

Three new species of haematozoans from freshwater teleosts (pisces)

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Abstract. Two new species of haematozoans, *Trypanosoma aori* (sp. nov.) and *Trypanoplasma mysti* (sp. nov.), were found harbouring the blood plasma of freshwater teleosts, *Mystus aor*, while *Trypanoplasma atti* (sp. nov.), was found in the plasma of another cat fish *Wallago attu*. The two hosts are new records for these parasites. All three species of the parasites described here showed characteristic polymorphism.

Keywords. Haematozoata; *Trypanosoma*; *Trypanoplasma*; blood; polymorphism.

1. Introduction

In the recent past quite a few new species of piscine haemoflagellate parasites have been described from various freshwater teleosts of India (Ray Chaudhuri and Misra 1973; Misra *et al* 1973; Tandon and Joshi 1973; Pandey and Pandey 1974; Mandal 1975, 1977, 1978, 1979 and Joshi 1976, 1978), besides the earlier reports (Lingard 1904, Demello and Valles 1936, Qadri 1955, 1962 and Hasan and Qasim 1962). In a recent paper Joshi (1979) reported occurrence of trypanosomes in thirteen species of freshwater teleosts of Lucknow. This paper describes three new species of haematozoan parasites from two freshwater teleosts viz. *Mystus aor* and *Wallago attu*.

2. Material and methods

Live specimens of *M. aor* and *W. attu* were obtained from river Gomati, transported to the laboratory, given rest for 12-14 hr in a large glass aquarium under laboratory conditions and then studied the blood smears, stained with Leishman and Wrights stains following the usual methods described earlier (Tandon and Joshi 1973 and Joshi 1978). Camera Lucida drawings were made of the parasites found in blood slides with precise details and measurements taken.

3. Observations

Histomorphological and morphometric studies made on the species of *Trypanosoma* and *Trypanoplasma* revealed the following characteristics, and accordingly

with the help of existing literature three new species of haemoflagellates/haematozoans are described here :

Parasite : *Trypanosoma aori* (sp. nov.)

Host : *Mystus aor*.

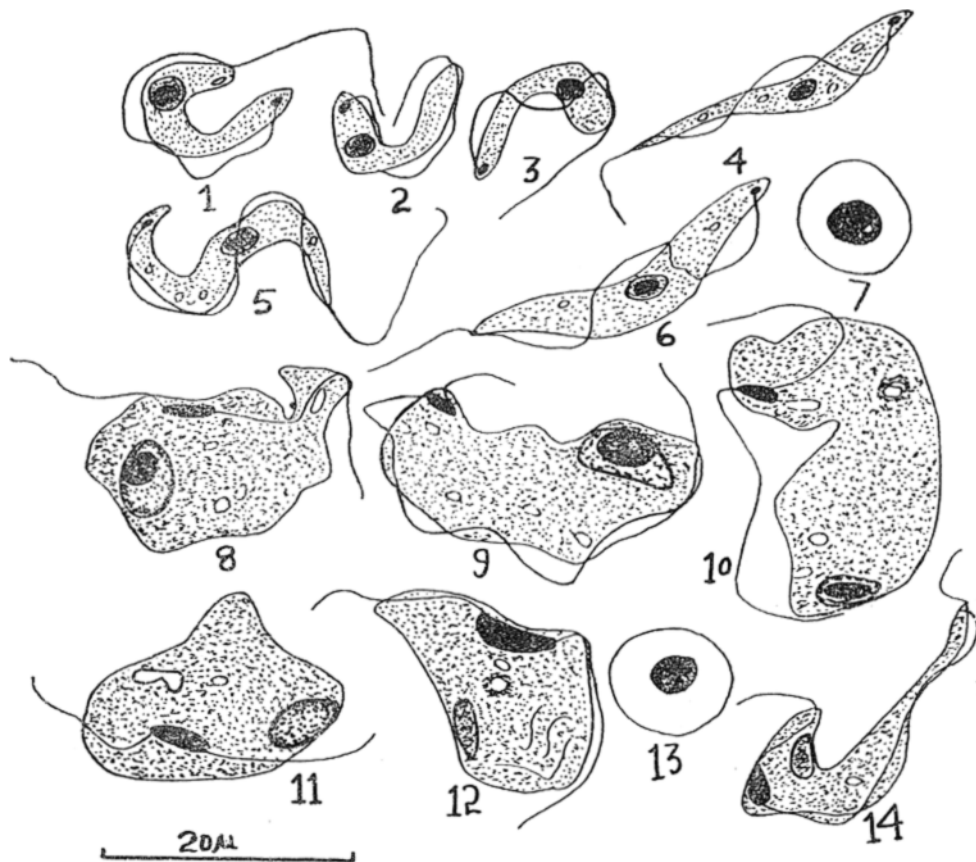
Location : Plasma of the host fish.

