

TAXONOMICAL ACCOUNTS OF PROTOZOAN PARASITES OF CLIMBING PERCH, ANABAS TESTUDINEUS IN BANGLADESH

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Abstract: The study was conducted to identify the protozoan parasites in a freshwater indigenous air breathing fish, *Anabas testudineus*. The host fish was collected during mid of the April 2018 to end of the March 2019 from freshwater bodies of Mymensingh, Kishoregonj, Faridpur, Jashore, Manikganj and Bogura districts of Bangladesh. Five species of phylum myxozoa namely *Henneguya gigas*, *Henneguya mystusia*, *Henneguyaa cerinae*, *Henneguya periophthalmusi* and *Myxidium* sp., three species of phylum ciliophora namely *Amphileptus disciformis*, *Epistylis woffi* and *Trichodina anabasi* and one species of phylum mastigophora namely *Trypanosoma anabasi* were identified in *A. testudineus*. Myxozoans (97.55%) were clearly dominant group than ciliophorans (2.45%).

Key words: Protozoa, Ecto-parasite, Taxonomy, *Anabas testudineus*, Bangladesh

INTRODUCTION

Fish and fishery resources are considered to be one of the most important economic sectors of Bangladesh. Particularly edible fishes contribute to the promotion of national health as being a vital source of nutrition along with maintaining a considerable contribution in trade and economy of our nation. Not only in our country but also in many other countries of the tropics and subtropics it plays a vital role in the proteinaceous diet of mass population where malnutrition is severely prevalent (Alune and Andrew 1996). As the human population is inevitably increasing, the demand for fish protein is also towards rising. In recent times, there has been tremendous increase in the development of fish farming and aquaculture which attributed to the increased demand for the affordable animal protein in the tropics (Davies *et al.* 2006). With the increasing interests in aquaculture, parasitic infestations are becoming threats for fish health management and aquatic crop production throughout the world. As both the wild and culture fishes are likely to be infested with parasites

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that can cause a considerable deterioration in the production of aquaculture; however significant damage or mortality cases are less reported. This may be largely due to the fact that this dimension is nearly unexplored. Therefore it is an essential area for proper attention to be given by the scientists for sustainable aquaculture production (Tavares-Dias *et al.* 2014).

A remarkable number of fish parasites are belonging to Sub-kingdom protozoa that can act as both ecto- and endo-parasite. Protozoan parasites cause serious losses in cultured fishes as well as in wild fishes and their pathological damage such as lesions, inflammations, growth retardation and weight loss; those generally deteriorate the market value of the fish resulting production loss. Protozoan parasites are known to infect several groups of fishes since many years and cause great damage to their host fish.

Anabas testudineus is reported to be infected with various groups of parasites along with protozoans in several studies. *A. testudineus* is a small grayish green colored, freshwater indigenous air breathing fish popularly known as climbing perch that can live without water for days and so were found alive on tree tops and hence the name as climbing perch. Sometimes they remain buried under the mud during dry season (Rahman 1989). It is very popular edible fish species with standard nutrient content. A considerable numbers of studies have been done on the metazoan parasites of *A. testudineus* in Bangladesh (Basirullah 1973, Ahmed and Begum 1978, Ahmed 1981, Banerjee and Chandra 1993, Akther 1995, Akhter *et al.* 2018, Parveen *et al.* 2006, Ghani *et al.* 2014 and Bhuiyan *et al.* 2014). But a little work has been found related to the protozoan infestation in *A. testudineus* (Anon 1993, Sanaullah 1996, Asmat *et al.* 2003, Kibria *et al.* 2011). Proposed research was an attempt to analyse the taxonomy of protozoan parasites to create a base line data of protozoan parasites of the wild fishes of *A. testudineus* in Bangladesh.

MATERIAL AND METHODS

Selection of species: *Anabas testudineus* (Bloch, 1792) is a fairly common fish species in Bangladesh that was selected as host species for conducting this study. A special characteristic of the fish is that it has additional respiratory organs, other than gills, that is known as accessory respiratory organs and by which it can receive oxygen directly from air and can survive long time in water with less oxygen or even without water. Simultaneously, our concerned protozoan parasites are very sensitive and cannot survive so prolonged except live fishes. This is the reason to select host fish with protracted survival capacity having accessory respiratory organ for collecting and transporting it from distant sampling place.

