International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 2; Issue 4; October 2017; Page No. 06-11

Record of new host, site of infection and locality of two *Myxobolus* infecting freshwater fishes in Ranjit Sagar wetland of Punjab (India)

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Abstract
In a study conducted from July, 2014 to August, 2015 on the myxosporean infections in freshwater fishes of Ranjit Sagar Wetland (Punjab), many species belonging to the genus *Myxobolus* were recorded. In this paper, new host, new site of infection and new locality of two myxobolid species viz., *Myxobolus saugati* Kaur and Singh, 2011 and *M. stomum* Ali *et al*., 2003 has been described along with their prevalence. Gill plasmodium index (GPI) and scale plasmodium index (SPI) have been also provided to measure the intensity of the infection. Morphometric variations recorded in the study have been given as remarks.

Keywords: *myxobolus*, myxosporea, freshwater fishes, Ranjit Sagar wetland, Punjab

Introduction

Myxozoans infect various organs of freshwater fishes such as scales, fins, gills, gall bladder, buccal cavity, nasal cavity, alimentary canal and other vital organs. They are detected as small-sized, whitish plasmodia on the infected organs. These are economically important group of microscopic metazoan parasites of fish harvested for food. Recently, many new myxosporean species have been described globally and there is substantial evidence that these threaten general health and vigor of the fish host. Many myxozoan infections are relatively benign, but some species are highly pathogenic and cause great damage (Yokoyama *et al*., 1996; Szekely *et al*., 2015) [24]. More than 2700 species of Myxozoa have been described so far and most of them are in teleost (bony fish) but a small number of species have also been found parasitizing marine fishes, amphibians, reptiles and birds (Lom & Dykova, 2006) [21].

Among myxosporeans, the genus *Myxobolus* includes the highest number of species. Eiras *et al.* (2005) [2] reported 744 valid species, while Lom & Dykova (2006) [21] counted 792 including 7 amphibian species. Kalavati and Nandi (2007) [4] gave significant details in a handbook on myxosporidian parasites of Indian fishes and reported 104 species of *Myxobolus* from Indian fishes in addition to 175 species belonging to genera in total. Earlier also a check list of about 80 species of myxosporidian was given by Kalavati *et al.* (1981) [5] which was the only compilation after Tripathi (1952). About 131 species of *Myxobolus* have been recorded in India given by Kaur & Singh (2012a) [12] and are differentiated by morphological, morphometric characteristics of myxospores, besides host and organ or tissue specificity.

Ranjit Sagar Wetland, also known as the Thein Dam is at the boundary of three states of Punjab, Jammu & Kashmir and Himachal Pradesh on river Ravi. Reservoirs are created by impoundments and are one of the great potential fishery resource. The Ranjit Sagar Wetland is a cold water wetland and occupies largest catchment area (6086 sq. km.) as compared to other wetlands in the Punjab state. It is a habitat for as many as 22 species of fishes which include *Tor*, *Catla*, *Cirrhinus*, *Puntius*, *Garra*, *Crossocheilus*, *Labeo*, *Cyprinus*, *Hypophthalmichthys*, etc. The study indicates that large variety of fishes in these wetlands are infested with myxozoan parasites. In North India many species of myxozoan parasites were recorded from freshwater fishes of wetlands of Punjab on morphological basis (Kaur and Singh, 2011a-f, 2012a,b; Kaur *et al*., 2014b, 2016; Kaur, 2014) [12, 13, 14]. Recently, molecular characterization of 18S rDNA gene has been done for the identification of myxozoa from northern part of India (Kaur and Attri, 2015; Kaur and Gupta, 2015, 2017a, b; Gupta and Kaur, 2016) [7, 8, 22]. The present study re-describes two myxosporeans infecting the freshwater fishes inhabiting Ranjit Sagar Wetland, Punjab, India.

Material and Methods

Live specimens of Indian major carps were collected from various catchment sites of Ranjit Sagar Wetland during the period from July, 2014 to August, 2015. The infected gills were removed with the help of forceps in a petridish containing 0.9% saline. The plasmodia were visible with the naked eye and appeared as creamish white pustules on the gills. The plasmodia were teased on a clean slide to liberate myxospores and were examined under the microscope. Fresh myxospores were treated with 8% KOH solution for the extrusion of polar filaments. For permanent preparation, air-dried smears were stained with Ziehl-Neelsen and Iron-Haematoxylin. Myxospores were measured with the help of a calibrated ocular micrometer. All measurements were recorded in microns (µm). The description of each species is

based on the guidelines of Lom and Arthur (1989) [20].

