.0–2.0)	1.2 (0.5–2.0)	
	Magna	Mean (range)	35.5 (30.2–52.0)	2.6 (2.0–3.6)	3.7 (1.0–6.0)	2.0 (1.0–4.0)	1.9 (0.5–2.0)	1.25 (0.5–2.0)	
<i>T. heteropneusti</i> (Gupta et al. 2006)	Small	Mean (range)	27.9 (23.5–30.5)	1.68 (1.45–1.95)	2.64 (1.41–3.35)	1.14 (1.1–1.2)	1.18 (1.07–1.35)	0.93 (0.90–0.95)	
	Large	Mean (range)	37.83 (33.1–43.8)	1.40 (1.3–1.5)	2.63 (1.51–3.30)	1.23 (0.95–1.25)	1.16 (0.8–1.35)	0.90 (0.8–0.95)	
<i>T. kashmirensis</i> n. sp (present study)	Small	Mean (range)	26.90 (25.92–27.88)	1.11 (0.99–1.31)	2.03 (1.88–2.18)	0.93 (0.76–1.23)	0.80 (0.66–0.89)	0.80 (0.68–0.95)	
	Large	Mean (range)	47.17 (39.20–50.80)	2.53 (1.90–3.19)	3.39 (2.77–4.21)	2.08 (1.56–2.78)	1.05 (0.76–1.68)	0.77 (0.62–0.97)	
		NA	KN	PK	PN	FL	NI	KI	FI
<i>T. aligaricus</i> (Gupta and Jairajpuri 1982)	Small	Mean (range)	–	–	–	10.87 (10.25–13.5)	–	–	2.16 ^b
	Large	Mean (range)	–	–	–	8.50 (7.82–9.4)	–	–	5.43 ^b
<i>T. aori</i> (Joshi 1982)	Small	Mean (range)	5.1 (3.2–11.0)	1.1 (0.7–1.3)	–	18.2 (15.0–19.6)	–	–	0.96 ^b
	Long	Mean (range)	12.0 (9.0–14.2)	1.4 (0.8–1.8)	–	11.0 (10–12.5)	–	–	2.96 ^b
<i>T. saulii</i> (Gupta et al. 2006)	Minuta	Mean (range)	–	–	–	6.6 (4.0–8.2)	1.62 ^a	1.72 ^a	2.80 ^b
	Magna	Mean (range)	–	–	–	8.8 (8.0–12.0)	–	–	3.20 ^b
<i>T. heteropneusti</i> (Gupta et al. 2006)	Small	Mean (range)	–	–	–	8.48 (6.95–11.4)	1.16 ^a	1.72 ^a	1.97 ^b
	Large	Mean (range)	–	–	–	16.05 (12.05–18.6)	–	–	1.41 ^b
<i>T. kashmirensis</i> n. sp (present study)	Small	Mean (range)	8.20 (7.49–8.91)	11.54 (10.73–12.35)	.72 (.70–.74)	13.26 (12.60–14.10)	5.86 (4.41–7.30)	1.83 (1.14–2.33)	1.09 (1.05–1.17)
	Large	Mean (range)	13.36 (10.77–15.22)	23.90 (22.68–25.40)	1.30 (1.03–1.66)	24.60 (19.64–26.43)	9.83 (8.63–10.98)	1.92 (1.62–2.47)	1.05 (1.03–1.07)

CB breadth of cell body, CL body length, flagellar index FI = CL/FL , FL free flagellum length, KB kinetoplast breadth, kinetoplast index KI = PN/KN , KL kinetoplast length, KV kinetoplast to mid-nucleus, NA mid-nucleus to anterior end, NB nucleus width, nuclear index NI, PN/NA, NL nucleus length, PK posterior end to kinetoplast, PN posterior end to mid-nucleus, TL total length with flagellum

^a Overall indices

^b Calculated from means given in references

Table 2 Haematological parameters (mean $\pm$ SD) of uninfected and naturally infected snow barbell (*Schizothorax plagiostomus* Heckel) by *Trypanosoma*

