



RESEARCH ARTICLE

REDESCRIPTION AND HISTOPATHOLOGY OF *MYXOBOLUS NANOKIENSIS* AND *M. SLENDRII* INFECTING GILLS OF FINGERLINGS OF INDIAN MAJOR CARPS

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ABSTRACT

During the present study, two myxosporean species, *M. nanokiensis* Kaur *et al.*, (2015) and *M. slendrii* Kaur and Singh (2010) have been described from the gills of *Cirrhinus mrigala* Hamilton vern. mrigal and *Labeo rohita* Hamilton vern. rohu at nursery ponds located in village Fagan majra, District Fatehgarh Sahib, Punjab, India. Earlier, *M. nanokiensis* were described from *L. rohita* and *M. slendrii* from *C. mrigala*. 40 specimens of *Labeo rohita* and 40 specimens of *Cirrhinus mrigala* were examined from November 2014 to April 2015. Out of which 33 and 21 fishes were infected respectively. The age of the fish was recorded as 2-3 months and length of the fish was 4-4.5 cm. The prevalence rate was more in *M. nanokiensis* (82.5%) than *M. slendrii* (52.5%). The histological effects of the pathogen were observed by light microscopy. The plasmodia of *M. nanokiensis* were located in the fine blood capillaries at the centre of gill lamella and plasmodia of *M. slendrii* were located within the arteries at the tip of the gill filaments. *M. nanokiensis* was highly pathogenic than *M. slendrii*. The plasmodia of *M. nanokiensis* were typed in "A" category caused complete necrosis of cellular elements and degeneration of gill lamellae and *M. slendrii* also in "A" category caused total destruction of the gill filament. The gill plasmodial index (GPI) was 1 for both species.

Key words: Gills, Myxobolus, Nursery ponds, Histology.

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INTRODUCTION

The study site Fagan Majra fish farm is located in the district of Fatehgarh Sahib managed by Fisheries Department, Government of Punjab consists of a total of 59 culture ponds. Out of which 14 are hatchery/ nursery ponds in which Indian major carps such as catla, rohu, mrigal, common carp, grass carp and silver carp are cultured in a semi-intensive polyculture system for supply to fish farmers all over the Punjab and adjoining states. Myxozoans are one of the economically important group of microscopic metazoan parasites as they infect fish harvested for food and most commonly parasitize invertebrates, typically oligochaetes, bryozoans, polychaetes 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). Developmental stages have also been found in waterfowl, but no myxozoan has been known to be hazardous to human health.

Myxozoan parasites can be located in every organ of the fish host, however, the gills are most commonly infested where they may cause mild or severe serious structural changes depending on the intensity of infection. Parasitic diseases are the most serious limiting factors in aquaculture in India because fish are usually polycultured in high density in a restricted water body, where fish pathogens can easily be transmitted amongst fish. Due to rich blood supply and a site of gaseous exchange, gills are prone to be more infected (Martins *et al.*, 1997, 2000, 2001). Myxozoan parasites are common in juvenile carps in nursery ponds and high mortality rates caused by their infections in the gills have raised serious concern among fish farmers. There are alarming economical losses due to *Myxobolus* spp. infestation of the major carp in the nursery ponds as reported by Sanaullah and Ahmed (1980).

MATERIALS 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 80 fish

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specimens belonging to two genera were examined for the presence of myxozoan infections. Out of which, 54 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 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 formula was applied.

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

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 8-10µm 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 percent 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).

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 µm)

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)

RESULTS

A. *Myxobolus nanokiensis* Kaur, Katoch, Dar and Singh, 2015

Plasmodia

Round, minute, creamish, visible under stereozoom binocular microscope, 0.20-8mm in diameter, attached to gill lamellae, histozoic, 1-4 in number per gill, 120-225 myxospores present per plasmodium. No clinical signs on the gills (Fig.1).

Taxonomic summary of *M. nanokiensis* Kaur, Katoch, Dar and Singh, 2015

Family: Myxobolidae

Type host: *Cirrhinus mrigala* Hamilton vern. mrigal

Family Cyprinidae

Age of the fish host: 2-3 months

Length of the fish: 6.5 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, Punjabi University, Patiala, India.

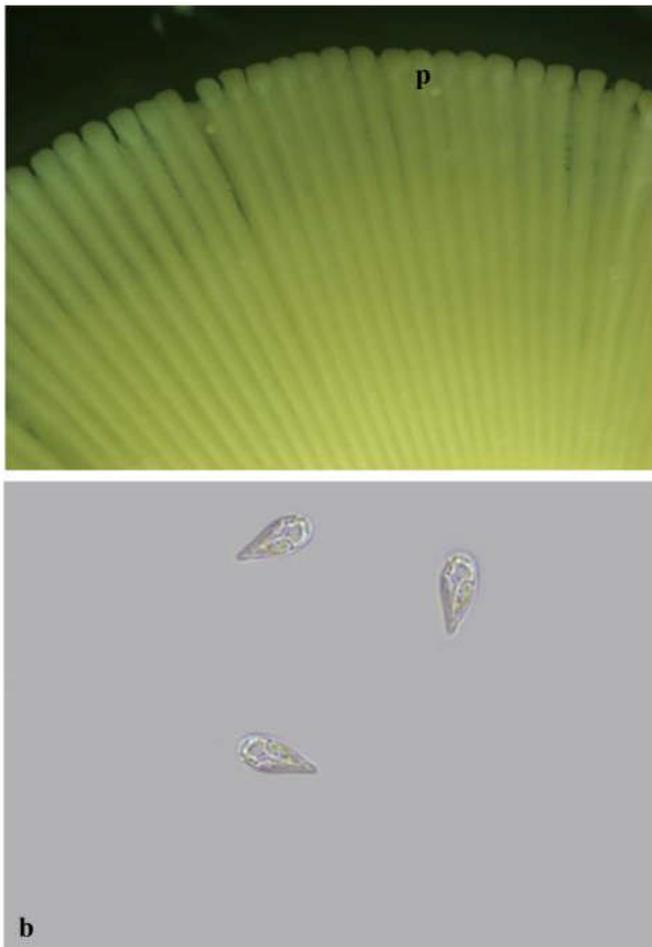


Fig. 1 (a-b)