Results

1. *Myxobolus saugati* Kaur and Singh, 2011

Vegetative stages (Plasmodia)

Round to irregular, large, white, 4-5 in number per scale, located within the scale in the form of a cavity, histozoic, measure 0.9 to 3.0 mm in diameter. 400-500 myxospores per plasmodium. Clinical signs on the scales were apparent. Highly symptomatic creamish white patches and mucous laded body surface (Fig. 1).

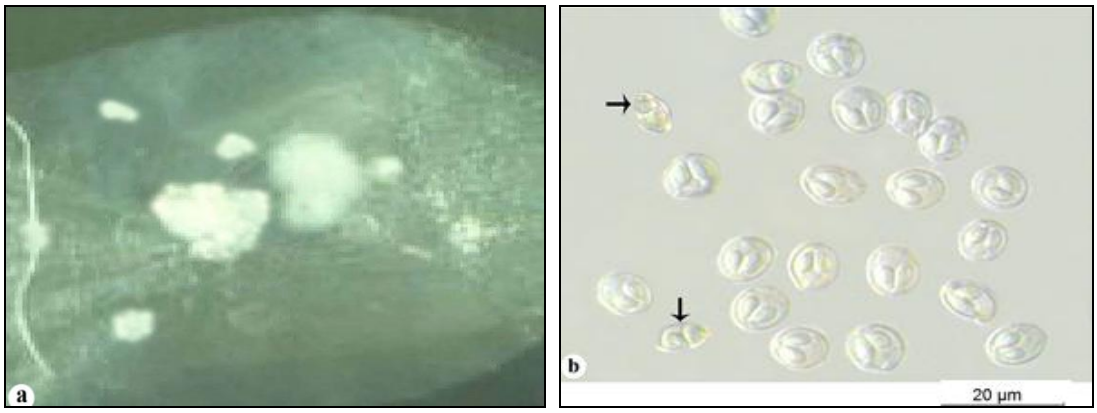

Fig 1: a. Scales of *Cirrhinus reba* showing plasmodia of *M. saugati* Kaur and Singh, 2011
b. Fresh myxospores in phase contrast (frontal view, sutural view ↑)

Taxonomic summary of *M. saugati* Kaur and Singh, 2011

Type host: *Cirrhinus reba* (Ham.) vern. reba carp, Family-Cyprinidae

Type locality: The fish specimens were obtained from Ranjit Sagar Wetland, Punjab, India. It lies at an altitude of about 540 msl at 32°26′30′′ N Latitude and 75°43′30′′ E Longitude and is spread over an area of 87.60 sq km

Site of organ development: Scales

Tissue location: Cavity within the scale pocket

Type material: Paratypes are myxospores stained by Ziehl-Neelsen and Iron-Haematoxylin, deposited with the supervisor. Slide no. M/ZN/22.09.2015 and M/IH/22.09.2015

Prevalence of infection: 51.25% (41/80)

Scale plasmodium index (SPI): 4 (Severe infection)

Category of plasmodium: Type C (visible with naked eye, size range 0.9-3.0 mm)

Clinical symptomatology: Highly symptomatic, creamish white patches and mucous laded body surface

Myxospore description

(Measurements based on 12-15 myxospores in frontal view)
Myxospores measure 8.05×6.80 µm, oval to spherical in frontal view having rounded anterior and posterior ends. Both the shell valves thick, symmetrical, 0.75 µm thick. Parietal folds absent. Polar capsules two, equal, pyriform, measure 4.01×2.55 µm with distinct neck at the anterior end. Polar filaments form 5-6 coils and arranged obliquely to the polar capsule axis, 4.5 µm in length when extruded. An intercapsular process absent. Two capsulogenic nuclei present beneath each of the polar capsule. Sporoplasm agranular, homogenous occupying whole of the extracapsular space behind the polar capsules and contain two nucleii and a large iodinophilous vacuole (Figs. 2, 3; Table 1).