Collection of host sample: According to the experimental design of the research, a total of 473 host fish species, *Anabas testudineus* were collected alive from the freshwater bodies of Kishoreganj (Kuliar char-24°10'40" N, 90°50'57" E and Pakundia-24°30'07"N, 90°67'71"E), Mymensingh (Ishawrganj-24°41'16" N, 90°35'58" E and Trishal- 24°57'18"N, 90°43'84"E), Faridpur (Modhukhali-23°32'50"N, 89°31'22"E and Boalmari-23°44'04"N, 89°66'84"E), Jashore (Purondorpur, Jhikorgacha upazila-23°5'51" N, 89°5'53" E and Monirampur-22°59'32"N, 89°11'53"E), Manikganj (Singair-23°81'45"N, 90°12'47"E and Ghior-23°93'74"N, 89°86'05"E) and Bogura (Sherpur-24°68'21"N, 89°41'47"E and Sadar-24°87'45"N, 89°38'34"E) with the help of fishermen during mid of the April 2018 to end of the March 2019. Sample size of fish collected from each area was not sharply equal.

Sample preparation: The fish were examined as soon as possible after capture. Immediately after collection, the external surface of the fish was observed carefully using a magnifying glass. External surface of the fish were examined and recorded for any abnormalities. After collecting the samples, their total length and weight were measured. Evidences were collected from the body slime, gill slime and blood of host fish which are the best suited micro-habited for protozoan parasites to get harbor. Smears of body slime, gill slime and blood were made on glass slides on the spot and fixed them in ethanol for further observation in the laboratory.

Klein's dry silver impregnation method: Klein's dry silver impregnation method was used for staining mobile peritrichs and other ciliates from the surface of fish. Mucous was scraped gently off gills and skin with a scalpel, spread thinly on a grease-free slide, and dry rapidly. The slide was covered with a 2% aqueous solution of silver nitrate (AgNO_3) for 8 min. After that they were rinsed thoroughly with distilled water and were placed facing up in a dish of distilled water and expose to bright sunlight for 1-2 hours. Finally they were allowed to dry and mount with a neutral medium, Canada balsam.

Giemsa's stain after acid hydrolysis: To detect the parasites in blood sample, the slides were stained using Giemsa stain and cover slipped by DPX mountant. During this process smears were fixed in Schaudinn's fluid and rinsed well in distilled water. After that they were hydrolysed for 8 min in 1N HCL at >60°C. Again they were rinsed for several times in distilled water and stained with stocked Giemsa's stain (diluted 1:20 with water at pH 7.0-7.2) for about 20 min and rinsed with tap water. Then they were allowed to dry directly and finally mounted with a neutral medium, Canada balsam. Giemsa's stain technique was used for rapid demonstration of nuclei in ciliates and in microsporidian spores.

Count: The slides were observed under microscope to note the presence or absence of protozoan parasites. Counts of parasites found in selective organs

were recorded. The numbers of observed parasites were counted for statistical analysis and microscopic photographs were captured for the identification of species with the help of 10-megapixel digital camera. Protozoans were identified according to the description of Lom and Dyková (1992), Sarkar (1985), Eiras (2002), Kalavati and Nandi (2007), Bashė and Abdullah (2010), Kibria *et al.* (2010). Some parasites could not be identified up to species level because these were not got matched with any of the available published description.

RESULTS AND DISCUSSION

A total of 473 specimens of host fish, named *Anabas testudineus* were collected from several districts and thoroughly examined to get the existence of protozoan parasites in all possible microhabitat (skin, gill and fin) of the host. The prevalence of protozoan parasites found in the present study was relatively higher than any other previous records in Bangladesh. Overall prevalence of the host, *A. testudineus* was found 66.60% (Table 1). A total of 9 species of parasite under three groups of protozoa were recorded during the study from *A. testudineus*. Among 9 parasite species in *A. testudineus*, there were 5 species under the phylum myxozoa, 3 species under the phylum ciliophora and 1 species under the phylum mastigophora (Table 2).

