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STUDIES ON THE PROTOZOAN PARASITES OF HILSA SHAD, *TENUALOSA ILISHA* IN BANGLADESH

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Abstract: Present investigation was conducted to study the protozoan parasites of hilsa shad, *Tenualosa ilisha* collected from Aricha Ghat, Dhaka. Thirty fishes were observed. Fishes were collected during months of August 2010 and September 2010. A total of 1099 individuals of protozoan parasite of eight genera/species were recorded during the present study. These parasites were found on body surface, gills and gall bladder of the fish. *Jirovecia piscicola* had the highest prevalence (53.33%) while *Coccomyxa baleswarensis* had the lowest prevalence (26.67%) of infection. Most of the host individuals had double (43%) and single (40%) species infection. Only a few (10%) host had triple species infection. No fish had more than three species of parasite at a time. *Glugea* sp. dominated over all other parasites. *J. piscicola, Zschokkella ilishae* and *Ceratomyxa hilsae* had almost similar abundance while *C. baleswarensis* had the lowest (5.64%) abundance. Abundance of *Sphaeromyxa dighae, Myxobolus* sp. and *Kudoa* sp. were comparatively low ranging from 9.36% to 17.35%.

Key words: Parasites, *Tenualosa ilisha,* Protozoa, Bangladesh.

INTRODUCTION

The hilsa (*Tenualosa ilisha*) is the largest single species contributor to the fisheries sector of Bangladesh (Rahman and Halder 1998). Parasitic infection can cause destruction of host tissues, abnormal growth as well as other biological activities. Some parasites damage the tissue of gills and destroy the respiratory system. So it seems important to have basic information on the parasite fauna of this economically important fish.

A considerable numbers of studies have been done on the metazoan parasites of hilsa in Bangladesh (Southwell and Prashad 1918, Bashirullah 1973, Bashirullah and D'Silva 1973, D'Silva and Khatoon 1997, Akhter *et al.* 2004, Bhuiyan 2006, Bhuiyan *et al.* 2007, Bhuiyan *et al.* 2009, D'Silva *et al.* 2012). So far no study has been carried out on the protozoan parasites of hilsa (*T. ilisha*) of Bangladesh. In India some studies have been made on the protozoan parasites of hilsa along with other fishes (Chakravarty 1939, Chakravarty 1943, Tripathi 1952, Sharkar and Majumder 1983, Sarkar 1995). Present study is the first attempt to study the protozoan parasites of hilsa in Bangladesh. Attention was paid to identification, estimation of the occurrence, and organ-wise distribution of protozoan parasites of hilsa.

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MATERIAL AND METHODS

Collection of host fish: Tenualosa ilisha were collected from river Padma at Aricha Ghat (100 Km from Dhaka city, 23°46′10″N 89°46′83″ E) twice during present study period. Each time a sample of 15 fish were collected directly from fishermen immediately after caught by them. Fishes were collected during months of August 2010 and September 2010.

Preparation for parasitological studies: Smears of body and gill slime were made on glass slides on the spot and fixed them in ethanol for observation in the laboratory. Blood smears on slides were also made and fixed in ethanol and brought to the laboratory. Content of gall bladder (bile) were collected in small glass vials and brought to the laboratory keeping them in an ice box. To maintain temperature well below 0°C for whole day ordinary salt (NaCl) was mixed with ice (1:3) following method described by Berland (2006). In the laboratory bile were centrifuged at 3000 rpm for 1 minute and sediments were observed putting on glass slide under compound microscope.

Methods of observation: Necroscopy - Immediately after collection the external surface of the fish were observed necroscopically using a magnifying glass. External surface of the fish were examined and recorded for any abnormalities. Attention was given to the presence of any deformities, external lesions, abnormal skin colouartion, abdominal swelling etc. Oral and opercular cavities, nares, gills and fins were carefully checked. Specimens were kept moist during examination by spraying them with a fine mist of water. Physiological saline was used when examining the specimens. *Giemsa's stain after acid hydrolysis*- During this process smears were fixed in Schaudinn's fluid; rinsed slides well in distilled water; hydrolysed for 8 min in 1N HCL; again rinsed several times in distilled water; stained with stock Giemsa's stain (diluted 1:20 with water at P^H 7.0-7.2); rinsed in tap water; allowed to dry directly from water or dehydrated through an ethanol series. *Klein's dry silver impregnation method*- Klein's dry silver impregnation method (Lom and Dyková 1992) were tried for ciliates in the gill and body slime of hilsa.

