

## A REPORT ON THREE MYXOZOAN PARASITES CAUSING GILL MYXOBOLIOSIS IN AQUACULTURE FISHES IN PUNJAB (INDIA)

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### ABSTRACT

During the present study two major carp fishes *Labeo rohita* (Ham.) vern. rohu and *Cirrhina mrigala* (Ham.) vern., mrigal were collected from aquaculture ponds in Patiala district and were examined for the presence of myxozoan parasites. Out of 64 examined, 28 were found infected (43.75%). In all the cases the infection was always recorded in the gills. Three already known species, *Myxobolus nchoutnounsensis* Nchoutpouen and Fomena (2011), *M. naini* Kaur and Singh (2008) and *M. slendrii* Kaur and Singh (2010) were recorded from the gill lamellae of *C. mrigala*. Spores of the first species, *M. nchoutnounsensis* Nchoutpouen and Fomena (2011), measuring 11.6x9.17µm with anterior flat and broad rounded posterior end. Polar capsules two, unequal, ovoid with notched anterior and rounded posterior end, larger 6.04x3.33µm and smaller 4.16x3.33µm in size, each having an independent opening. A triangular medium-sized intercapsular process (ICP) present. In the present study, this species has been recorded for the first time in India. Spores of the second species, *M. naini*, Kaur and Singh (2008) measuring 12.50x7.45µm with rounded anterior and posterior ends. Polar capsules two, unequal, oval to spherical in shape, larger 4.16x1.67 µm and smaller 3.34x1.67µm in size. Spores of the third species, *M. slendrii* Kaur and Singh (2010) measuring 10.83x2.50µm, pyriform, highly elongated, much slender in valvular view having pointed anterior end and rounded posterior end. Polar capsules two, equal, elongated, pyriform measuring 5.00x1.66µm in size.

**KEY WORDS:** Aquaculture fish, Gills, India, *Myxobolus*

### INTRODUCTION

Fish is an important source of animal protein in the world. Fish culture is a fast-developing branch of agriculture in Punjab. As natural stocks have declined, aquaculture has become increasingly important as a source of fishery products. A large variety of fishes in these aquaculture ponds are vulnerable to various parasitic infections, out of which Myxozoa is emerging as a major group. The healthy population of any species depends on the control of disease and the maintenance of a healthy relationship between living creatures and their environment. In order to increase profitability, health care based on the knowledge of organisms, their ecology, and application of the knowledge in the control of diseases is essential (Snieszko, 1983). Therefore, contribution to the knowledge of fish parasites is a prerequisite for the rapid and correct diagnosis of the disease. Early diagnosis can lead to preventive measures which is the best way to reduce outbreak of disease (Bylund *et al.*, 1980; Abdel-Ghaffar *et al.*, 2012; Morsy *et al.*, 2012). Myxosporidia are an economically important group of fish parasites and have 52 genera (Kent *et al.*, 2001). So far, about 800 *Myxobolus* species have been described (Eiras *et al.*, 2005, Lom and Dykova, 2006), most of them from cyprinid fishes. According to Kalavati and Nandi (2007) in culture ponds the gill myxoboliosis was the most widely distributed disease in many states of India and was found in all the three species of major carps. Heavy mortality was reported in Andhra Pradesh during November-December 2000. Pathogenic effects included hypertrophy of secondary gill lamellae and atrophy of branchial arches.

The present study was aimed to examine the aquaculture fishes in district Patiala, India and to identify the myxobolids infecting them.

