

## Redescription and Histopathology of Two Species of *Myxozoans* Infecting Gills of Fingerlings of Indian Major Carps

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### Abstract:

During the present study, two myxosporean species, *M. potularis* Madhavan et al. (2013) and *M. rocatlae* Basu and Haldar (2002) have been described from the gills of *Labeo rohita* Hamilton vern. rohu and *Cirrhinus mrigala* Hamilton vern. mrigala at nursery ponds located in village Fagan majra, District Fategarh Sahib, Punjab, India. Earlier, both the species *M. potularis* and *M. rocatlae* were described from West Bengal. 30 specimens of *Labeo rohita* and 40 specimens of *Cirrhinus mrigala* were examined. Out of which 23 and 21 fishes were infected respectively. The prevalence rate was more in *M. potularis* (76.66%) than *M. rocatlae* (52.5%). At the time of collection various physico-chemical parameters like temperature, pH, TDS, DO etc. were also recorded. The histological effects of the pathogen were observed by light microscopy. The plasmodia of *M. potularis* were located in the afferent artery of the gill filament and plasmodia of *M. rocatlae* were located in the fine blood capillaries at the tip of the gill lamellae. *M. potularis* was highly pathogenic than *M. rocatlae*. The plasmodia of *M. potularis* were typed in "D" category caused complete destruction of all the cellular elements of gills and caused inflammation and abscesses. When present in large number and regarded as highly pathogenic parasite infects fingerlings in aquaculture. The gill plasmodial index (GPI) was 4 for *M. potularis* n. sp. indicated severe infection and 1 for *M. rocatlae* indicated light infection.

**Keywords:** Myxobolus; Gills; Nursery ponds; Parameters

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## Introduction

Myxozoans are microscopic metazoan endoparasites that most commonly parasitize invertebrates, typically oligochaetes, polychaetes, bryozoans and poikilotherms, primarily fishes but also reptiles and amphibians (Kent et al., 2001; Canning and Okamura, 2004; Lom and Dykova, 2006). They have also been found in homeotherms including birds, moles and humans (Boreham et al., 1998; Friedrich et al., 2000; Moncade et al., 2001; Lowenstine et al., 2002; Dykova et al., 2007). A large variety of fishes in aquaculture ponds are vulnerable to various parasitic infections, out of which Myxozoa is emerging as a major group. Myxozoans are one of the economically important group of microscopic metazoan parasites as they infect fish harvested for food. New myxosporean pathogens are continually emerging and threatening the development of pisciculture all over the world. Myxozoan parasites are widely dispersed in native and pond-reared fish populations. Most infections in fish create minimal problems, but heavy infestations can become serious, especially in fingerlings.

Nursery ponds for rearing fry and fingerlings are enough in Punjab. Management practices in the available nursery ponds of both the government and private sectors are at a standard level. But fish farmers often raise questions about the quality of fry obtained from nursery ponds, where they have observed reduced growth performance. The poor growth of fingerlings depends on many factors. Myxozoan parasites are common in juvenile carp in nursery ponds. High mortality rates caused by myxosporidians infection in the gills have raised serious concern among fish farmers.

However, histopathology has been successfully used to diagnose the pathogenicity of carps throughout the world. Davis (1923) studied the development and sporulation of cysts of myxosporidian species. There are alarming economic losses due to *Myxobolus* spp. infestation of the major carp in the nursery ponds as reported by Sanaullah and Ahmed (1980). The present work was therefore undertaken to investigate the pathogenicity of the gills of fingerlings to clarify the prevalence rate and nature of damage caused by myxosporidian parasites.

## Material and Methods

During the survey, live fingerlings of two species were collected from nursery ponds located in the village Fagan Majra, District Fatehgarh Sahib, Punjab, India. The fish species examined included native carps *Labeo rohita* Hamilton vern. rohu and *Cirrhinus mrigala* Hamilton vern. mrigal. The live specimens were collected and brought to the laboratory in oxygenated bags for further investigation. A total of 70 fish specimens belonging to two genera were examined for the presence of myxozoan infections. Out of which, 44 fishes were found to be infected with myxozoan parasites in the form of plasmodia. Prevalence of infection was recorded to be 62.85%.