Table 1. Community structure of protozoan parasites in *A. testudineus*

Factors	Number/ Percentage
Number of fish examined	473
Number of fish infected	315
Prevalence of infestation	66.60%
Total number of parasite individuals recorded	2805
Total number of parasite species	9
Mean intensity \pm SD	8.90 \pm 13.87
Prevalence of infestation in male host	74.35%
Prevalence of infestation in female host	61.35%
Intensity of infestation in male host	9.17 \pm 14.19
Intensity of infestation in female host	8.69 \pm 7.49

The identification with a brief description of each species followed by remarks has been presented as follows:

Henneguya gigas (Chen and Hsieh, 1960)

Description: Cysts of *Henneguya gigas* were found in the gills of host fish. Mature spores are rounded in shape with a curved caudal projection. The polar capsules were elongated and equal in size and found to occupy less than half of the spore body. Spores were 16.1 (13.2-21.0) μm in length and 6.2 (5.9-6.6) μm

in width. The length of the shorter tail was found 6.7(4.4-10.3) μm which extended behind the spore. The overall length was recorded 16.1 (13.2-21.0) μm in total length. Two equal capsules were observed pyriform that was tapering toward their anterior end and occupying nearly half of the spore (Plate. 1a).

Table 2. List of protozoan parasites encountered during this study

Host	Group	Parasites
<i>Anabus testudineus</i>	Myxozoa	<i>Henneguy agigas</i> <i>Henneguya acerinae</i> <i>Henneguyamystusia</i> <i>Henneguya periophthalmusi</i> <i>Myxidium sp.</i>
	Ciliophora	<i>Amphileptus disciformis</i> <i>Epistylis lwoffii</i> <i>Trichodina anabasi</i>
	Mastigophora	<i>Trypanosoma anabasi</i>

Systemic position

Phylum: Myxozoa, Class: Myxosporea, Order: Bivalvulida, Family: Myxobolidae,
Genus: *Henneguya*, Species: *H. gigas*

Microhabitat: Gill

Macrohabitat: Faridpur, Kishoreganj, Mymensingh and Jashore

Remarks: This parasite was first reported by Chen and Hsieh (1960) from the gill, ovary and intestine of *Channa argus* in China. The same myxophoridian species was observed in the present study from the body slime of *A. testudineus*. This is the new host and locality for this parasite to be recorded.

Henneguy aacerinae (Schroder, 1906)

Description: *Henneguya acerinae* is generally longer than most other *Henneguya* species with a wide spore body. Mature spore body was found elongated ranging from 20-22 μm in valvular view with bluntly pointed narrow anterior end in the present host. Posterior end was narrowly rounded. Spore body was prolonged by two very filiform, narrow and long extensions; total length was measured 70-82 μm . Caudal appendages were separated from each other and 50-60 μm long. Two pyriform to straight short polar capsules were visible in the anterior part of spore. Polar capsules were elongated (length 8-9 μm , width 2-3 μm) and of equal size. Polar filaments were thin and coiled that were aligned perpendicularly to longitudinal axis of capsule. The length of the polar filament was 80-90 μm (Plate. 1b).

Systematic position

Phylum: Myxozoa, Class: Myxosporea, Order: Bivalvulida, Suborder: Variisporina, Family: Myxobolidae, Genus: *Henneguya*, Species: *H. acerinae*
Microhabitat: Gill

Macrohabitat: Manikganj, Kishoreganj, Mymensingh, Bogura and Jashore

Remarks: *Henneguya acerinae* was first described by Schroder (1906) from *Gymnocephalus cernua*, and later from pike perch by Nemeček (1911). Nemeček (1911) also recorded another species designated as *H. gigantean* from pike perch. The species described by Cohn (1896) as *H. texta* and *H. minuta* were reported from perch, *Perca fluviatilis*. Donets and Shulman (1984) later synonymized the species *H. acerinae* with *H. creplini*, whereas, after Tripathi (1953), they declared the new species described by Nemeček (1911) as *H. acerinae* to be a distinct species designated *H. nemečeki*. *A. testudineus* would be a new host for *Henneguya acerinae* and the present study would be the new locality record for this parasite in Bangladesh.

***Henneguya mystusia* (Sarkar, 1985)**

Description: Mature spores were observed elongated, biconvex and oval with bluntly rounded anterior end and gradually tapering posterior end with a caudal prolongation. The length and breadth of the spore without prolongation was found 13 µm and 3.5 µm respectively. Length of the caudal prolongation was found 19.3 µm. The caudal prolongation was found bifurcated at the tip. The total length of the spore was recorded 32.3 µm. There were two polar capsules which were equal in length. It generally looks pyriform in shape and is bluntly pointed at both ends. The length of the polar capsule was recorded 5.5 µm and breadth was found 1.0-1.3 µm occupying nearly half of the spore body (Fig. 1c). The length of the cyst was recorded 0.1-0.2 mm. Extra-capsular region was occupied with granular sporoplasm. The sporoplasm was found slightly raised up between the two polar capsules. The size of the sporoplasm varied and contained 1 to 2 small sporoplasmic nuclei. In some spore there was no sporoplasmic nucleus.