Counts of parasites found in each organ were recorded. Camera lucida sketches were made both from temporary and permanent preparations. Measurements were taken with the help of an ocular micrometer and a stage micrometer. Identification of protozoans were made mainly with the help of Lom and Dyková (1992), Lom and Dyková (2006), Chakravarty (1943), Sarkar (1995) and Tripathi (1952). Margolis *et al.* (1982) were followed for the terms used for the calculation of parasite distribution.

RESULTS AND DISCUSSION

The parasitic protozoa identified from body slime, gill slime and gall bladder of *Tenualosa ilisha* are described below. Three protozoans could not be identified up to species level. More detailed observations are necessary to identify them to the species level. Since all the parasites are already described, detailed descriptions and discussion has not been intended. Description of each parasite is followed by remarks on it.

***Glugea* sp.** Thélohan, 1891 (Microsporea: Microsporidia) (Fig. 1a).

Microhabitat: Body slime, gill slime.

Description: Xenomas single, dot-like and pea-sized. Spores in the posterior vacuole. One to many spores present within membrane-walled sporophorous vacuoles. Subcutaneous xenoma. Up to 20 uninucleate sporoblast mother cells. Spores concentrate in the centre of the xenoma.

Remarks: Lom and Dyková (1992) listed 31 species under the genus *Glugea.* There is no record of *Glugea* sp. in Bangladesh or from *T. ilisha.* Therefore this is a new locality and new host record of the parasite. Literature on *Glugea* spp. is vast. There have been numerous studies on their biology, development and pathogenecity of many species. Most of the species have been described in the skin and the subcutaneous tissue as well as the intestine of fishes but are known to invade other organs in heavy infections.

No conspicuous malformations were observed in *T. ilisha* from which *Glugea* was spotted in this study. However, it is a harmful genus that may proliferate and proved to be detrimental to *T. ilisha* as it is to other fishes.

Jirovecia piscicola Cépède, 1924 (Microsporea: Microsporidia) (Fig. 1b).

Microhabitat: Body slime.

Description: Long, cylindrical spores with a polar filament. Basal part of polar filament thickened like a rigid rod termed the manubrium. Spores were within a xenoma. Spores measures 20 μm × 6 μm.

Remarks: Normally species of the genus *Jirovecia* are found in arthropods and oligocheates. *Jirovecia piscicola* is the only species identified from fish. It was first found in *Odontogadus merlangus* (whiting) in France (Cépède 1924). However Lom and Dyková (1992) is suspicious about the record. The present study confirms that this is a parasite of fish. Information on the development and pathogenecity of this microsporodia is scanty. *J. piscicola* in this study is both a new locality and host record.

Zschokkella ilishae Chakravarty, 1943 (Myxosporea: Bivalvulida) (Fig. 1c)

Microhabitat: Gall bladder.

Description: Spore ellipsoidal in sutural view and slightly bent in valvular view, with bluntly pointed ends. Spore size 12.36 µm × 6.18 µm. Shell valve thin with longitudinal striations. Sutural line straight. Polar capsule almost spherical, equal, 4.26 µm in diameter, opening subterminally and both to one side.

Remarks: Chakravarty (1943) reported this species from the gall bladder of *T. ilisha*. The record of *Zschokkella ilishae* has not been reported by any one else until this study. *Z. ilishae* differs from other species of *Zschokkella.* So far 49 species of *Zschokkella* have been reported from freshwater and marine fishes with a few species from amphibians and reptiles (Lom and Dyková 1992). Species of this genus are commonly found in the gall bladder, urinary tract and other internal organs of fishes (Lom and Dyková 1992). Little is known about the biology and the effect of *Z. ilishae* on the host.

Coccomyxa baleswarensis Sarkar, 1995 (Myxosporea: Bivalvulida) (Fig. 1d).

Microhabitat: Gall bladder.

Description: Ellipsoidal spores seen from both valvular and sutural view. Suture thin and sigmoid. Each polar capsule (5.20 µm×2.43 µm) single, broadly pyriform and obliquely placed and has 3-4 turns of polar filament. Spore ellipsoidal (11.36 µm×5.17 µm).