Various organs such as skin, gills, heart, fins, scales, stomach, intestine and kidney were examined under stereozoom binocular microscope for the presence of plasmodia. The infection was

recorded in the gills only in the form of minute to large-sized plasmodia. The infected gills containing plasmodia were fixed in Bouin's fixative and preserved in 10% formalin for further study. For fresh myxospores, each plasmodium was ruptured in normal saline (0.85%) with the help of a fine needle on a clean slide and examined under light microscope for the presence of myxospores. The fresh myxospores were photographed under phase contrast microscope (Image Processing Unit Magnus MLX Model No. 12G961) in the Parasitology Laboratory, Department of Zoology and Environmental Sciences, Punjabi University, Patiala. For dry preparations, thin smear was made on a clean slide, air dried, fixed in methanol. In case of permanent (wet) preparation, smear was fixed in Bouin's fixative. The stains such as Heidenhain's Iron haematoxylin and modified Ziehl-Neelsen were used to study the myxospore morphology as per the protocol given by Kaur and Singh (2008). Slides were mounted in DPX. Ziehl-Neelsen stained the myxozoan myxospores bright red in colour and was useful to count the number of coils of polar filament inside the polar capsule. Similarly, Iron-haematoxylin stain proved useful to show the presence or absence of intercapsular process and number of capsulogenic and sporoplasmic nuclei.

Myxospores were measured with the help of calibrated ocular micrometer according to the guidelines of Lom and Arthur (1989). Photography of fresh myxospores was done under the phase contrast microscope (Image Processing Unit Magnus MLX Model No. 12G961) in the Parasitology Laboratory, Department of Zoology and Environmental Sciences, Punjabi University, Patiala and of stained myxospores was done under Leica photographic unit at Sophisticated Instrumentation Center, Punjabi University, Patiala. Line drawings were made from stained material with the aid of camera lucida.

For calculations of prevalence, the following formulae were applied.

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fish}}{\text{Total number of fish examined}} \times 100$$

The pathogenic effects caused by myxozoan parasites have been studied with the help of histological sections of the infected organs.

### Light microscopy

For light microscopy, infected organs were cut into small pieces and fixed in Bouin's fixative. For histology, the tissue samples were dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 8-10  $\mu\text{m}$  and stained with Luna's method (Luna, 1968) and haematoxylin and eosin (H+E).

### Gill plasmodial index (GPI) (Kaur and Attri, 2015)

GPI was calculated on the basis of number of plasmodia present per gill (one side) visible under the stereozoom binocular microscope. 0-0 (no infection-0); 1-5 (light infection-1); 5-10 (moderate infection-2); 10-20 (heavy infection-3); 20-50 (severe infection-4).

### Localization of plasmodia in the gill tissue

The location of myxosporean plasmodia in various tissues of the gills was determined with the help of histological sections stained with Luna's method and were categorized into types according to the guidelines of Molnar (2002).

**Intralamellar-epithelial type (LE-LE1, LE2):** Small plasmodia (LE1) and large plasmodium deforming several gill lamellae (LE2).

#### Intralamellar-vascular type (LV -LV1, LV2, LV3):

- **LV1:** Plasmodium located centrally in the gill lamellae.
- **LV2:** Plasmodium protruding from one side of the gill lamellae
- **LV3:** Large plasmodium deforming several gill lamellae

**Intrafilamental-vascular type (FV-FV1, FV2):** Small round or elongated plasmodia in the afferent artery (FV1) and large plasmodia formed by the fusion of several plasmodia near the end of the gill filament (FV2).

**Plasmodium located in the gill arch (AC, AV, AE):** Plasmodia developing in the cartilaginous structure of the gill arch (AC). In AV plasmodia are developing in the blood vessels of the gill arch and in AE type plasmodia developing in the epithelial or connective tissue elements of the gill arch.

**Type of plasmodia according to size:** Type of Plasmodia were categorized into three types

- **Type A:** Plasmodia visible under binocular microscope (size range=40-200  $\mu$ m)
- **Type B:** Plasmodia visible under stereozoom microscope (size range=0.2-0.9 mm)
- **Type C:** Plasmodia visible with naked eye (size range=0.9-3.0 mm)
- **Type D:** Plasmodia of very large-sized (size range=3.0-10 mm)

Various water parameters were analyzed such as water temperature, pH, conductivity, TDS and dissolved oxygen (DO). All the parameters were recorded on the spot with the help of portable water testing kit.

## Results and Discussions

### *Myxobolus potularis* (Madhavan et al., 2013)

**Plasmodia:** Large, creamish, elongated in shape, visible with naked eye, 3-10 mm in diameter, attached to the gill filament, histozoic, 15-25 in number per gill, 1500-2000 myxospores present per plasmodium. Highly symptomatic (gills full of abscesses, haemorrhagic) (Figure 1a).