Systematic position

Phylum: Myxozoa, Class: Myxosporea, Order: Bivalvulida, Suborder: Variisporina, Family: Myxobolidae, Genus: *Henneguya*, Species: *H. mystusia*.
Microhabitat: Gill

Macrohabitat: Manikganj, Faridpur, Kishoreganj, Mymensingh and Jashore

Remarks: Sarkar (1985) described protozoan parasite *Henneguya mystusia* from gill filaments of *Mystus* sp. from India. Krishna Kumar (2000) described this species from Kerala infecting host *Aplocheilus lineatus* and noticed the dilatation

of capillaries of primary gill lamellae and atrophy of secondary gill lamellae evidently causing functional disruption with the *A. lineatus* infection. Rupture of the parasitic cyst was resulted in hemorrhage. As far as our concern, *A. testudineus* might be the new host for this parasite to be reported in these localities in Bangladesh.

Henneguya periophthalmusi (Wu *et al.*, 1993)

Description: Cysts of *Henneguya periophthalmusi* were found in gill filaments of host species *A. testudineus*. The short white spore was measured approximately 0.2-0.3 mm in length. The length, width and breadth of the spore body was found 37.7 (26.4-39.6) μm , 6.1 (4.8-6.2) μm and 4.5 (4.4-4.6) μm respectively. The spore was found with two equal polar capsules with the measurement of 5.8 (4.7-7.3) μm in length and 2.3 (2.0-2.3) μm in width. The length of the tail was found 6.7 (4.4-10.3) μm . The number of coils of the polar filament was counted 7-8 (Fig. 1d).

Systematic position

Phylum: Myxozoa, Class: Myxosporea, Order: Bivalvulida, Family: Myxobolidae,

Genus: *Henneguya*, Species: *H. periophthalmusi*

Microhabitat: Gill

Macrohabitat: Manikganj and Bogura

Remarks: The parasite was first reported by Wu *et al.*, (1993) from the gills of *Periophthalmus cantonensis* in China. The same myxophoridian was also observed in the present study at the gill of *A. testudineus* which is novel to be recorded for this parasite in Bangladesh.

Myxidium sp. (Butschli, 1882)

Description: Genus *Myxidium* was identified first by Butschli (1882) for a myxosporean parasite *M. lieberkuhni* from the urinary bladder of *Esox lucius* and *Lotalota*. About 149 species of *Myxidium* had been reported from different parts of the world (Lom and Dykova, 1992). In India, 17 species of *Myxidium* were reported as parasites of fishes. The major characteristics of the parasite *Myxidium* (Butschli, 1882) which were found to be reported in the present studies were as follows: Spores were as a rule fusiform, straight and slightly crescent, with pointed ends; shell valves were smooth with suture line bisecting the spore; two mostly pyriform polar capsules were situated one at each end of the spore; capsular foramina lied in the sutural plane, at the end of the spore and open mostly in opposite directions (Fig. 1e). One binucleate sporoplasm was located as a rule between the polar capsules (Lom and Dykova, 1992).

Systematic position

Phylum: Myxozoa, Class: Myxosporea, Order: Bivalvulida, Suborder: Variisporina, Family: Myxidiidae, Genus: *Myxidium*, Species: *Myxidium* sp.

Microhabitat: Body slime

Macrohabitat: Kishoreganj and Mymensingh

Remarks: The shape of the spores of the parasite reported in the present study did not resemble any known species of *Myxidium* so far described, but as per the size of the spores the reported parasite species approach to form such as *M. Leiberkuhni* (Butschli, 1881). The spores show affinities to those of *M. giardi* (Cepede), described by Schaferna and Jirovec (1934). However, they are not appeared to be inferred up to species level in the present study. In fact, this is the new locality record for this parasite in the host *A. testudineus*.

Amphileptus disciformis (Chen, 1955)

Description: Flat, leaf or lancet-like body with one side containing longitudinal of strongly arched ciliary rows and left side contained arcuate that was almost concentric kineties. Length was recorded 50 µm. Formed cysts were embedded in the surface tissue of skin or gills with which the ciliate attached its anterior end, including the ciliary rows and became spirally twisted. Two rounded or oval macronucleus and a single micronucleus were observed (Plate.