Parameters	Uninfected fish (n = 35)	Naturally infected fish (n = 55)	p value
Haemoglobin (g/dl)	Mean 10.60 $\pm$ 0.99 Range 9.40–11.60	8.30 $\pm$ 0.53 7.90–8.90	.016 ^a
Haematocrit (%)	Mean 33.63 $\pm$ 2.64 Range 29.80–35.72	25.29 $\pm$ 3.30 21.70–28.10	.013 ^a
RBC ($\times 10^6/\text{mm}^3$)	Mean 1.87 $\pm$ 0.29 Range 1.45–2.13	1.21 $\pm$ 0.12 1.10–1.34	.016 ^a
MCV (fl)	Mean 178.36 $\pm$ 10.04 Range 167.70–191.91	207.47 $\pm$ 9.30 197.27–215.45	.011 ^a
MCH (pg)	Mean 57.25 $\pm$ 5.25 Range 53.13–64.83	68.39 $\pm$ 2.98 66.41–71.82	.023 ^a
MCHC (g/dl)	Mean 31.50 $\pm$ 1.12 Range 29.92–32.47	33.05 $\pm$ 2.92 33.05 $\pm$ 2.92	.368 ^{ns}
WBC ($\times 10^3/\text{mm}^3$)	Mean 16.68 $\pm$ 2.50 Range 13.86–19.80	30.35 $\pm$ 4.97 25.74–35.61	.005 ^a

^a Significant ($p < 0.05$)^{ns} Not significant ($p > 0.05$)

Taxonomic summary

Phylum:	Sarcomastigophora Honigberg and Balamuth 1963
Class:	Kinetoplastidea Honigberg 1963
Order:	Kinetoplastida Honigberg 1963
Family:	Trypanosomatidae Doflein 1911
Genus:	Trypanosoma Gruby 1843
Species:	<i>Trypanosoma kashmirensis</i> n.sp. (Fig. 2 a, b, c, d)

Parasite profile

Type host:	<i>S. plagiostomus</i> Heckel (Cypriniformes: Cyprinidae)
Type locality:	Srinagar, Jammu and Kashmir, India. (34°5'N; 74°47'E)
Site of infection:	Blood
Prevalence:	61.1 %
Intensity:	1–9 forms/100 RBCs
Etymology:	The specific name is derived from the place of species' geographical location

Deposition of the type slides: Holotype (KU/ZM/TP/SP-1) and paratype (KU/ZM/TP/SP-2) have been deposited in the Zoology Museum, Department of Zoology, University of Kashmir, Srinagar, J&K, India.

Parasite description: Two pleomorphic forms: 'small' and 'large'. Body narrow and elongated with attenuated posterior and anterior ends. Few vacuoles and many deeply stained granules in cytoplasm. Nucleus generally oval but

may be spherical and located in the anterior part of body. Few, shallow waves of the undulating membrane and nucleus with no distinct karyosome in small forms; the large forms with prominent karyosome in nucleus. Dark staining prominent kinetoplast lies close to posterior end of body in all forms. Small forms possess spherical kinetoplast; In larger forms, the organelle may even be elongated. Single flagellum arises from dark stained kinetoplast on posterior side forming undulating membrane, extending along one side of body and continues as free flagellum anteriorly. Flagellum stains poorly as compared to cytoplasm. No evidence of dividing trypomastigotes.

Haematological parameters of infected and non-infected fish are given in Table 2. Hb Hct and red blood cell counts (RBC) showed a significant decrease ($p < 0.05$), while the white blood cell counts (WBC) showed a significant increase ($p < 0.05$) in the infected fish as compared to the non-infected. MCV and mean corpuscular haemoglobin (MCH) increased significantly ($p < 0.05$) in the infected fish, but no significant change ($p > 0.05$) was observed in the mean corpuscular haemoglobin concentration (MCHC) of the infected fish compared to non-infected ones.

Discussion

A dimorphic trypanosome species was discovered from the blood smears of *S. plagiostomus* inhabiting the river Jhelum in Kashmir. The only report of a piscine trypanosome from Kashmir has been that of *Trypanosoma mukasai* (Shahi et al. 2013). The species discovered in the present study differs from *T. mukasai* due to its dimorphic nature