#### Myxospore description

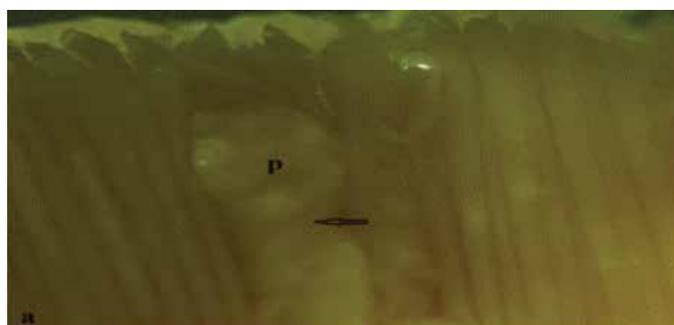
This is clearly mentioned in Table 1 and Figures 1b-3c.

**Measurements based on 10-12 myxospores in frontal view:** Myxospores measure  $7.83 \times 4.79 \mu$ m, oval in frontal view bluntly

pointed with prominent knob at the anterior end and greatly rounded pot-like posterior end. Shell valves measure  $0.30 \mu$ m thick. Parietal folds absent. Polar capsules two, equal, pyriform, measure  $4.00 \times 1.23 \mu$ m sharply pointed at the anterior end and rounded posterior end, occupy three fourth of the myxospore body cavity, placed towards the anterior proximity. Polar filament coils 7-9 in number, arranged perpendicular to the polar capsule axis. Polar filaments thread-like when extruded, measure  $25 \mu$ m in length. Intercapsular process (ICP) absent. Sporoplasm agranular, homogenous and cup-shaped with two nuclei,  $1.50-1.25 \mu$ m in diameter and an iodophilous vacuole present,  $0.70 \mu$ m in diameter.

#### Taxonomic summary of *M. potularis* (Madhavan et al., 2013)

**Family:** Myxobolidae



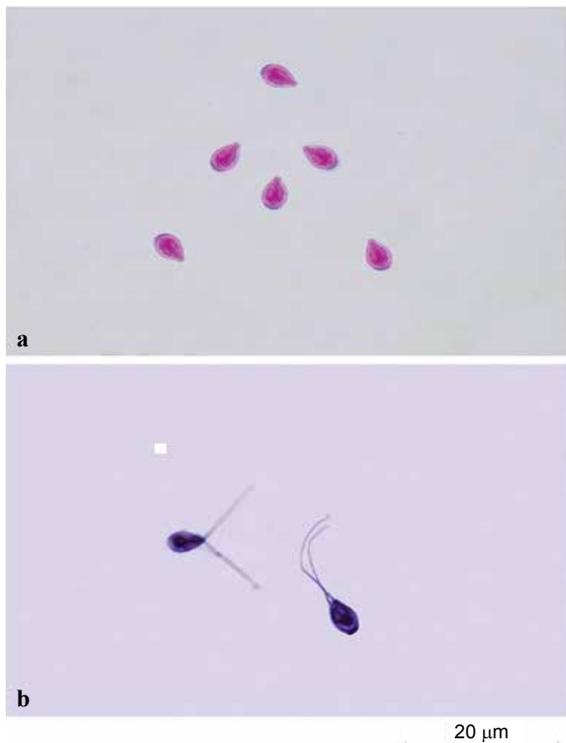
**Figure 1a:** Infected gills of *Labeo rohita* under stereozoom microscope showing plasmodia of *M. potularis* Madhavan et al., 2013.