1f).

Systemic position

Phylum: Ciliophora, Class: Kinetophragminophorea, Order: Pleurostomata,

Family: Amphileptidae, Genus: *Amphileptus*, Species: *A. disciformis*

Microhabitat: Gill and Body slime

Macrohabitat: Faridpur and Mymensingh

Remarks: Among the known *Amphileptus* species, there were only two species which were rounded to ovoid in shape with two macronuclear nodules. These species were *A. disciformis* recorded by Chen (1955) and *A. branchiarum* described by Mitchell and Smith (1988). Comparing with the first ciliate, the present parasite had a smaller size and had only one micronucleus which is similar with *A. disciformis*. It is clearly evident that the current parasite should be *Amphileptus disciformis* and conclusively, *A. testudineus* would be the new host and new locality record for this parasite.

Epistylis lwoffii (Faure-Fremiet, 1943)

Description: *Epistylis lwoffii* is an ectoparasite of various freshwater fishes infecting the gills and skin. *Epistylis* are colonial ciliates with bell-shaped body

provided with long peduncle in the apex that was found to be located in the zooid cell with a nucleus, contractile vacuoles and cilia (Lom and Dykova, 1992). Similar to *Apisoma*, this sessile peritrichid uses fish as a substrate for attachment and feeds on suspended particles in the water (Padua *et al.*, 2012). The length of the grown-up zooids was about 81µm, the greatest width was recorded in the oral third of the body and the average width was recorded approximately 33µm. The scopula was about 8µm broad and the stalk was about 5-5.5µm. The peristomial lip was well developed, its height was amounting to 8.4µm on the infundibular and 4.7µm on the opposite side. The peristomial lip was found slightly vaulted and was elevated some 5 µm above the mouth of the infundibulum (Plate. 1g).

Systemic Position:

Phylum: Ciliophora, Class: Oligohymenophorea, Order: Peritrichida, Family:

Epistylididae, Genus: *Epistylis*, Species: *E. lwoffii*

Microhabitat: Body slime

Macrohabitat: Manikganj and Faridpur

Remarks: These unicellular protozoans possess mobile cilia involving the external body surface in some stage of their life cycle. Cytostome, macronucleus and micronucleus are present. They use fish only for attachment and do not invade the epithelial cells, thus feeding by filtration of suspended material in the water. *A. testudineus* was new host and new locality to be recorded for this parasite.

Trichodina anabasi (Asmat *et al.*, 2003)

Description: *Trichodina anabasi* is a large, cup shaped ciliate parasite. Diameter of body was found 38.2-82.5 (63.2±6.4) µm, diameter of central area was measured 8.0-33.2 (17.0±3.9) µm and width of central part was 2.5-5.6 (3.2±0.6) µm. The adhesive disc was concave, 44.4-63.4 (55.2±6.2) µm and contained a centre whose texture was similar to the rest of adhesive disc. Diameter of adhesive disc was 30.0-74.1 (55.4±6.2) µm. The adoral zone of cilia formed a spiral of 385-390°. The denticulate ring was composed of 21-27 (24.4±1.7) large denticles. Diameter of denticulate ring was 20.3-47.7 (32.8±3.7) µm. There were 5-10 (6.8±0.8) radial pins to one denticle. Length of denticle was measured 4.5-12.6 (7.5±1.1) µm and span of denticle was recorded 10.2-26.6 (16.6±2.7) µm. Diameter of radial pins/denticle was found 5-10 (6.9±0.9) µm.

The blade of a denticle was observed broad. The distal margin of blade was angular, slightly rounded, situated higher than the tangent point and laid close to the border membrane. Diameter of blade was measured 4.8-9.8 (6.9±0.9) μm . The tangent to the y-1 axis was found flat which formed a small line rather than pointed or sometimes rounded. The anterior and posterior margins were almost parallel. The anterior blade apophysis was prominent, but posterior one was not clearly visible. The posterior margin formed L-shaped curve with deepest point at the lower and at the same level as apex. The blade connection was found thin. The central part of the denticle was stout, cylindrical with bluntly rounded point which extended slightly more than halfway to the y axis fitting tightly into the corresponding denticle. The section of the central part above and below the xaxis was recorded similar in shape. The ray connection was short and thin with ray apophysis that was situated a bit high and pointed towards upward direction. The indentation on the lower central part was not clearly visible. The ray was not considerably longer than blade, but slender, almost of equal thickness, sometimes with gradually pointed tip. Typically, the ray was found slightly curved in the posterior direction with anterior margin parallel to the y+1 axis. The central groove in the ray was prominent in most cases (Plate. 1h).