and also the larger forms have lesser cell breadth (2.53 ± 0.47), nuclear length (3.39 ± 0.43), kinetoplast index (1.05 ± 0.01) and greater nuclear index (1.92 ± 0.28) than the reported *T. mukasai*. Morpho-taxonomic grounds are adequate for the description of a new species of trypanosome (Bennett 1989) however, the characters need to be significantly different and the new species has to be morphologically compared in depth to all the similar species and to other species in the same major group of hosts (Gupta 2006). The important morphological characters at the light microscopical level that are useful to distinguish different species include; size, PK region extent, position of nucleus (i.e. NI), and flagellar-lengths (Diamond 1965; Hoare 1972; Gupta 2006; Karlsbakk and Nylund 2006). Various dimorphic trypanosome species have been reported from around the world including India. Some of them include; large and small forms of *T. remarki* (Laveran and Mesnil 1907); short and slender forms of *T. gachuii* (Misra et al. 1973); slender and stumpy forms of *T. mukundi* (Raychaudhuri and Misra 1973); small and large forms of *T. aligaricus* (Gupta and Jairajpuri 1982) small and long forms of *T. aori* (Joshi 1982); small and large forms of *T. artii* (Gupta et al. 2002); minuta and magna forms of *T. sauli* (Gupta et al. 2006); small and large forms of *T. heteropneusti* (Gupta et al. 2006). A comparison of the size and morphometric characteristics of the present trypanosome species with the related species is presented in Table 1. The trypanosome species in the present study differed significantly from the related species in almost all morphometric and cytomorphic characters. Cell length (CL) of the present species is greater than *T. heteropneusti* and less than *T. aligaricus*. The present species also differs from *T. aori* in having lesser cell breadth (CB). The present species is comparable to *T. sauli* in its cell size, nuclear size and flagellar length, but differs from it in having a smaller kinetosome in the ‘small’ forms than the corresponding ‘minuta’ forms of *T. sauli*. Presence of vacuoles in the cytoplasm of the present species also sets it apart from *T. sauli*. Kinetoplast indices (KI) of the present species, *T. sauli* and *T. heteropneusti* show a marked difference. The present species also differs from *T. heteropneusti*, *T. aori* and *T. sauli* in having a higher flagellar index (FI). Moreover, there are no previous records of *Trypanosoma* spp. infecting *S. plagiostomus*. It is therefore, proposed that the parasite discovered from the blood of *S. plagiostomus* Heckel, collected from the river Jhelum in Kashmir be designated as a new species and accordingly, the name *T. kashmirensis* n. sp is proposed.

Alteration of host physiology is one of the manifestations of parasitization by blood parasites that depend on the blood of their hosts (von Brand 1973). In aquaculture, haematology is gaining importance because of the ease and reliability in monitoring the health status of cultured

species (Hrubec et al. 2000). Blood haematological investigations have proved valuable for fisheries biologists for quick detection of changes in fish health and these changes may precede changes in fish behavior and visible lesions (Kavitha et al. 2012). One of the pathological impacts related to piscine trypanosomiasis is anemia (Becker 1977; Khan 1985; Lom 1979; Islam and Woo 1991; Gupta and Gupta 2010), which is directly related to parasitaemia (Khan 1977; Khan et al. 1980; Islam and Woo 1991) in the blood. Infections may be chronic or acute and severe parasitaemia can result in anaemia and death (Bruno et al. 2006). The blood cells of fish are produced in hemopoietic tissues located in the spleen and kidney (Heath 1995) and any impact on these hemopoietic organs would lead to alteration of haematological parameters. Dykova and Lom (1979) found severe histopathological changes in hemopoietic organs (i.e. spleen and kidney) in goldfish infected with *T. danilewskyi* and consequently, suggested that anemia in fish was due to the malfunctioning of the spleen. Khan (1985) also observed severe anemia in *T. murmanensis* infected fishes and associated it with an inactive rather than an impaired hemopoietic system. According to Islam and Woo (1991) there are two factors which contribute to anemia in *Trypanosoma* infected fish hemolytic factor (hemolysin) present in the secretory/excretory product of the trypanosome and the hemodilution.