**Table 1:** Measurements ( $\mu$ m) and ratio of *M. potularis* Madhavan, Bandyopadhyay and Santosh, 2013.

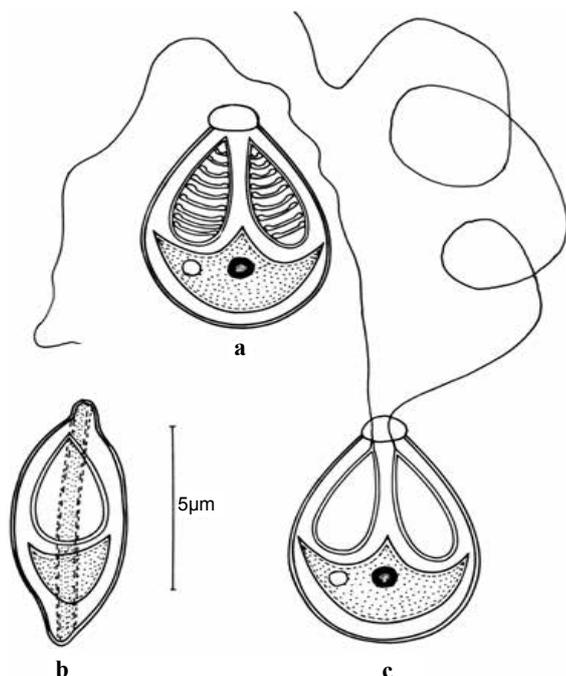
Characters	Range	Mean Values	SD
LS	6.75-8.91	7.83	0.71
WS	3.80-5.78	4.79	0.58
LPS	3.0-5.0	4	0.32
WPS	0.95-1.51	1.23	0.28
Ratio: LS/WS		1.63	
ICP		Absent	
NC		07-Sep	
Parietal Folds		Absent	



**Figure 1b:** Myxospores of *M. potularis* Madhavan et al., 2013 in 425 fresh preparations (Bright field) in frontal view and sutural view ( $\uparrow$ ).



**Figure 2:** a) Myxospores of *M. potularis* Madhavan et al., 2013 stained in Ziehl-Neelsen. b) Myxospores of *M. potularis* Madhavan et al., 2013 stained in Iron haematoxylin with extruded polar filaments.



**Figure 3:** a) Line drawing of fresh mature myxospore of *M. potularis* Madhavan et al., 2013 infecting gills of *L. rohita* (Frontal view). b) Line drawing of fresh mature myxospore of *M. potularis* Madhavan et al., 2013 infecting gills of *L. rohita* (Sutural view). c) Line drawing of fresh mature myxospore of *M. potularis* Madhavan et al., 2013 infecting gills of *L. rohita* (Frontal view with extruded polar filaments).

**Type host :** *Labeo rohita* Hamilton vern. Rohu

Family: Cyprinidae

**Age of the fish:** 2-3 months

**Length of the fish:** 4.2 cm

**Type locality:** Nursery Pond Fagan Majra, District Fatehgarh Sahib, Punjab (India)

**Type specimen:** Paratypes are myxospores stained in Ziehl-Neelsen and Iron-haematoxylin deposited in the Parasitology Laboratory, Department of Zoology and Environmental sciences, Punjabi University, Patiala, India. Slide no. LR/ZN/15.10.2015 and LR/IH/15.10.2015

**Site of infection :** Gill filament (Intrafilamental vascular type FV2)

**Type of Plasmodia :** Type C (visible with naked eye)

**Prevalence of infection (%):** 76.66% (23/30)

**Pathogenicity:** Highly pathogenic, necrosis of the gills

**Gill plasmodial index (GPI):** 4 (15-25 plasmodia per gill) indicating severe infection

**Clinical symptomatology :** Highly symptomatic, gills full of abscesses, mucous laden, pale haemorrhagic gills

### Remarks

The observations on the specimens of *M. potularis* Madhavan et al. (2013) are in conformity with the original description except for some minor variations in the size of the myxospore, polar capsules and number of coils in the polar capsule. Earlier, the parasite was recorded from gill filaments of *C. reba*, *L. calbasu*, *L. bata* and *L. gonius* in West Bengal (India). A new locality-Nursery Pond, Fagan Majra, District Fatehgarh Sahab, Punjab (India) and a new host- *Labeo rohita* are recorded for this parasite in the present study (Table 2). In addition, gill plasmodial index (GPI) and histopathogenesis are provided in the present study.

### Myxobolus rocatlae Basu and Haldar, 2002

**Plasmodia:** Small, microscopic, round to oval, white, 0.5-1.2 mm in diameter, attached to the gill lamellae, histozoic, 1-5 in number per plasmodium. Mucous laden gills and pale haemorrhagic (Figure 4a).

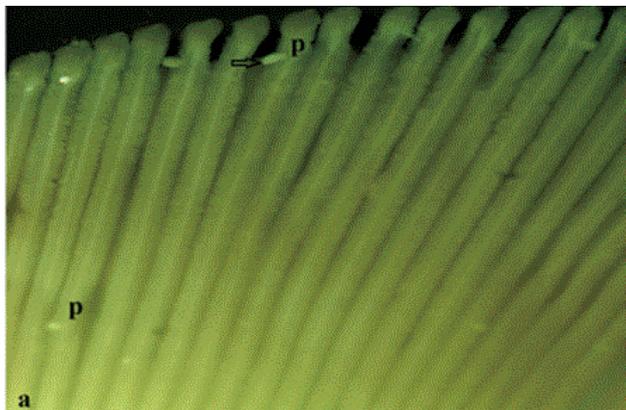
### Myxospore description

This is clearly mentioned in Table 3 and Figures 4b-6c.