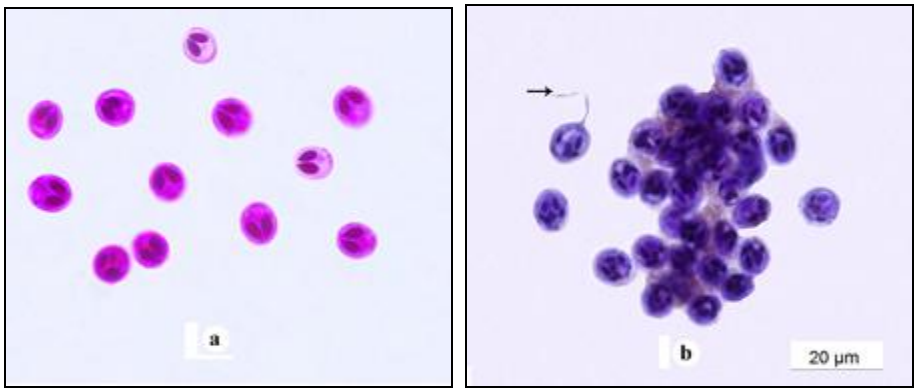

Fig 2: a. Myxospores stained in Ziehl-Neelsen (frontal view)
b. Myxospores stained in Iron-Haematoxylin with extruded polar filament ↑ (frontal view)

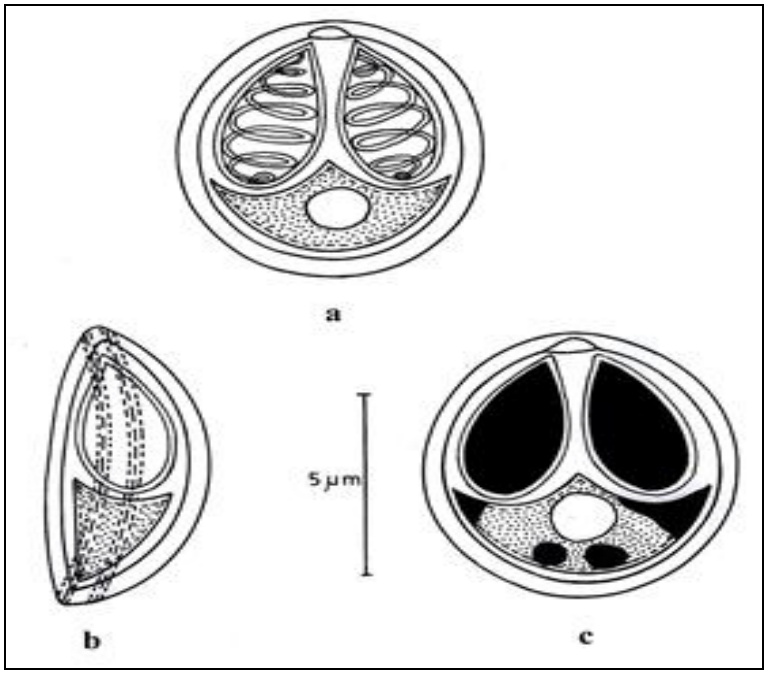

Fig 3: Line drawing of myxospores showing
a. Ziehl-Neelsen stain (frontal view)
b. Ziehl-Neelsen stain (sutural view)
c. Iron-Haematoxylin stain (frontal view)

Table 1: Measurements (µm) and ratio of *M. saugati* Kaur and Singh, 2011

Characters	Range	Mean Values	SD	CV
LS	8.01-8.09	8.05	0.05	0.00
WS	6.76-6.84	6.80	0.05	0.01
LPC	3.97-4.05	4.01	0.07	0.02
WPC	2.50-2.60	2.55	0.07	0.01
Ratio: LS/WS	-	1.34	-	
ICP	-	Absent	-	
NC	-	5-6	-	
Parietal folds	-	-	-	

Remarks
The present observations were in conformity with the original description of *M. saugati* Kaur and Singh, 2011 except some variations in the size of the myxospores (LS/WS: 1.25 vs 1.34), and polar capsules. An intercapsular process and parietal folds were also absent in the present species as in the original specimens. Earlier, the parasite was recorded from the scales of *Labeo rohita* in Kanjali Wetland of Punjab. A new host, *Cirrhinus reba* has been reported for this parasite in the present study (Table 2). In addition, scale plasmodium index (SPI) and type of plasmodium have been provided in the present study.