Locality : River Gomati, Lucknow.

Diagnosis and descriptions : (figures 1-6) table 1.

Body : Parasites were short (figures 1-3), elongated and partly stumpy. Out of many forms seen in three stained preparations, some typically large elongated forms were also found (figures 4-6). These forms mainly had both ends blunt or rounded, while few forms had pointed or beak shaped extremities.

Figures 7 and 13 are those of the RBC's of the host fishes to give a comparative idea of the blood cell size and the parasite.



Figures 1-14. 1-3. Small sized forms of *T. aori* (sp. nov.). 4-6. Large sized form of *T. aori*. 8, 9, and 10. *T. mysti* (sp. nov.) 7. RBC of *M. aor*, the host fish. 11, 12 and 14. Polymorphic forms of *T. atti* (sp. nov.). 13. RBC of *W. attu*, the host fish.

Cytoplasm : It stained bluish purple, with fine azurophilic dusty granules and granulation appeared denser in the smaller forms, than in the larger ones, which also showed vacuoles. Myonemes were not seen in either of the forms.

Nucleus : It was distinct in all the forms, situated towards either of the two extremities in smaller forms (figures 1-3) or almost in the centre (figures 4-6). In most of the forms, it was rounded or oval, with a distinct karyosome. Karyosome contained, more dense and hyperbasophilic contents than the surrounding karyoplasm.

Kinetoplast : It was present almost at the posterior terminal end and was rounded or slightly elongated.

Flagellum : It always arises from the inner end of the kinetoplast, runs towards the anterior end bordering the undulating membrane before being free at the anterior extremity. In most cases it took light basophilic stain. The free flagellum showed much variation in size.

Undulating membrane : This structure was conspicuously present in all elongated forms and was well differentiated from the body.

Remark : A distinct polymorphism existed, with high parasitemia and low instance of infection.

Parasite : *Trypanoplasma mysti* (sp. nov.)

Host : *Mystus aor.*

Location : Plasma of the host fish.

Locality : River Gomati, Lucknow.

Diagnosis and description : (figures 8, 9 and 10) table 2.

Body : The trypanoplasmid forms were stoutly elongated and irregularly curved (figures 8, 9 and 10). All forms showed conspicuous body width (table 1). Both ends of the parasite were blunt and wide.

Cytoplasm : The cytoplasm is densely packed with fine to coarse granules. It took deep bluish black stain. Vacuoles were frequently present and at places were dense, surrounded by cytoplasmic granules (figure 10).

Nucleus : There occurred marked variation in the shape, size and position of the nucleus of this new species of *Trypanoplasma*. In few forms it was mid-anterior (figure 8) and parallel to kinetoplast, while in others it was situated at either of the two ends (figures 9, 10), being quite away from the kinetoplast. The nucleus always showed a distinct and deeply stained karyosome, which occupied much space within the nucleus. Nuclear chromatin around the karyosome was thinly scattered. The karyosome always took a deep bluish black stain, whereas the nuclear chromatin was purple blue. The nuclear shape varied from ovoid (figure 8), reniform (figure 9) to drop shaped (figure 10).

Kinetoplast : Like that of the nucleus, it also revealed conspicuous variations in the shape, size and position. It was rod shaped in few forms (figures 8, 9) and rounded (figure 10) in others. It is situated either at extreme terminal end

Table 1. Morphometric comparison of *T. aori* (sp. nov.) with other trypanosomes described from the freshwater siluroide hos from the Indian subcontinent.