Remarks: There are 7 known species of *Coccomyxa* (Sarkar 1995). *Coccomyxa baleswarensis* differs from other species of *Coccomyxa* chiefly in the number of spirals in the polar filament in the polar capsule. It was first reported from the Bay of Bengal near Baleswar, Orissa in India (Sarkar 1995). Like other species of *Coccomyxa* it inhabits the gall bladder of the host fish. The only species of *Coccomyxa* to be found in an organ other than the gall bladder is *C. hoffmani* (Cheung and Nigrelli 1990) which was collected from the gill of *Plotosus anguillaris*. Sarkar (1995) found numerous spores of *C. baleswarensis* floating in the bile of the infected gall bladder. Any pathogenecity due to this parasite is not apparent.

Ceratomyxa hilsae Chakravarty, 1939 (Myxosporea: Bivalvulida) (Fig. 1e).

Microhabitat: Gall bladder.

Description: Elongated arcuate spore with conical shell valves which are longer than the diameter of the spore. Breadth of the spore 25-40 µm. Sutural plane prominent. Sutural diameter 10 µm. Polar capsules spherical, equal in size, 5 µm in diameter. Polar filament 35-40 µm long. The polar capsule open at

opposite sides of the central sutural line. The binucleate sporoplasm does not completely fill up the spore cavity.

Remarks: Species of *Ceratomyxa* are coelozoic in marine fishes, rarely found in fresh water fishes and usually not histozoic (Lom and Dyková 1992). According to Lom and Dyková (1992) there are 135 species of *Ceratomyxa*. Since then two more species (Yokoama and Fukuda 2001) have been identified to date from this genus. The species *Ceratomyxa hilsae* was first reported by Chakravarty (1939) from the gall bladder of *T. ilisha*.

Sphaeromyxa dighae Sarkar and Majumdar, 1983 (Myxosporea: Bivalvulida) (Fig. 1f).

Microhabitat: Gall bladder.

Description: Spores broad and bent in valvular view are spherical with rounded pyramidal anterior end. Two polar capsules lie in opposite, tapering and truncate ends of the spore. Both polar capsules open at the anterior end and are situated perpendicular to the sutural line. One binucleate sporoplasm.

Remarks: This is the first report of *Sphaeromyxa* sp. in Bangladesh. *Sphaeromyxa dighae* was first described by Sarkar and Majumdar (1983) in the gall bladder of *T. ilisha* sampled from the Digha Coast off the Bay of Bengal.

***Myxobolus* sp.** Bütschli, 1882 (Myxosporea: Bivalvulida) (Fig. 1g).

Synonym: *Myxosoma* Thélohan, 1892.

Lentospora Plehn, 1905.

Facieplatycauda Wyatt, 1979.

Rudicapsula Kalavati and Narasimhamurti, 1984.

Microhabitat: Gall bladder.

Description: Spore ellipsoidal, ovoid or rounded in valvular view. Biconvex in sutural view. Shell valves as a rule smooth. Two polar capsules are pyriform. Binucleate sporoplasm. Sporoplasm with a conspicuous iodinophile vacuole.

Remarks: A total of 444 species has been reported from fish under the genus *Myxobolus* (Landsberg and Lom 1991). These parasites are mostly histozoic in freshwater fishes and in a few marine (but mostly estuarine) fishes. Few species are very specific to host and site of infection. Most species infect wide range of host and also various tissues of host (Lom and Dyková 1992). This is the first report of *Myxobolus* sp. in *T. ilisha*.

***Kudoa* sp.** Meglitsch, 1947 (Myxosporea: Multivalvulida) (Fig. 1h).

Synonym: *Tetraspina* Xie and Chen, 1988.

Microhabitat: Gill slime.

Description: Spores quadrate in apical view. Sutural line indistinct. Polar capsule pyriform. Two uninucleate sporoplasms, one enclosing other.

Remarks: Species of *Kudoa* are commonly found in marine fishes. They are histozoic, mostly intracellular in muscle with a few coelozoic instances (Lom and Dyková 1992). Thirty seven species have been recorded under this genus (Lom and Dyková 1992). This is the first record of *Kudoa* sp. from *T. ilisha*.