Table 2: Comparative description of *M. saugati* Kaur and Singh, 2011 with the original description of species (measurements in micrometer)

Species	Host	Site of infection	Locality	Myxospores	Polar capsule
M. saugati Kaur and Singh, 2011 (Present study)	*Cirrhinus reba*	Scales	Punjab (India)	8.05×6.80	4.01×2.55
M. saugati Kaur and Singh, 2011	*Labeo rohita*	Scales	Punjab (India)	8.3×6.6	4.0×2.4

2. *Myxobolus stomum* Ali, Abdel-Baki, Sakran, Entzeroth and Abdel- Ghaffar, 2003
Vegetative stages (Plasmodia)
Minute, visible under binocular microscope, round to oval, creamish white, measure 0.04-0.05 mm in diameter, histozoic, 3-5 plasmodia in number per gill, 50-60 myxospores per plasmodium. No clinical signs on gills (Fig. 4).

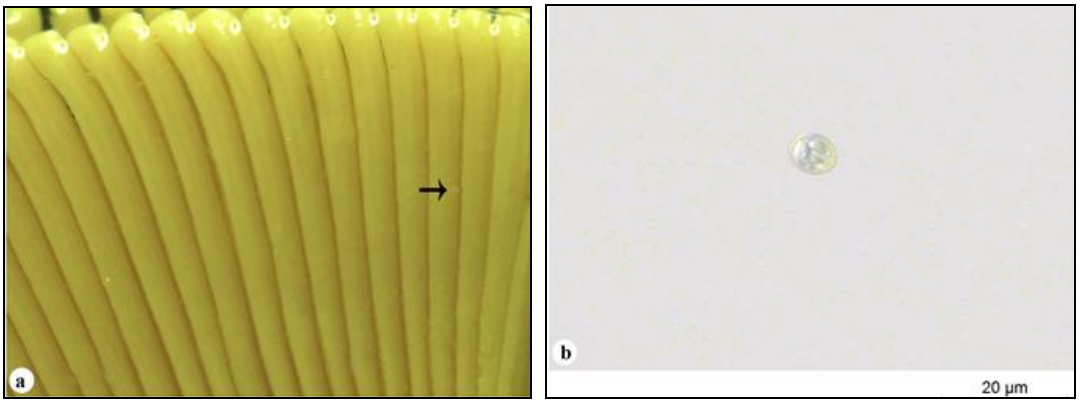

Fig 4: a. Gills of *Cirrhinus reba* showing plasmodia of *M. stomum* Ali *et al*., 2003
b. Fresh myxospores in phase contrast (frontal view)

Taxonomic summary of *M. stomum* Ali, Abdel-Baki, Sakran, Entzeroth and Abdel- Ghaffar, 2003
Type host: *Cirrhinus reba* (Ham.) vern reba carp, Family-Cyprinidae

Type locality: The fish specimens were obtained from Ranjit Sagar Wetland, Punjab, India. It lies at an altitude of about 540 msl at 32˚26′30′′ N Latitude and 75˚43′30′′ E Longitude and is spread over an area of 87.60 sq km

Site of organ development: Gills
Tissue location: Gill lamellae

Type material: Paratypes are myxospores stained by Ziehl-Neelsen and Iron-Haematoxylin, deposited with the supervisor. Slide no. M/ZN/12.11.2015 and M/IH/12.11.2015

Prevalence of infection: 53.33% (32/60)

Gill plasmodium index (GPI): 1 (Light infection)

Category of plasmodium: Type B (visible under stereo zoom, size range 0.6-0.87 mm)
Clinical symptomatology: Moderately symptomatic, creamish white pustules on the gills and mucous laded

Pathogenicity: Degeneration of gill lamellae and cellular elements

Myxospore description
(Measurements based on 10-12 myxospores in frontal view)
Myxospores measure 8.20×5.8 µm, round to sub-spherical in frontal view having rounded anterior and posterior ends. Sutural line distinct. Both shell valves smooth, symmetrical, thick, measure 0.60 µm in thickness. Parietal folds absent. Polar capsules two, equal, pear-shaped, anteriorly situated, measure 5.20×2.10 µm, converge slightly towards the anterior end but diverge apart posteriorly occupying more than half of the myxospore body cavity. Polar filaments arranged perpendicular to polar capsule axis, 6-7 in number. Polar filaments when extruded, thin, thread-like, equal, measure 15.8 µm in length. Intercapsular process absent. Sporoplasm agranular and homogeneous, occupy whole of the extracapsular space behind the polar capsules. Sporoplasm nuclei one, measure 0.65 µm in diameter. Capsulogenic nucleus one, measure 0.80-0.95 µm in diameter. Large, ovoidal iodinophilous vacuole measure 1.6-1.8 µm in diameter (Figs. 5, 6; Table 3).