Particulars	Species	<i>T. bairrachi</i> (Qadri 1962)	<i>T. sacco-branchi</i> (1962)	<i>T. Mukundi</i> (Raychoudhri and Mishra 1973)	<i>T. baigulensis</i> (Pandey and Pandey 1978)	<i>T. maguri</i> (Tandon and Joshi 1973)	<i>T. vittati</i> (Joshi 1976)	<i>T. seenghali</i> (Joshi 1976)	<i>T. tandoni</i> (Mandal 1980)	<i>T. aori</i> (sp. nov.) Small form	<i>T. aori</i> (sp. nov.) Long form
1. Length of free flagellum		9-14*	11-15	4-6.5 ¹	4-4.5 ¹	4.2-21.0	6.5-14.0	8.5** (4.0-12.0)	14.5 (11.5-18.5)	18.2 (15.0-19.6)	11.0 (10-12.5)
2. Length of body		22-29	38-47	9-15 ² 18-23.5 24-28.5	5-5.6* 10.2-12.3 18.1-20.5	8.2-35.3	18.2-34.8	24.5 (17.8-28.0)	23.5 (20.0-25.0)	17.6 (14.8-20.2)	32.6 (29.5-30.0)
3. Total body length		31-33	38-47	22-28.5 32.5-43.5	12.1-14.5 20.3-22.5	26.1-53.8	29.7-47.4	33.0 (27.5-38.0)	38.0 —	35.8 (30.0-39.8)	43.6 (38.5-48.0)
4. Width of body at the centre of nucleus		1.5-3.5	1-1.5	1.9-2.5 2.0-2.5	2-2.1 1.8-2.5	1.1-3.0	1.3-2.3	1.71 (1.2-2.5)	1.5 (1.3-1.8)	3.0 (2.6-4.2)	4.6 (4.0-5.2)
5. Width of undulating membrane		×	×	×	0.8-1.0 0.6-1.0	0.2-1.8	0.6-1.4	1.4 (0.9-2.0)	0.6 (0.3-0.95)	1.4 (1.0-2.5)	1.9 (1.2-2.8)
6. Long axis of the nucleus		2-3	2.75-3.2	2.25-3.75 2.25-3.75	1.1-1.6 1.0-1.5	1.2-5.0	1.9-4.0	2.75 (1.5-3.5)	2.5 (2.25-3.0)	1.9 (1.3-3.2)	3.1 (2.2-4.4)
7. Short axis of the nucleus		1-1.5	0.7-1.0	1.75-2.25 1.75-2.25	1.2-1.5 1.7-2.0	0.7-1.6	0.7-1.8	1.7 (1.2-2.3)	0.75 (0.5-1.0)	1.5 (1.0-2.5)	1.4 (0.9-2.2)

8. Distance of nucleus from a flagellar end	x	x	x	x	x	6-8 7-10	3-2-15.6	8.0-14.0	11.4 (8.5-15.0)	10.0 (9-11)	5.1 (3.2-11.0) 1.6	12.0 (9.0-14.2) 1.2
9. Length of karyosome	x	x	x	x	x	x	x	x	x	x	(0.6-2.0)	(0.9-1.5)
10. Width of karyosome	x	x	x	x	x	x	x	x	x	x	1.2 (0.9-1.9)	0.8 (0.6-1.2)
11. Diameter of kinetoplast	1.1	0.75-1.0	0.75	x	x	x	x	x	x	x
12. Length of kinetoplast	x	x	x	x	x	x	0.4-1.6	0.9-1.7	0.9 (0.3-1.2)	1.5 (1.0-1.75)	0.9 (0.6-1.2)	1.0 (0.6-1.3)
13. Width of kinetoplast	x	x	x	x	x	0.2-0.6	0.3-1.8	0.8-1.8	0.5 (0.3-1.2)	0.76 (0.5-1.0)	0.65 (0.4-0.9)	0.4 (0.2-0.9)
14. Post kinetoplast distance	x	x	x	x	x	0.2-0.8	0-1.2	1.1-2.0	1.20 (0.4-2.0)	1.5 (0.5-2.0)	1.1 (0.7-1.3)	1.4 (0.8-1.8)

Values are quoted as presented by their authors. These are either ranges* or mean and range**. Numbers 1 and 2 denote the size of the polymorphic forms. All measurements are in microns. (x) cross signs denote the values not given by the author.