### MATERIALS AND METHODS

Fish specimens were procured from local ponds in district Patiala (Punjab), freezed in ice-box and were brought to the laboratory for further investigation. Fishes were 8-15 months old and 46 were male and 18 were female specimens. The fishes were examined and dissected under the stereoscopic microscope. The organs examined were gills, liver, intestine, stomach, kidneys, gall bladder, scales and fins. Plasmodium was removed, teased on a clean microscopic slide and examined under the light microscope at 100X oil objective (Magnus inclined Trinocular microscope MLX-Tr) for the presence of myxospores. The fresh spores were treated with 8% KOH solution to evert the polar filaments. For permanent preparations, air dried smears were stained with Ziehl-Neelsen, Giemsa and Iron haematoxylin. Identification up to generic level was done with the help of key given by Kaur and Singh (2012). Complete description of the species was prepared according to the guidelines of Lom and Arthur (1989). The spore characteristics such as shape and size of the spores and polar capsules, presence or absence of the intercapsular appendix and iodophilous vacuole etc were taken into consideration. The abbreviations used in the paper are as follows:- LS: Length of spore; WS: Width of spore; LPC: Length of polar capsule; WPC: Width of polar capsule; ICP: Intercapsular process; TS: Thickness of shell valves; NC: Number of coils of polar filaments; SD: Standard deviation.

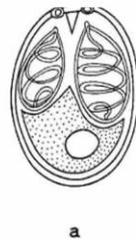
**RESULTS AND DISCUSSION**

**Sp. I**

*M. nchoutnounensis* Nchoutpouen and Fomena (2011) (Figures 1a,b;2a,b)

**Plasmodia**

Minute, attached on the mucous membrane around gill lamellae. Spores 5-10 per plasmodium.



a

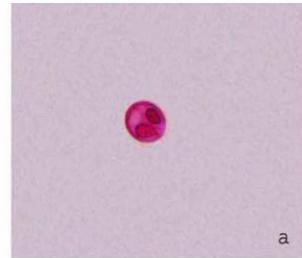


b



Fig. 1

a-b Spore stained in Ziehl-Neelsen



a



b

2



a Spore stained in Ziehl-Neelsen

b Fresh spore

**Figures 1a, b; 2a, b. *M. nchoutnounensis* Nchoutpouen and Fomena (2011)**

**Spore description (Table 1)**

(Measurements based on 12-14 spores in frontal view)

Spores histozoic, oval to rounded in valvular view, measuring 11.6x9.17µm having anterior end flat and broad rounded posterior end. Shell valves thick, smooth, symmetrical and measuring 0.67µm in thickness. Parietal folds absent. Polar capsules two, unequal, ovoid with notched anterior end and rounded posterior end, each having an independent opening. Both the polar capsules converged anteriorly and diverged apart posteriorly. Larger polar capsule measuring 6.04x3.33µm and smaller one measuring 4.16x3.33µm in size. Polar filaments form 4-5 coils in larger and 3-4 in smaller polar capsule arranged perpendicular to the polar capsule axis. An intercapsular process (ICP) of medium-size, and triangular in shape present. Sporoplasm agranular, homogenous having one nucleus measuring 0.17µm in diameter. An iodophilous vacuole measuring 3.33µm in diameter.

**Taxonomic summary**

- Host** : *Cirrhina mrigala* (Ham.) vern. mrigal
- Locality** : Mallu matra Pond, Patiala (India)
- Site of infection** : Gill lamellae
- Prevalence of infection** : 21.4% (6/28)
- Age of the fish** : 8-12 months old, 17 Males and 11 Females
- Symptoms** : Mucous laden with haemorrhagic gills
- Remarks**

The observations on the specimens of *M. nchoutnounensis* Nchoutpouen and Fomena (2011) under study were in conformity with the original description except for some minor variations in the size of the spore, polar capsules and number of coils. Both the polar capsules were longer and wider (LLPC:6.04 and LSPC:4.16;WLPC:3.33 and WSPC:3.33) with 4 and 3 number of coils in comparison to the original specimens (LLPC:4 and LSPC:2.9; WLPC: 1.5 and WSPC:1.6) containing 6-9 and 3-4 number of coils. Two prominent openings were present at the anterior end of the spores under study. Earlier, it was recorded from South Africa (Cameroon) infecting various organs such as gills, fins, scales and cornea of *Labeo parvus*. *M. nchoutnounensi* is a new record from India. A new host i.e. *C. mrigala* has been recorded for this parasite (Table 2).