**Measurements based on 10-12 myxospores in frontal view:** Myxospores measure  $15.62 \times 5.1 \mu\text{m}$ , elongately pyriform in frontal view having anterior end sharply pointed and slightly broad posterior end. Sutural line straight. Both shell valves uniformly thick, smooth, symmetrical,  $0.9 \mu\text{m}$  thickness. Parietal folds absent. Polar capsules rarely two, equal, sometimes unequal, elongately pyriform, running parallel to each other, measure  $10.0 \times 1.3 \mu\text{m}$  (larger polar capsules) and  $7.0 \times 1.30 \mu\text{m}$  (smaller polar capsules), having blunt anterior and rounded posterior end, occupying three fourth of the myxospore body cavity. Polar

**Table 2:** Comparison of *M. potularis* Madhavan, Bandyopadhyay and Santosh, 2013 with the original descriptions (measurements in micrometer).

Species	Host	Site of Infection	Locality	Myxospore	Polar Capsule
<i>M. potularis</i> (Present study)	<i>Labeo rohita</i>	Gill filament	Nursery Pond, Fagan Majra, Punjab (India)	7.83×4.79	4.00×1.23
<i>M. potularis</i> Madhavan, Bandyopadhyay and Santosh, 2013	<i>Labeo calbasu</i> , <i>L. bata</i> , <i>L. gonius</i> , <i>Cirrhinus reba</i>	Gill filament	West Bengal (India)	9.0×6.0	5.4×2.2



**Figure 4a:** Gills of *Cirrhinus mrigala* under stereozoom microscope showing plasmodia of *M. rocatlae* Basu and Haldar, 2002.

**Table 3:** Measurements ( $\mu\text{m}$ ) and ratio of *M. rocatlae* Basu and Haldar, 2002.

Characters	Range	Mean Values	SD
LS	14.62-16.62	15.62	0.56
WS	4.60-6.60	5.1	0.44
LPC	9.0-11.0	10	0.28
WPC	0.95-1.65	1.3	0.22
Ratio: LS/WS		3.06	
ICP		Absent	
NC		09-Oct	
Parietal Folds		Absent	

filament coils arranged perpendicular to the polar capsule axis, 9-10 in larger and 6-7 in smaller polar capsule. Intercapsular process (ICP) absent. Sporoplasm agranular and homogenous, occupy rest of the myxospore body cavity. Sporoplasmic nuclei two, 0.2-0.2  $\mu\text{m}$  in diameter. Iodinophilous vacuole absent.

#### Taxonomic summary of *M. rocatlae* Basu and Haldar, 2002

**Family :** Myxobolidae

**Type host:** *Cirrhinus mrigala* Hamilton vern. Mrigal

**Family:** Cyprinidae

**Age of the fish host :** 1-2 months

**Length of the fish:** 4.4 cm

**Type locality:** Nursery Pond, Fagan Majra, District Fatehgarh Sahib, Punjab, India

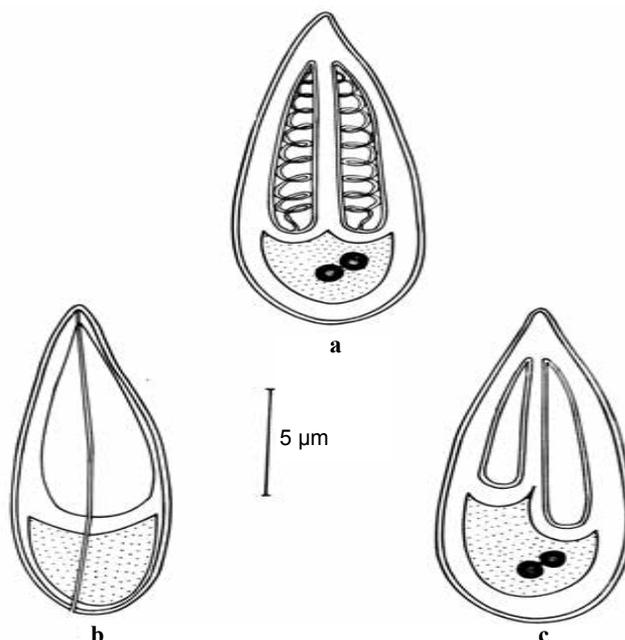
**Type specimen:** Paratypes are myxospores stained in Ziehl-Neelsen and Iron-haematoxylin, deposited in the Parasitology, Laboratory, Department of Zoology and Environmental Sciences, Punjabi University, Patiala, India. Slide no. CM/ZN/10.07.2015 and CM/IH/10.07.2015.