In the present study, a significant decrease in Hb, Hct, and RBC counts was observed in trypanosome-infected fishes. These results are in agreement with Joshi and Dabral (1981) in *T. maguri*-infected *Heteropneustes fossilis*. These workers found a significant decrease in Hb concentration (31–44 %) and RBC values (30–45 %) in the infected fishes. The decrease in Hb content probably may be brought about by the inhibition of Hb synthesis (Gupta and Gupta 2010) or due to the poor mobilization of Hb from the spleen and other hemopoietic organs (Scott and Rogers 1981), which leads to impairment of the overall oxygen transport ability of the blood to various tissues, resulting in decrease of RBC number through haemolysis (Larsson et al. 1985; Ahmad et al. 1995; Atamanalp and Yanik 2003; Harikrishnan et al. 2012). Accelerated erythrocyte lysis due to parasitic stress leads to a reduction in Hct value (Celik and Aydin 2006). The blood Hct rates in marine fish infested with *T. murmanensis* was found to decrease (Khan 1987). A reduction in Hb and Hct has also been reported by the infections of *T. vittati* in *Mystus vittatus* and *T. maguri* in *C. batrachus* (Tandon and Joshi 1973), *T. batrachi* in *C. batrachus* (Gupta and Gupta 1985, 2010), *T. aligaricus* in *Channa punctatus* (Gupta and Gupta 1985), *Trypanosoma* sp. in hybrid *C. macrocephalus* x *C. gariepinus* (Supamattaya et al. 2005), *T. danilewskyi* in *C. batrachus* (Saha et al. 2007).

The fluctuation in MCV and MCH values in the infected fishes observed in the present study clearly shows that the concentration of Hb in the RBCs was altered in parasite infected fishes, indicating macrocytic hypochromic anemia. This kind of anemia is classified as regenerative with large and young RBCs which are not fully haemoglobinized (Tvedten 2010) and has been reported in *Trypanosoma*-infected fishes (Tandon and Joshi 1973; Gupta and Gupta 2010). Leucocytes play an important role in protecting fish body and preventing diseases (Qiang et al. 2013). These cells orchestrate the initial line of defense against pathogens (Stoskopf 1993). It is well recognized that leukocyte cells are normally lower in healthy fish while, under stress (due to infection), the number of cells increases and hence, WBC count can be used as a significant indicator of health status in fish (Roberts 1978; Hayatbakhsh et al. 2013). In the present study, the total leucocyte count showed a significant increase in the infected fishes, which has also been reported by Joshi and Tandon (1980); Gupta and Gupta (1985) in *Trypanosoma* infected fishes.

In summary, this paper reports the first record of *Trypanosoma* infection in snow barbell, *S. plagiostomus* Heckel from Kashmir, which was found acting as host to a dimorphic trypanosome species. The geographical location, morphometrics, cytological peculiarities, host status and comparative study of this dimorphic trypanosome puts it in contrast against other related species. Therefore, it is proposed to consider it as a distinct species and as such, the name *T. kashmirensis* n. sp. is proposed for it. The haematological values of parasitized fish showed significant variations and therefore could provide useful parameters for evaluating the physiological effects of *Trypanosoma* infestation, but the application of these are subjected to detailed laboratory study, in order to validate them as established biomarkers.