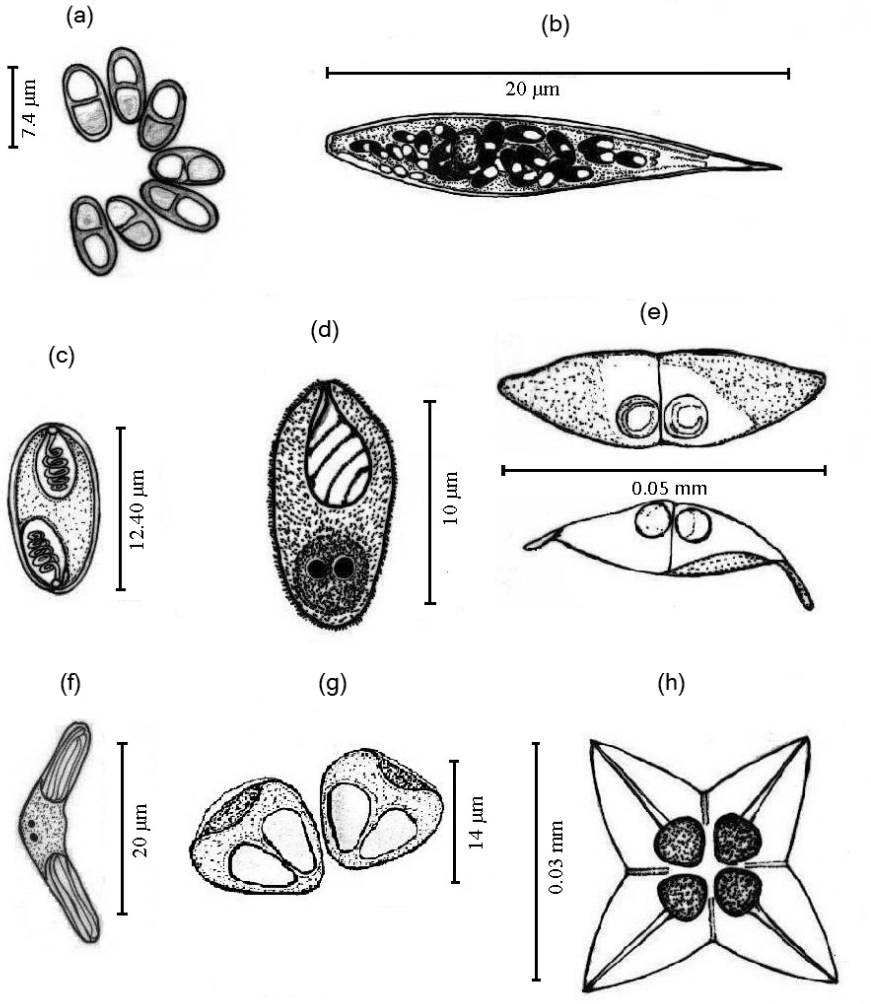

Fig. 1. Line drawings of spores of protozoan parasites found in or on *T. ilisha*: (a) *Glugea* sp. Thélohan 1891, (b) *Jirovecia piscicola* Cépède 1924, (c) *Zschokkella ilishae* Chakravarty 1943, (d) *Coccomyxa baleswarensis* Sarkar 1995, (e) *Ceratomyxa hilsae* Chakravarty 1930, (f) *Sphaeromyxa dighae* Sarkar and Majumdar 1983, (g) *Myxobolus* sp. Bütschli 1882, (h) *Kudoa* sp. Meglitsch 1947.

Infection characteristics: A total of 1099 individuals of protozoan parasites were recorded during the present study. Some 8 protozoa were identified (Table 1). Among the protozoans found *Myxobolus* sp., *Glugea* sp. and *Kudoa* sp. could not be identified to the species level. Most of the parasites were collected from body slime and gall bladder while only one parasite was found in gill slime of *T. ilisha* (Table 1).

Table 1. List of protozoan parasites found in different organs of *T. ilisha*.