**Figure 4b:** Myxospores of *M. rocatlae* Basu and Haldar, 2002 in fresh preparation (Phase contrast) in frontal view.



**Figure 5:** a) Myxospores of *M. rocatlae* Basu and Haldar, 2002 stained in Ziehl-Neelsen. b) Myxospores of *M. rocatlae* Basu and Haldar, 2002 stained in Iron haematoxylin with extruded polar filaments.



**Figure 6:** a) Line drawing of fresh mature myxospore of *M. rocatlae* Basu and Haldar, 2002 stained in Ziehl-Neelsen (Frontal view). b) Line drawing of fresh mature myxospore of *M. rocatlae* Basu and Haldar, 2002 (Sutural view). c) Line drawing of fresh mature myxospore of *M. rocatlae* Basu and Haldar, 2002 stained in Ironhaematoxylin (Frontal view).

**Table 4:** Comparison of *M. rocatlae* Basu and Haldar, 2002 with the original description (measurements in micrometer).

Species	Host	Site of Infection	Locality	Myxospore	Polar Capsule
<i>M. rocatlae</i> (Present study)	<i>Cirrhinus mrigala</i>	Gill lamellae	Nursery Pond, Fagan Majra, Punjab (India)	15.62×5.10	10.0×1.30
<i>M. rocatlae</i> Basu and Haldar, 2002	Catla-Rohu hybrid	Gills, Gut	West Bengal (India)	18.3×6.0	12.6×2.8

**Site of infection:** Gill lamellae (Intralumellar vascular type LV1)

**Type of Plasmodia:** Type A (visible under binocular microscope)

**Prevalence of infection (%):** 52.5% (21/40)

**Pathogenicity:** Hypertrophy and hyperplasia of cellular elements of the gill lamellae located at its tip

**Gill plasmodial index (GPI):** 1 (1-5 plasmodia per gill) indicating light infection

**Clinical symptomatology:** Mucous laden and pale haemorrhagic gills

### Remarks

The observations on the specimens of *M. rocatlae* Basu and Haldar (2002) are in conformity with the original description except for some minor variations in the size of the myxospore and polar capsules. In the present study, myxospores with equal polar capsules are recorded. Earlier, the parasite was recorded from gills and gut of Catla-Rohu hybrid in West Bengal (India). In the present study, a new locality- Nursery Pond, Fagan Majra, District Fatehgarh Sahib, Punjab (India) and new host- *Cirrhinus mrigala* are recorded for this parasite (Table 4). In addition, gill

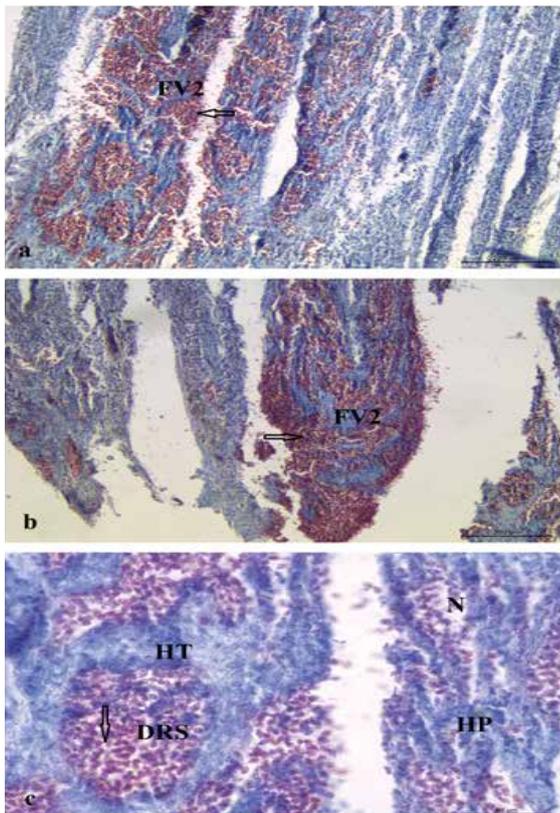
plasmodial index (GPI), tissue location and histopathogenesis are provided in the present study.