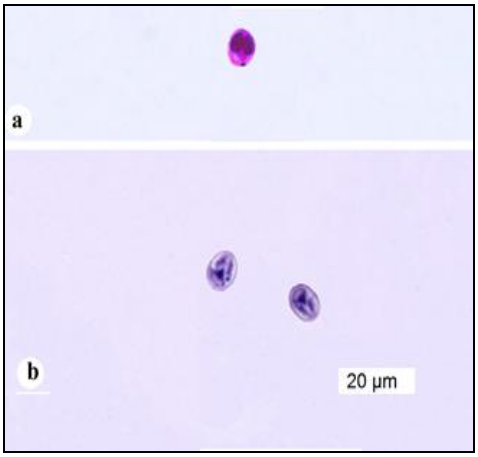

Fig 5: a. Myxospore stained in Ziehl-Neelsen (frontal view)
b. Myxospores stained in Iron-Haematoxylin (frontal view)

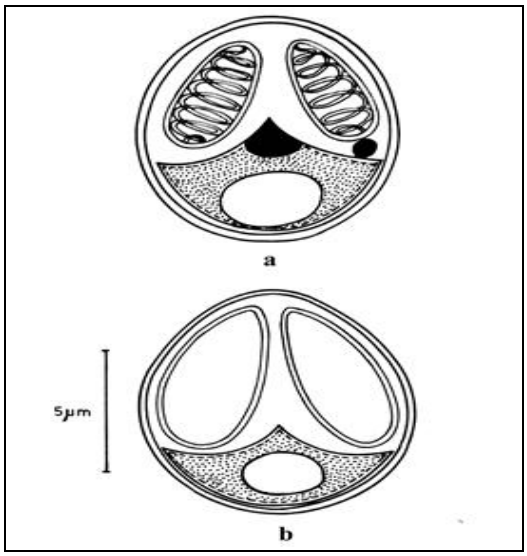

Fig. 6 Line drawing of myxospores showing
a. Ziehl-Neelsen stain (frontal view)
b. Fresh myxospore (frontal view)

Table 3: Measurements (in µm) and ratio of *M. stomum* Ali, Abdel-Baki, Sakran, Entzeroth and Abdel- Ghaffar, 2003

Characters	Range	Mean Values	SD	CV
LS	8.10-8.30	8.20	0.12	0.01
WS	5.75-5.86	5.80	0.04	0.00
LPC	5.12-5.38	5.20	0.11	0.01
WPC	2.05-2.15	2.10	0.05	0.00
Ratio: LS/WS		1.41		
ICP		Absent		
Number of filament turns		6-7		
Parietal folds		Absent		

Remarks

The present observations on *M. stomum* Ali *et al*., 2003 were in conformity with the original description except for some minor variations in the size of the myxospore and polar capsules (LS/WS= 1.30 vs 1.42). Parietal folds and intercapsular process were also absent. Earlier, the parasite was recorded in the oral cavity and lips of *Plectorhynchus gaterinus* from Egypt. A new locality- Ranjit Sagar Wetland (Punjab) and a new site of infection- gills have been recorded for this parasite (Table 4).

Table 4: Comparative description of *M. stomum* Ali, Abdel-Baki, Sakran, Entzeroth and Abdel- Ghaffar, 2003 with original species (measurements are in micrometer)

Species	Host	Site of infection	Locality	Myxospores	Polar capsule
M. stomum Ali *et al*., 2003 (Present study)	*Cirrhinus reba*	Gills	Punjab (India)	8.2×5.8	5.2×2.1
M. stomum Ali *et al*., 2003	*Plectorhynicus gaterinus*	Oral cavity and lips	Egypt	8.5×6.5	4.4×2.4

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