Parasite	Group	Site of infection
Glugea sp. Thélohan 1891	Microsporea: Microsporidia	Body slime/gill slime
Jirovecia piscicola Cépède 1924	Microsporea: Microsporidia	Body slime
Zschokkella ilishae Chakravarty 1943	Myxosporea: Bivalvulida	Gall bladder
Coccomyxa baleswarensis Sarkar 1995	Myxosporea: Bivalvulida	Gall bladder
Ceratomyxa hilsae Chakravarty 1930	Myxosporea: Bivalvulida	Gall bladder
Sphaeromyxa dighae Sarkar and Majumdar 1983	Myxosporea: Bivalvulida	Gall bladder
Myxobolus sp. Bütschli 1882	Myxosporea: Bivalvulida	Gall bladder
Kudoa sp. Meglitsch 1947	Myxosporea: Multivalvulida	Gill slime

Infection status of each species of parasite have been depicted in table 2. Prevalence of infection by any protozoan parasite was considerably high in *T. ilisha* (Table 2). *J. piscicola* had the highest prevalence (53.33%) and *C. baleswarensis* had the lowest prevalence (26.67%). Among the other species of protozoa, prevalence of infection did not vary greatly (28.24%-51.33%). *Glugea* sp. had the highest mean intensity (22.08 ± 5.74) while *C. baleswarensis* had the lowest (7.75 ± 3.39). Of the other parasites *Myxobolus* sp., *Z. ilishae, C. hilsae* and *J. piscicola* had considerably higher intensity of infection; while *Kudoa* sp., *S. dighae* had comparatively lower intensity.

In terms of abundance of parasite individuals *Glugea* sp. dominated over all other parasites. *Jirovecia piscicola, Zschokkella ilishae* and *Ceratomyxa hilsae* had almost similar abundance while *Coccomyxa baleswarensis* had the lowest (5.64%) abundance (Table 2).

Table 2. Overall infection characteristics of parasitic protozoan in or on hilsa shad, *T. ilisha*.

Parasite	Prevalence (%)	Intensity (± SD)	Abundance (%)
Glugea sp.	43.30	22.08 (±5.74)	26.11
Jirovecia piscicola	53.33	12.43 (±5.91)	18.10
Zschokkella ilishae	36.67	18.27 (±6.25)	18.29
Coccomyxa baleswarensis	26.67	7.75 (±3.39)	5.64
Ceratomyxa hilsae	50.00	14.33 (±4.74)	19.56
Sphaeromyxa dighae	46.67	9.64 (±2.35)	12.28
Myxobolus sp.	51.33	19.97 (±5.35)	17.35
Kudoa sp.	28.24	9.87 (±4.35)	9.36

To observe the nature of multiple infections, the number of parasite species found in each host at a time was recorded and is present in the figure 2. Single and double species infections were higher in *T. ilisha*. Ten percent host fish had 3 species of parasites at a time, while 7% had no infection and none of the host had more than 3 species at a time.

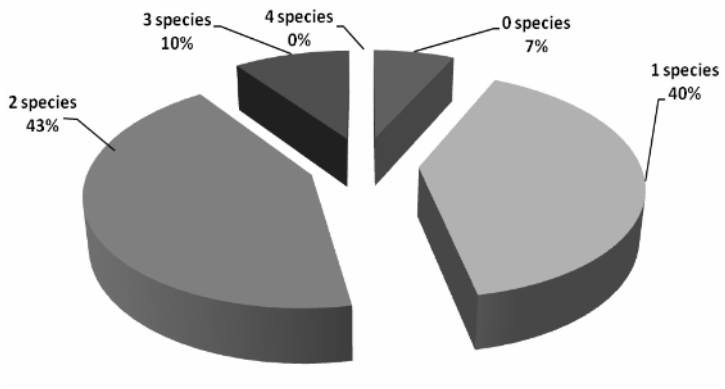

Fig. 2. Relative proportion of infection by species of protozoan parasites in hilsa (*T. ilisha*) of Bangladesh.

This is the first study on protozoan parasites of *T. ilisha* in Bangladesh. Some of the parasites recorded during present study were reported earlier from India. Others are new host records. The present study indicates a rich protozoan parasite fauna exist in *T. ilisha* in Bangladesh than previously recorded from neighboring country, India. Of the 8 genera/species 2 Microspora and rest are Myxozoa.

No data on infection of protozoan parasites are available. So it was not possible to compare results of present finding with others.

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