Table 2. Comparative morphometric characteristics of the new species and allied species of the *Trypanoplasma* (*Cryptobia*).

Particulars	Species Author	<i>C. borreli</i> (Meshil 1901)	<i>C. salmositica</i> (Katz 1951)	<i>C. cataractai</i> (Putz 1972)	<i>C. indica</i> (Mandal 1979)	<i>T. mysti</i> (sps. nov.)	<i>T. atti</i> (sps. nov.) longform	Ovoid form
Length of anterior flagellum		×	16.5	(9.6-13.2)	25.0 (21.0-30.0)	13.8 (10.0-22.0)	9.6 (7.5-12.0)	7.5 (6.0-9.0)
Length of posterior flagellum		×	8.96	×	10.5 (9.0-12.0)	11.2 (8.0-15.0)	8.3 (7.0-10.5)	9.0 (6.0-15.0)
Body length		20-25	14.9	17.0 (14.17-18.9)	28.5 (25.0-30.5)	28.2 (24.0-34.2)	35.3 (26.0-50.0)	22.2 (18.0-27.0)
Body width		3-4	2.0-9.6	2.0 (1.5-2.2)	8.5 (6.0-10.5)	10.9 (8.8-13.0)	6.8 (5.0-8.0)	12.0 (9.0-14.0)
Length of the nucleus		×	3.5	×	7.5	6.5 (5.0-8.0)	5.5 (3.5-7.0)	4.9 (4.0-6.0)
Width of the nucleus		×	1.5	×	5.0	3.4 (2.3-4.0)	1.9 (1.0-3.0)	2.9 (1.5-3.5)
Length of the kinetoplast		×	4.58	5-6	5.0	1.3 (0.5-2.5)	4.0 (3.0-5.0)	5.5 (4.5-9.4)
Width of the kinetoplast		×	0.78	0.75-2.0	1.5	0.9 (0.4-1.5)	1.4 (0.9-2.0)	2.5 (1.8-3.7)
Length of karyosome		×	×	×	×	3.0 (2.2-4.5)	—	—
Width of karyosome		×	×	×	×	3.0 (1.0-3.2)	—	—

All values are in microns and are mean and range: Sign of cross (×) denotes the value not given by the author and a dash (—) means absence of the organoid.

Note: All measurements for the new species of *Trypanoplasma*, described here, are for 7 observations each.

(figure 9) or nearly towards the central periphery of the cell. It always stained deep bluish black.

Flagellum : All the forms possessed two free flagella, which arise from the two ends of the same kinetoplast. Anterior flagellum was usually larger than the posterior (table 2). The posterior flagellum either runs through the cytosome (figure 8) or borders the undulating membrane (figure 9), before being freed at the post extremity.

Undulating membrane : It was not seen in few forms (figure 8), but distinctly present in others (figures 9, 10).

Remark : This newly described species of *Trypanoplasma aori* was found harbouring the same specimen of the host species, *M. aor*, to which the *T. aori* (sp. nov.) harboured. The host species thus showed a multispecies parasitemia of high intensity. Only one specimen of the host fish, out of 40 observed was found to be parasitized by these haematozoans (Joshi 1979).

Parasite : *Trypanoplasma atti* (sp. nov.)

Host : *Wallago attu*

Location : Plasma of the host fish

Locality : River Gomati, Lucknow.

Diagnosis and descriptions : (figures 11, 12 and 14).

Body : The unicellular body showed marked variations, with characteristic undulations (figure 14), while few others were ovo-triangular (figures 11, 12), with irregular shapes, hence conspicuous differences were noted in body size (table 2).

Cytoplasm : It was homogeneous and densely filled with coarse cytoplasmic granules. The cytoplasmic contents took most hyperbasophilic stain than in any other form described. Vacuoles were present in almost all forms, besides myonemic striations were also seen in few forms (figure 12).