Systematic position:

Phylum: Ciliophora, Class: Oligohymenophorea, Order: Spirotrichida, Family:

Trichodinidae, Genus: *Trichodina*, Species: *T. anabasi*

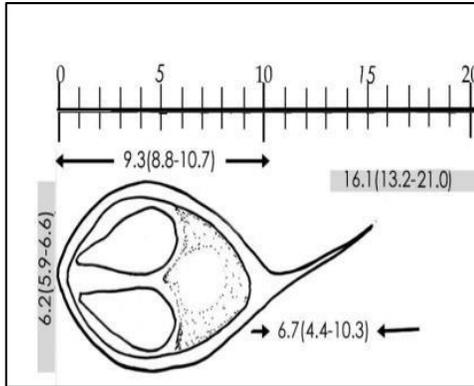
Microhabitat: Gill

Macrohabitat: Manikganj, Kishoreganj, Bogura and Jashore

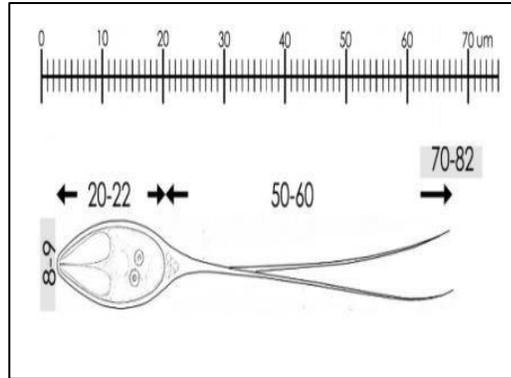
Remarks: *Trichodina anabasi* is parasitic on the climbing perch, *A. testudineus*. Asmat et al. (2003) recorded *Trichodina anabasi* from *A. testudineus* in Chittagong. It remains attached to the gills and widely distributed in Bangladesh.

Trypanosoma anabasi (Mandal, 1978)

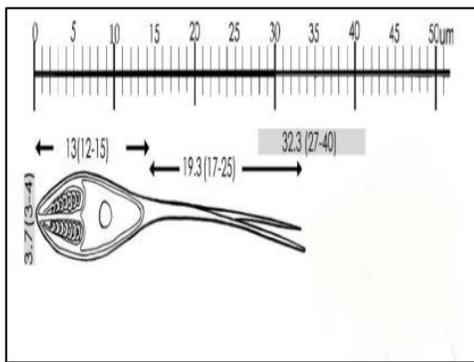
Description: *Trypanosoma anabasi* were elongated and gradually tapering at both ends measuring 29.5 (26.5-38.5) μm in length. No polymorphism was noted. No division stages had so far been encountered in blood or any other organ-smear preparations. In one smear the number of individual varied from 530. The cytoplasmic granules stained deep blue in giemsa but they did not form any striation or got localized in any particular place in the body of the parasites. Nucleus were observed nearly oval, situated about the middle and did not