### Histopathogenesis

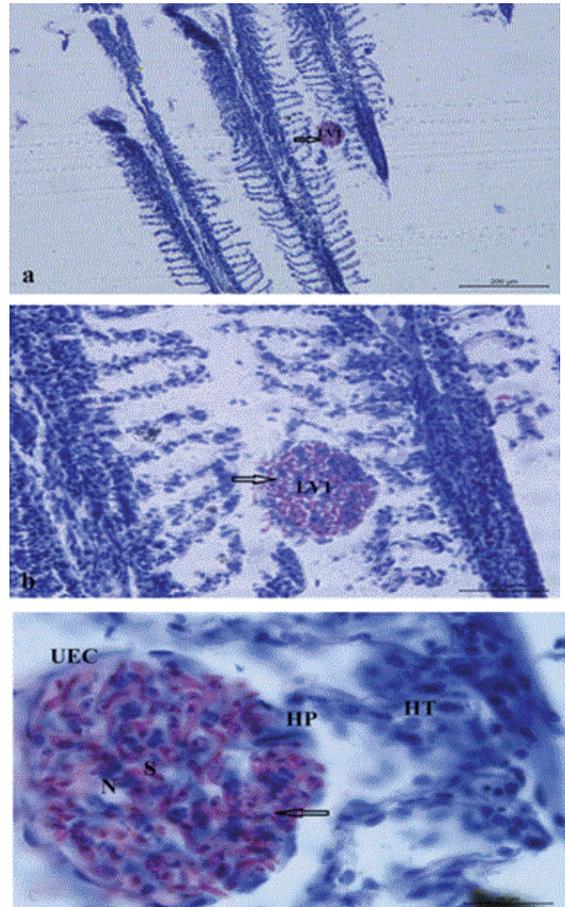
The plasmodia of *M. potularis* are located in the afferent artery of the gill filament of *L. rohita*. The large-sized abscesses on the gills of *L. rohita* are formed by the fusion of several plasmodia and are typed as intrafilamental vascular type of plasmodium FV2. The plasmodium is located inside the gill filament, elongated in shape and size ranging from 3-10 mm in diameter. Kaur et al. (2014) also reported that large-sized plasmodia damaged more than 50% of the gills causing respiratory distress and suffocation. Similar studies have been made by Schulman (1957), Current and Janovy (1978), Dykova and Lom (1978), Shariff (1982), Bowser and Conroy (1985), Rukayani (1990), Martins et al. (1997), Adriano et al. (2009), Chavda et al. (2010), de Campos et al. (2011) and Kaur and Katoch (2014). The histological section show severe infection damaging almost 95% of the gill filament and also overlying gill lamellae due to the hypertrophy, hyperplasia, vacuolization of the stratified epithelium and vascular endothelium of the filament. Necrosis of cellular elements of gill lamellae has been recorded due to severe infection. The observations clearly indicated that the destruction of respiratory surface and interruption of the vascular supply which may result in the suffocation of the fish due to lack of oxygen and can ultimately lead to the death of the

fish (Figures 7a-7c). According to Lebelo et al. (2001) structural damage and surface inflammation of gills leading to difficulties in osmoregulation and respiration causing decrease in oxygen uptake that causes hypoxia. Eissa (2002) and Sabri et al. (2010) described that such damages make gills and accessory respiratory organs less functional by reducing the respiratory surfaces. Histopathological changes derived from *Myxobolus* spp. infection in the gills of *Labeo rohita* and *Cirrhinus mrigala* are in accordance with the findings of Dey et al. (1988), Sanaullah and Ahmed (1980), and Dykova and Lom (1978). Kalavati and Narasimhamurti, (1985) and Kaur and Katoch (2014) observed that rupturing of cysts can also lead to hemorrhages, and may result in considerable loss of respiratory surface.

The plasmodium of *M. rocatlae* is located in the fine blood capillaries at the tip of the gill lamella of *C. mrigala* and is typed as intralamellar vascular type of plasmodium LV1 (Figures 8a and 8b). The plasmodia are round to oval and size ranging from 0.5-1.2 mm in diameter. The plasmodium occupies only single gill lamella at its tip and displaying the adjacent gill lamellae. Histological sections also reveal hypertrophy, lifting and hyperplasia of epithelial cells, goblet cells and pillar cells



**Figure 7:** a) Sagittal sections of infected gills of *L. rohita* showing plasmodia of *M. potularis* Madhavan et al., 2013 located in the gill filament. b) Sagittal sections of infected gills of *L. rohita* showing FV2 type of plasmodia of *M. potularis*. c) Magnified view of infected gills of *L. rohita* showing histopathological effects. FV2: Intrafilamental Vascular, HT: Hypertrophy, HP: Hyperplasia, N: Necrosis, DRS: Destruction of Respiratory Surface.