Nucleus : It was short, cylindrical or rod like (figures 11, 12), reniform or ovoid (figure 14). Karyosome was not seen in any form of this species. Nuclear chromatin loosely distributed within the karyoplasm which stained deep purple blue (figures 11, 12, 14) to light reddish purple.

Kinetoplast : It was larger in size than in *T. mysti* (sp. nov.) described above (table 2), and was usually situated towards either of the extremities within the cell (figures 11, 14). It always stained bluish black.

Flagellum : One anterior and one posterior flagella were always present in all forms. In few forms anterior flagellum was longer (figures 11, 12) while in others, posterior (figure 14). In some forms both the flagella free abruptly, after traversing through the cytosome (figures 11 and 14), while in others it borders the outer margin of the cell or the undulating membrane (figure 12) and then frees.

Undulating membrane : It was present in few forms (figure 14) and not distinct in others (figures 11, 12).

Remark : Two specimens of the host fish out of 65 observed were found harbouring this parasite.

4. Discussion

Despite the fact that the above description is characteristic to the new species of the haematozoans described, the problem of new speciation remains complicated for the haemoflagellates from fish, as also encountered earlier by various authors (Baker 1960 ; Becker 1970 ; Putz 1972 ; Joshi 1978 and Mandal 1979). The problem becomes more complicated when a particular species of these haematozoan parasites show a great degree of polymorphism (Laired 1948, 1951 ; Tandon and Joshi 1973 and Joshi 1978). Besides, experimental studies have also revealed that many of these haematozoans are euryhostpatalic (Becker 1977). Further, recently Froes *et al* (1978, 1979) and Grogl *et al* (1980) have described six and one new species of the trypanosomes, respectively, from seven new host fish. They have also used the same criteria of species specificity and varying morphometric characteristics in all cases to create new speciation.

In the present case *T. aori* (sp. nov.) is not only different in having a new host fish, hitherto not described, but also in various cytomorphological and meristic characteristics. A high degree of polymorphism was also evident. The morphometric differences from the twentytwo species of the trypanosomes described earlier from the Indian freshwater teleosts viz, four species by Qadri (1955, 1962), one by Hasan and Qasim (1962), one by Misra *et al* (1973), two by Tandon and Joshi (1973), two by Ray-Chaudhury and Misra (1973), one by Pandey and Pandey (1964), seven by Mandal (1975, 1977, 1978 and 1979) and four by Joshi (1976 and 1978). The new species, *T. aori* is also conspicuously different from many other forms described from various freshwater host species by Dutton *et al* (1906), Hoare (1932), Baker (1960), Smirnova (1970) and Abolarin (1970). The degree of polymorphism encountered here is well comparable with those described by Laired (1952) for several species of trypanosomes.

Table I provides a comparative account of meristic morphological characteristics of eight species of trypanosome described from the siluroid hosts inhabiting the freshwater realms of the Indian subcontinent. Interestingly, when linear overlaps of all these species including the new species described here, are compared (as suggested by Mayr 1969), then at least 10-30% of all the measurements given for a species are overlapped by one or the others. However, despite these facts, the present new species *T. aori*, described here, have two major differences than the others described earlier. These are (i) small forms are most characteristic in appearance (acquiring a twisted grub-like structure) and (ii) these forms have usually maximum body width at the centre of the nucleus. Besides, the flagellar length, post nuclear distance, presence of the karyosome within the nucleus and comparatively blunt posterior extremity are its species characteristics.

Two new species of trypanoplasma viz., *T. mysti* and *T. atti*, differed not only from *C. indica* (Mandal 1979), described from *M. vittatus*, but also from related species like *C. borsoli* (Laveran and Mesnil 1901), *C. salmositica* (Katz 1951) and *C. cataractae* (Putz 1972), in almost all morphometric and cytomorphic character. It is important to mention that till recently the diflagellate haemotozoans

harbouring in the blood stream of the fishes were described under the genus *Cryptobia* (viz., Katz 1951) and since recently it has been resolved that all the diflagellate haematozoans be described under the generic name of *Trypanoplasma*, as pointed out by Woo (1979).

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