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References

- Ahmad F, Ali SS, Shakoori AR (1995) Sublethal effects of danitor (fenpropathrin), a synthetic pyrethroid on fresh water Chinese grass carp, *Ctenopharyngodon idella*. *Folia Biol* 43:151–159
- APHA (1992) Standard methods for the examination of water and wastewater, 18th edn. American Public Health Association, Washington
- Atamanalp M, Yanik T (2003) Alterations in hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. *Turk J Vet Anim Sci* 27:1213–1217
- Becker CD (1977) Flagellate parasites of fish. In: Kreier JP (ed) *Parasitic Protozoa*, vol 1. Academic Press, Amsterdam, pp 358–416
- Bennett G (1989) The use of morphotaxonomy in describing new species of Haematozoa. *J Bengal Nat Hist Soc* 8:3–4
- Brassard P, Rau ME, Curtis MA (1982) Parasite-induced susceptibility to predation in diplostomiasis. *Parasitology* 85:495–501
- Bruno DW, Nowak B, Elliott DG (2006) Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. *Dis Aquat Org* 70:1–36
- Celik ES, Aydin S (2006) Effect of *Trachelobdella lubrica* (Hirudinea: Piscicolidae) on biochemical and haematological characteristics of black scorpion fish (*Scorpaena porcus*, Linnaeus 1758). *Fish Physiol Biochem* 32:255–260
- Clauss TM, Dove AD, Arnold JE (2008) Hematologic disorders of fish. *Vet Clin North Am Exot Anim Pract* 11:445–462
- Crowden AE, Boom DM (1980) Effects of the eyefluke, *Diplostomum spathaceum*, on the behavior of dace (*Leuciscus leuciscus*). *Anim Behav* 28:287–294
- Dacie S, Lewis S (1991) Practical haematology, 7th edn. Churchill Livingstone, London
- Davies AJ, Gibson W, Ferris V, Basson L, Smit NJ (2005) Two genotypic groups of morphologically similar fish trypanosomes from the Okavango Delta, Botswana. *Dis Aquat Org* 66:215–220
- Diamond LS (1965) A study of the morphology, biology and taxonomy of the trypanosomes of Anura. *Wildl Dis* 44:1–82
- Drabkin DL (1946) Spectrometric studies. XIV. The crystallographic and optimal properties of the hemoglobin of man in comparison with those of other species. *J Biol Chem* 164:703–723
- Dykova I, Lom J (1979) Histopathological changes in *Trypanosoma danilewskyi* Laveran & Mesnil, 1904 and *Trypanoplasma borelli* Laveran & Mesnil, 1902 infections of goldfish. *Carassius auratus* (L.). *J Fish Dis* 2:381–390
- FAO (2005) Nutritional elements of fish. <http://www.fao.org/fishery/topic/12319/en>. Accessed 25 July 2013
- FAO (2012) The state of world fisheries and aquaculture. Fisheries and Aquaculture Department, FAO
- Gupta N (2006) Historical review of piscine trypanosomiasis and survey of Indian *Trypanosoma*. *J Parasit Dis* 30:101–115
- Gupta N, Gupta DK (1985) Haematological changes due to *Trypanosoma batrachi* and *T. aligaricus* infection in two fresh water teleosts. *Angew Parasitol* 26:193–196
- Gupta DK, Gupta N (2010) A Hemogram of *Clarias batrachus* parasitized by two species of hemoflagellates (*Trypanosoma batrachi* Qadri, 1962 and *Trypanoplasma haematialis* n. sp.) as an indicator of infectivity. *Rev Fish Sci* 18:177–182
- Gupta N, Gupta DK (2012) Erythropenia in piscine trypanosomiasis. *Trends Parasitol* 1:1–6
- Gupta N, Jairajpuri DS (1982) *Trypanosoma aligaricus* n. sp. from the freshwater murrel. *Ophiocephalus punctatus* Bloch Arch Protistenkd 125:109–114
- Gupta DK, Gupta N, Gupta A (2002) Dimorphism of *Trypanosoma* in *Heteropneustes fossilis* and its role in inducing haematological aberrations. *Flora Fauna* 8:3–6
- Gupta DK, Gupta N, Gangwar R (2006) Two new species of *Trypanosoma* from freshwater fish (*Heteropneustes fossilis* and *Channa punctatus*) from Bareilly, India. *Parasit Dis* 30:58–63
- Gupta DK, Gupta N, Yadav P (2003) *Trypanosoma piscidium* n. sp. and its role in inducing anemia in *Colisa fasciatus*. In: Pandey BN, Choudhary RK, Singh BK (eds) Biodiversity conservation, environmental pollution and ecology, vol II. APH Publishing Corporation, New Delhi, pp 127–133
- Harikrishnan R, Kim JS, Kim MC, Balasundaram C, Heo MS (2012) Pomegranate enriched diet enhances the hematology, innate immune response, and disease resistance in olive flounder against *Philasterides dicentrarchi*. *Vet Parasitol* 187:147–156