**Figure 8:** a) Sagittal sections of infected gills of *C. mrigala* showing plasmodia of *M. rocatlae* Basu and Haldar, 2002 located in the gill lamellae. b) Sagittal sections of infected gills of *C. mrigala* showing LV1 type of plasmodia of *M. rocatlae*. c) Magnified view of infected gills of *C. mrigala* showing histopathological effects. LV1 Intralamellar vascular, HT: Hypertrophy, HP: Hyperplasia, LEC: Lifting of Epithelial Cells.

within the gill lamellae thereby damaging and reducing the respiratory surface leading to stress conditions due to the lack of oxygen supply (Figure 8c). Similar observations were made by (Adriano et al., 2009) that the presence of plasmodia affect the gill functions and drastically reduces the respiratory surface. The location of plasmodia in the intralamellar site was recorded to be associated with hypertrophy and inflammation. (Kaur et al., 2013; Kaur and Katoch, 2014) reported cellular changes leads to the fusion of adjoining secondary lamellae. The presence of plasmodia exerted pressure on adjacent gill tissue and reduced the respiratory surface, particularly in cases of heavy infection with macroscopic cysts, or those with microscopic plasmodia in the lamellae. According to Longshaw et al., (2005) some species can affect growth, reproduction and cause death of the host and economic losses caused by these parasites in aquaculture have been well documented by Lom and Dykova (2006). In the present study, small cysts were observed in the gill lamellae infecting with *M. rocatlae*. Similarly, Szekely et al. (2015) reported the development of small cysts in the gill lamellae formed by *M.*

*kalavatae*, *M. meerutensis*, *M. bhadrensis* and *M. catlae*. The size of plasmodia varied with the species of the myxozoan parasite.

At the time of collection period, various parameters were also recorded. The mean value of Temperature, pH, Dissolved oxygen, Conductivity and TDS were 21.4, 8.21, 9.14, 1.33 and 891 respectively. All the parameters were recorded with the help of portable water testing kit. Nearly all the parameters measured were within the range of optimal values for carp production (Albaster and Lloyd, 1982; Boyd, 1982; Piper et al., 1982 and Svobodova et al., 1993). Awal et al. (2001) and Saha et al. (2012) affirm the role of water temperature and dissolved oxygen in inducing myxozoan infection in carps. Also Banerjee and Bandyopadhyay (2010) observed water temperature, pH, and DO an important water parameters that are related to disease infestation as they fluctuate more rapidly. During the entire sampling period, age and length of the fingerlings was recorded. The age of the fingerlings was recorded as 1-3 months and length ranged from 4-4.4 cm. The study clearly indicated light to severe infection in fingerlings as indicated by the gill plasmodial index (GPI). Gill plasmodial index (GPI) was recorded for both of *Myxobolus* species and ranged from 1-4 as calculated on the basis of number of plasmodia present per gill (one side) visible under the stereozoom binocular microscope and with naked eye (Kaur and Attri, 2015). Maximum number of plasmodia was recorded in *Labeo rohita* with *M. potularis* with GPI of 4 indicating severe infection followed by *M. rocatlae* in *Cirrhinus mrigala* with GPI of 1 indicating light infection. Fish fingerlings become more susceptible to myxozoan infection because of their immature immune system as discussed by Anderson (1974).

## Conclusion

The present study represents the first time data of myxozoan parasites infecting fingerlings in nursery ponds in Punjab, India. During the study, two already known species *M. potularis* Madhavan et al. (2013) and *M. rocatlae* Basu and Haldar (2002) were redescribed morphologically. In addition to this histopathogenesis have been also described. Plasmodia of *M. potularis* are located in the afferent artery of the gill filament by the fusion of several plasmodia and are typed as Intrafilamental vascular type (FV2) resulting necrosis, hypertrophy and hyperplasia of cellular elements of the gill lamellae. In *M. rocatlae*, Plasmodia is of LV1 type showed hypertrophy, lifting and hyperplasia of epithelial cells, goblet cells and pillar cells within the gill lamellae. GPI was calculated as per Kaur and Attri (2015). *M. potularis* showed much more intensity of infection than *M. rocatlae*. Also some parameters like temperature, pH, DO and conductivity were recorded at the sampling time.

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