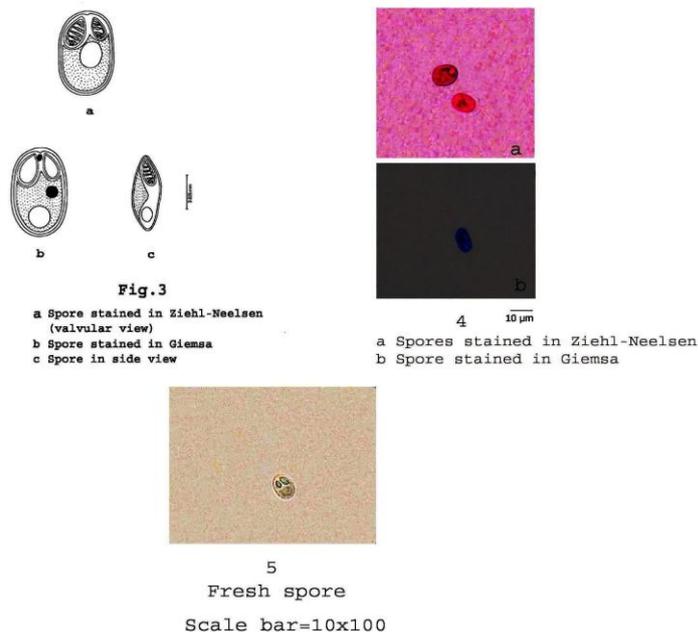
## Sp. II

*M. naini* Kaur and Singh (2008) (Figures 3a-c; 4a,b-5)

### Plasmodia

Minute, attached on the mucous membrane around gill lamellae. Spores 10-15 per plasmodium.

*M. naini* Kaur and Singh, 2008



Figures 3a, b, c; 4a, b; 5. *M. naini* Kaur and Singh (2008)

### Spore description (Table 3)

(Measurements based on 8-10 spores in frontal view)

Spores histozoic, oblong to oval in valvular view measuring  $12.50 \times 7.45 \mu\text{m}$  having rounded anterior and posterior ends. Shell valves thick, smooth and symmetrical measuring  $0.83 \mu\text{m}$  in thickness. No parietal folds were present. Polar capsules two, unequal, oval to spherical in shape and placed anteriorly in the spore body cavity. Larger polar capsule measuring  $4.16 \times 1.67 \mu\text{m}$  and smaller one  $3.34 \times 1.67 \mu\text{m}$  in size. Both the polar capsules converge closely anteriorly but diverge apart posteriorly occupying more than one third of the spore body cavity. Polar filaments form 5-7 coils in the larger and 4-5 coils in the smaller polar capsule, arranged perpendicular to the polar capsule axis. An intercapsular process (ICP) of small-size present. Sporoplasm agranular, homogenous and occupying whole of the extracapsular space behind the polar capsules. Sporoplasmic and capsulogenic nuclei measuring  $0.50 \mu\text{m}$  and  $0.17 \mu\text{m}$  in diameter respectively. An iodophilous vacuole measuring  $3.33 \mu\text{m}$  in diameter.

### Taxonomic summary

<b>Host</b>	: <i>Cirrhina mrigala</i> (Ham.) vern. mrigal
<b>Locality</b>	: Dhindsa pond Patiala
<b>Site of infection</b>	: Gill lamellae
<b>Prevalence of infection</b>	: 83.33% (10/12)
<b>Age of the fish</b>	: 1 year, 10 Males and 3 Females
<b>Symptoms</b>	: Mucous laden with haemorrhagic gills

### Remarks

The observations on the specimens of *M. naini* Kaur and Singh (2008) were in conformity with the original description except for some minor variations in the size of spore, polar capsules and number of coils in the polar capsules. Earlier, the parasite was recorded from gills of *C. mrigala* in Kanjali wetland of Punjab. A new locality- Dhindsa pond Patiala, Punjab (India) has been recorded for this parasite (Table 4).