- Hayatbakhsh MR, Khara H, Movahed R, Sayadborani M, Rohi JD, Ahmadnezhad M, Rad AS (2013) Haematological characteristics associated with parasitism in bream. *J Parasit Dis, Abramis brama orientalis*. doi:10.1007/s12639-013-0256-y
- Heath AG (1995) *Water Pollution and Fish Physiology*. CRC Press, Boca Raton
- Hoare CA (1972) *The trypanosomes of mammals: A geological monograph*. Blackwell Publications, Oxford
- Hoare CA, Wallace FG (1966) Developmental stages of trypanosomatid flagellates: a new terminology. *Nature* 212:1385–1386
- Hrubec TC, Cardinale JL, Smith SA (2000) Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). *Vet Clin Pathol* 29:7–12
- Islam AKN, Woo PT (1991) Anemia and its mechanism in goldfish *Carassius auratus* infected with *Trypanosoma danilewskyi*. *Dis Aquat Org* 11:37–43
- Joshi BD (1982) Three new species of trypanosomes from freshwater teleosts (Pisces). *Proc Indian Acad Sci* 91:397–406
- Joshi BD, Dabral R (1981) Some haematological changes in a freshwater catfish *Heteropneustes fossilis*, infected with the trypanosome, *Trypanosoma maguri*. *Proc Anim Sci* 90:295–301
- Joshi BD, Tandon RS (1980) Effects of trypanosome infection on some haematologic values in ten species of fresh water teleosts. *J Anim Morphol Physiol* 27:39–44
- Karlsbakk E, Nylund A (2006) Trypanosomes infecting cod *Gadus morhua* L. in the North Atlantic: a resurrection of *Trypanosoma pleuronectidium* Robertson, 1906 and delimitation of *T. murmanense* Nikitin, 1927 (emend.), with a review of other trypanosomes from North Atlantic and Mediterranean teleosts. *Syst Parasitol* 65:175–203
- Kavitha C, Ramesh M, Kumaran SS, Lakshmi SA (2012) Toxicity of *Moringa oleifera* seed extract on some hematological and biochemical profiles in a freshwater fish, *Cyprinus carpio*. *Exp Toxicol Pathol* 64:681–687
- Kennedy CR (1994) Foreword. In: Pike AW, Lewis JW (eds) *Parasitic diseases of fish*. Samara Publishers, Tresaith, pp 1–2
- Khan RA (1977) Blood changes in Atlantic cod (*Gadus morhua*) infected with *Trypanosoma murmanensis*. *J Fish Res Board Can* 34:2185–2192
- Khan RA (1985) Pathogenesis of *Trypanosoma murmanensis* in marine fish of the northwestern Atlantic following experimental transmission. *Can J Zool* 63:2141–2144
- Khan RA (1987) Effects of chronic exposure to petroleum hydrocarbons of two species of marine fish infected with a hemoprotozoan, *Trypanosoma murmanensis*. *Can J Zool* 65:2703–2709
- Khan RA, Barrett M, Campbell J (1980) *Trypanosoma murmanensis*: its effect on the longhorn sculpin, *Myoxocephalus octodecemspinosus*. *J Wildl Dis* 16:359–361
- Kori-Siakpere O, Ubogu EO (2008) Sub-lethal haematological effects of zinc on the freshwater fish, *Heteroclaris* sp. (Osteichthyes: Clariidae). *Afr J Biotechnol* 7:2068–2073
- Kumaraguru AK, Beamish FWH, Woo PTK (1995) Impact of a pathogenic haemoflagellate, *Cryptobia salmositica* on the metabolism, and swimming performance of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis* 18:297–305
- Larsson A, Haux C, Sjobeck ML (1985) Fish physiology and metal pollution: results and experiences from laboratory and field studies. *Ecotoxicol Environ Safe* 9:250–281
- Laveran A, Mesnil F (1907) *Trypanosomes and trypanosomiasis*. Baillière, Tindall and Cox
- Lingard A (1904) A short account of the various Trypanosomata found to date in the blood of some of the lower animals and fish. *Indian Med Gaz* 39:445
- Lom J (1979) Biology of trypanosomes and trypanoplasms of fish. In: Lumsden WHR, Evans DA (eds) *Biology of the Kinetoplastida*. Academic Press, London, pp 269–337
- Lom J, Dyková I (1992) Protozoan parasites of fishes. *Developments in aquaculture and fisheries science*, vol. 26. Elsevier
- Misra KK, Chandra AK, Choudhury A (1973) *Trypanosoma gachuii* n. sp. from a fresh water teleost fish *Ophicephalus gachua* Ham. *Arch Protistenkd* 115:18–21
- Nandi NC, Nandi R, Mandal AK (2002) Index catalogue and bibliography of protozoan parasites from Indian fishes. *Zoological Survey of India, Kolkata*, p 65