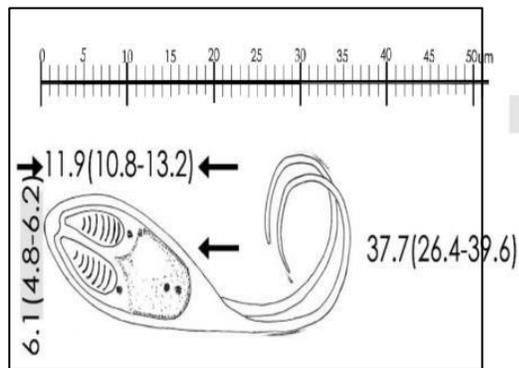
a) *Henneguya gigas*



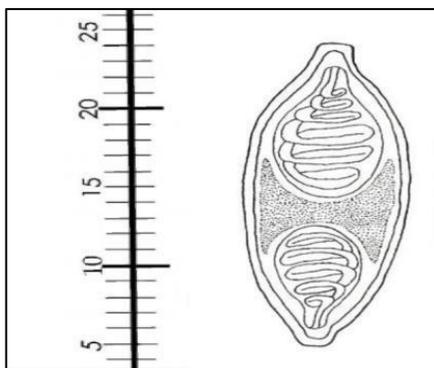
b) *Henneguya acerinae*



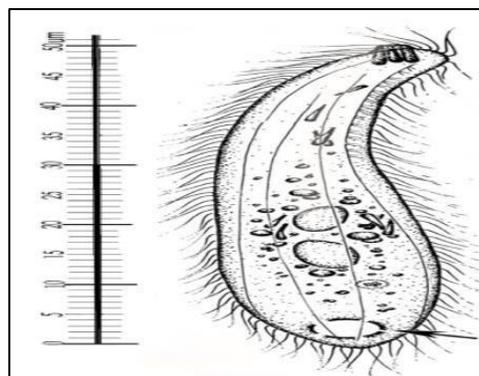
c) *Henneguya mystusia*



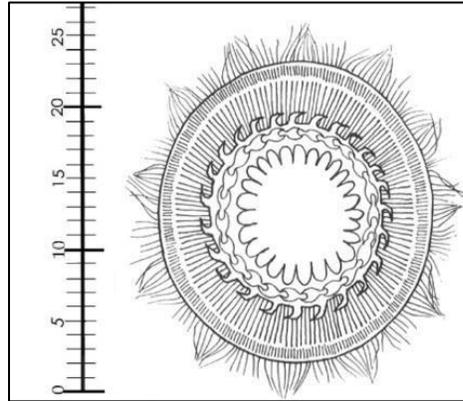
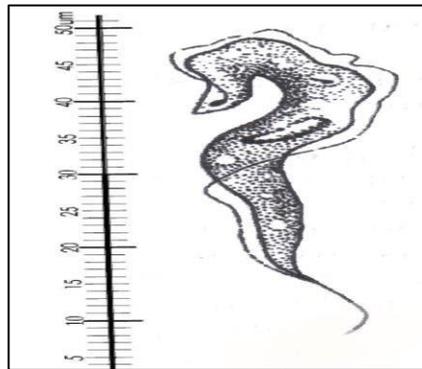
d) *H. periophthalmusi*



e) *Myxidium* sp.



f) *Amphileptus disciformis*

g) *Epistylis lwoffi*h) *Trichodina anabasi*i) *Trypanosoma anabasi*Plate-1: Diagrams of protozoan parasites found in *Anabas testudineus*.

occupy the entire width of the body. The chromatin granules were arranged in the form of inverted S (“S”) and take a dark blue stain with Giemsa. Length of the nucleus was found 3.3 (3.00-4.5) μm and width of the nucleus was found 1.3 (1.00-2.5) μm . Distance from anterior end of the nucleus to anterior end of the body was 13.3 (12.5-15.5) μm . Kinetoplast was found spherical which was stained very deep blue and always darker than the nucleus. Diameter of the kinetoplast was measured 0.80 (0.5-0.9) μm . Distance from kinetoplast to posterior tip was 1.3 (1.0-2.3) μm and distance from posterior end of the nucleus to kinetoplast was measured 11.6 (10.5-13.5) μm . From the point of origin, the flagellum was trailed anteriorly bordering the undulating membrane and extended beyond the body as a free flagellum. The conspicuous undulating membrane was recorded having 6-8 large folds. Length of the free flagellum was recorded 15.5 (10.5-17.5) μm and width of the undulating membrane was found 0.2 μm (Plate-1i).

Systematic position

Phylum: Mastigophora, Class: Zoomastigophora, Order: Kinetoplastida, Family: Trypanoplasmiidae, Genus: *Trypanosoma*, Species: *T. anabasi*

Microhabitat: Blood

Macrohabitat: Kishoreganj, Mymensingh and Bogura

Remarks: The present species has shorter flagella and body length than *T. neinavana* (Fattohy, 1978) although the nuclear position varied, nuclear index of the former being much less than the latter. The present species is somewhat comparable to *T. mugiiicola* (Baker, 1960) in its size but again differs drastically in nuclear index which is only 1.1-1.2 in the trypanosome under study whereas 3.6 in a fore mentioned species. The form under study is separable from *T. singhii* and *T. atti* (Gupta and Jairajpuri, 1981) in its peculiar granulation pattern, nuclear position and differences in statistical measurements.

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