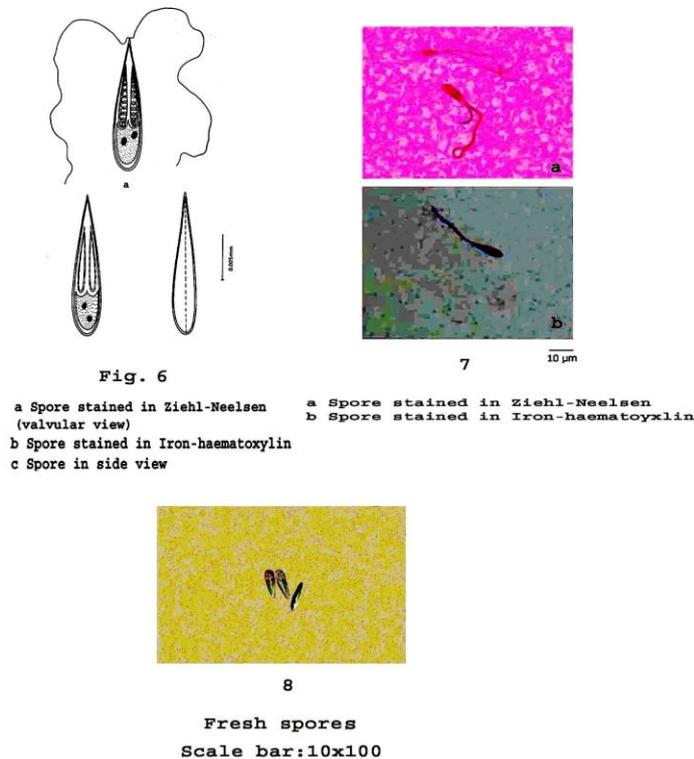
## Sp. III

***M. splendrii* Kaur and Singh (2010) (Figs.6a-c; 7a,b;-8)**

**Plasmodia**

Minute, attached on the mucous membrane around gill lamellae. Spores 20-25 per plasmodium.

*M. splendrii* Kaur and Singh, 2010



**Figures 6a, b, c; 7a, b; 8. *M. splendrii* Kaur and Singh (2010)**

**Spore description (Table 5)**

(Measurements based on 10-15 spores in frontal view)

Spores histozoic, measuring 10.83×2.50µm, pyriform, highly elongated, much slender in valvular view, having pointed anterior and rounded posterior ends. Shell valves thin, smooth, symmetrical, measuring 0.17µm in thickness. Shell valves at the posterior end of the spore appeared thicker than the rest of the spore body. Polar capsules two, equal, pyriform, highly elongated measuring 5.00×1.66µm. Both polar capsules placed posteriorly from the tip of the spore, parallel to each other and each containing 10-11 coils arranged perpendicular to the polar capsule axis. Polar filaments measuring 28 µm in length when extruded. Intercapsular process absent. Sporoplasm agranular and homogenous occupying whole of the extracapsular space behind the polar capsules. Sporoplasmic nuclei two, each measuring 1.33 µm in diameter. Iodinophilous vacuole absent.

**Taxonomic summary**

<b>Host</b>	: <i>Cirrhina mrigala</i> (Ham.) vern. mrigal
<b>Locality</b>	: Dhindsa pond and Mallu matra pond Patiala
<b>Site of infection</b>	: Gill lamellae
<b>Prevalence of infection</b>	: 83.33% (10/12)
<b>Age</b>	: 1 year, 10 Males and 3 Females
<b>Symptoms</b>	: Mucous laden with haemorrhagic gills

**Remarks**

The observations on the specimens of *M. splendrii* Kaur and Singh (2010) (LS/WS: 4.33) were in conformity with the original description (LS/WS:4.35) except some variations in the size of the spore and polar capsules as indicated by LS/WS ratio. Two new localities- Dhindsa pond and Mallu matra pond, Patiala, Punjab (India) were recorded for this parasite (Table 6).