- Natt MP, Herrick CA (1952) A new blood diluent for counting erythrocytes and leukocytes of the chicken. *Poul Sci* 31:735–738
- O’Neal CC, Weirich CR (2001) Effects of low level salinity on production and haematological parameters of channel catfish (*Ictalurus punctatus*) reared in multi crop ponds. In: Book of abstract, aquaculture international triennial conference of world aquaculture society, Honolulu, pp 21–25
- Olufayo MO (2009) Hematological characteristics of *Clarias gariepinus* (Burchell 1822) juveniles exposed to *Derris elliptica* root powder. *Afr J Food Agric Nutr Dev* 9:920–933
- Padua SBD, Ishikawa MM, Satake F, Jeronimo GT, Pilarski F (2011) First record of *Trypanosoma* sp. (Protozoa: Kinetoplastida) in *tuvira* (*Gymnotus aff. inaequilabiatus*) in the Pantanal wetland, Mato Grosso do Sul State Brazil. *Rev Bras Parasitol Vet* 20:85–87
- Qiang J, Yang H, Wang H, Kpundeh MD, Xu P (2013) Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles, *Oreochromis niloticus* (L.) and mortality under *Streptococcus iniae* infection. *Fish Shellfish Immunol* 34:8–16
- Rainza-Paiva MJT, Ishikawa CM, Das Eiras AA, Felizardo NN (2000) Haematological analysis of ‘chara’ *Pseudoplatystoma fasciatum* in captivity. *Aqua* 2000. Responsible aquaculture in the new millennium. European Aquaculture Society Special Publication, France
- Raychaudhuri S, Misra KK (1973) Two new fish trypanosomes from India. *Arch Protistenkd*. 115:10–17
- Roberts RJ (1978) The pathophysiology and systemic pathology of teleosts. In: Roberts RJ (ed) *Fish Pathology*. Bailliere Tindall, London, pp 55–91
- Saha J, Saha NC, Mukhopadhyay TK, Midya T, Sarkar NK (2007) Trypanosome Infection and Some Associated Physiological Changes in an Indian Cat-fish, *Clarias batrachus* (Linnaeus). In: Kumar A (ed) *Disaster: ecology and environment*. Daya Publishing House, Delhi, pp 30–35
- Scott AL, Rogers WA (1981) Hematological effects of prolonged sublethal hypoxia on channel catfish *Ictalurus punctatus* (Rafinesque). *J Fish Biol* 18:591–601
- Shahi N, Yousuf AR, Rather MI, Ahmad F, Yaseen T (2013) First report of blood parasites in fishes from Kashmir and their effect on the haematological profile. *Open Vet J* 3:89–95
- Smit NJ, Van As JG, Davies AJ (2004) Fish trypanosomes from the Okavango Delta, Botswana. *Folia Parasitol* 51:299–303
- Stoskopf MK (1993) *Fish Medicine*. Saunders, Philadelphia
- Supamattaya K, Ruangsri J, Ruggamol R, Songpradit A, Bhuvanath S, Promkhunthong W (2005) Trypanosomiasis in hybrid catfish (*Clarias macrocephalus* x *Clarias gariepinus*) and other freshwater fishes. *Songklanakarin J Sci Technol* 27:321–332
- Tandon RS, Joshi BD (1973) Studies on the physiopathology of blood of freshwater fishes infected with two new forms of trypanosomes. *Z Wiss Zool* 185:207–221
- Tavares-Dias M, Moraes FR (2003) Haematological evaluation of *Tilapia rendalli* Boulenger, 1896 (Osteichthyes: Cichlidae) captured in a fee fishing farm in Franca, Sao Paulo state, Brazil. *Biosci J* 19:103–110
- Tvedten H (2010) Laboratory and clinical diagnosis of anemia. In: Weiss DJ, Wardrop KJ (eds) *Schalm’s Veterinary Hematology*, 6th edn. Blackwell Publishing Ltd, Ames, pp 152–161

- von Brand T (1973) Biochemistry of parasites, 2nd edn. Academic Press, London
- Williams HH, Jones A (1994) Parasitic worms of fish. Taylor and Francis, London
- Woo PTK (1969) The haematocrit centrifuge for the detection of trypanosomes in blood. *Can J Zool* 47:921–923
- Woo PTK (1995) Fish diseases and disorders protozoan and metazoan infections, vol I. CAB International, Oxon
- Woo PTK (2006) Diplomonadida (Phylum Parabasalia) and Kinetoplastea (Phylum Euglenozoa) In: Woo PTK (ed) Fish diseases and disorders, Vol. 1: Protozoan and metazoan infections, 2nd edition. CABI, Wallingfordpp, pp 46–115
- Zaragoza ODR, Rodriguez MH, Bückle-Ramirez LF (2008) Thermal stress effect on tilapia *Oreochromis mossambicus* (Pisces: Cichlidae) blood parameters. *Mar Freshw Behav Physiol* 41:79–89