**Table 1. Measurement (in  $\mu\text{m}$ ) and ratio of *M. nchoutnounsensis* Nchoutpouen and Fomena (2011)**

Characters	Range	Mean value	SD
LS	10.66-12.66	11.66	1.49
WS	8.17-10.17	9.17	0.91
LLPC	5.06-7.02	6.04	0.80
WLPC	2.31-4.37		3.33
LSPC	3.20-5.15	4.16	4.86
WSPC	2.30-4.37	3.33	4.86
Ratio: LS/WS		1.27	
ICP		Medium sized	
NC		4 in larger and 3 in smaller polar capsule	
Parietal folds		Absent	

**Table 2. Comparative description of *M. nchoutnounsensis* Nchoutpouen and Fomena (2011) with original species (measurements are in micrometer)**

Species	Host	Site of infection	Locality	Spore	Polar capsules
<i>M. nchoutnounsensis</i> (present study)	<i>Cirrhina mrigala</i>	gill lamellae	Mallu matra pond, Patiala (India)	11.66x9.17	6.04x3.33 and 4.16x3.33
<i>M. nchoutnounsensis</i> Nchoutpouen and Fomena (2011)	<i>Labeo parvus</i>	gills, fins, scales and cornea of eye	Cameroon (South Africa)	11.8x9.2	4x1.5 and 2.9x1.6

**Table 3. Measurements (in  $\mu\text{m}$ ) and ratio of *M. naini* Kaur and Singh (2008)**

Characters	Range	Mean value	SD
LS	11.35-13.12	12.22	0.86
WS	6.02-7.72	6.86	0.72
LLPC	3.57-5.30	4.44	0.86
WLPC	1.04-2.30	1.67	0.89
LSPC	2.83-3.83	3.33	0.70
WSPC	1.04-2.30	1.67	0.89
Ratio: LS/WS		1.67	
ICP		Small sized	
NC		5-7 in larger and 4-5 in smaller polar capsule	
Parietal folds		Absent	

**Table 4. Comparative description of *M. naini* Kaur and Singh (2008) with original species (measurements are in micrometer)**

Species	Host	Site of infection	Locality	Spore	Polar capsules
<i>M. naini</i> (present study)	<i>Cirrhina mrigala</i>	gill lamellae	Dhindsa pond Patiala (India)	12.50x7.45	4.16x1.67 and 3.33x1.67
<i>M. naini</i> Kaur and Singh (2008)	<i>C. mrigala</i>	gill lamellae	Kanjali wetland Punjab (India)	12.9x8.2	4.9x3.1 and 3.33x1.63

**Table 5. Measurements (in  $\mu\text{m}$ ) and ratio of *M. slendrii* Kaur and Singh (2010)**

Characters	Range	Mean value	SD
LS	10.25-11.40	10.83	0.81
WS	1.66-3.33	2.50	1.18
LPC	4.0-6.0	5.00	1.41
WPC	1.15-2.20	1.66	0.74
Ratio: LS/WS		4.33	
ICP		Absent	
NC		10-11	
Parietal folds		Absent	

**Table 6. Comparative description of *M. splendrii* Kaur and Singh (2010) with original species (measurements are in micrometer)**

Species	Host	Site of infection	Locality	Spore	Polar capsules
<i>M. splendrii</i> (present study)	<i>Cirrhina mrigala</i>	gill lamellae	Dhindsa pond Patiala (India)	10.83x2.50	5.00x1.66
<i>M. splendrii</i> Kaur and Singh (2010)	<i>C. mrigala</i>	gill lamellae	Ropar wetland Punjab (India)	14.87x3.4	5.74x1.48

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