20 µm

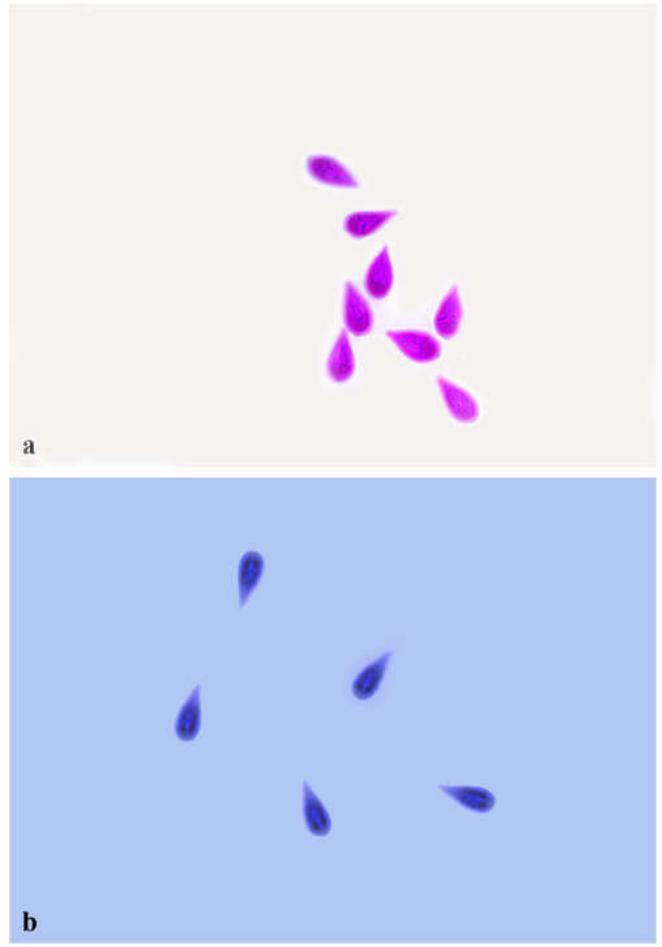


Fig. 2 (a,b)

20 µm

Fig. 1. (a) Gills of *Cirrhinus mrigala* infected with myxozoan plasmodia *Myxobolus nanokiensis* (b) Fresh myxospores of *M. nanokiensis* (Phase contrast)

Fig. 2. (a) *Myxobolus nanokiensis* stained with Ziehl-Neelsen stain (20µm) (b) *M. nanokiensis* stained with Iron Haematoxylin (20µm)

Slide no. CM/ZN/12.05.2015 HM/IH/12.05.2015

Site of infection: Gill lamellae (Intralamellar vascular type LV3)

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

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

Pathogenicity: Hypertrophy and hyperplasia of infected gill lamellae and distortion of adjacent lamellae

Gill Plasmodial Index (GPI): 1 (1-4 plasmodia per gill) indicating light infection

Clinical symptomatology: No clinical signs

Myxospore description (Table 1)

(Measurements based on 10-12 myxospores in frontal view)

Myxospores measure $9.91 \times 4.61 \mu\text{m}$, pyriform with bluntly pointed anterior end and rounded posterior end. In sutural view anterior end slightly bent. Sutural line straight. Both shell valves thin, smooth, symmetrical, $0.2 \mu\text{m}$ thick. Parietal folds absent.

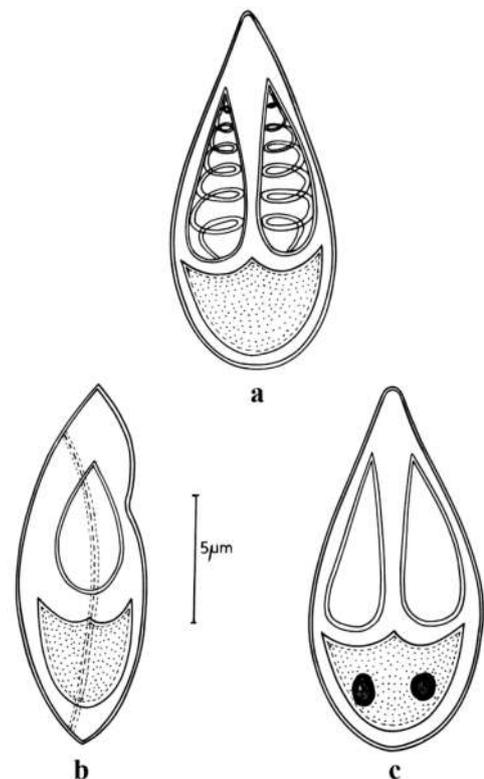


Fig. 3. (a-c) Line drawings of myxospores infected with *M. nanokiensis*

Table 1. Measurements (μm) and ratio of *M. nanokiensis* Kaur, Katoch, Dar and Singh, 2015

| Characters | Range | Mean Values | SD |
|----------------|------------|-------------|------|
| LS | 8.96-10.86 | 9.91 | 0.82 |
| WS | 3.61-5.61 | 4.61 | 0.60 |
| LPC | 4.94-6.64 | 5.79 | 0.52 |
| WPC | 0.76-2.76 | 1.76 | 0.32 |
| Ratio: LS/WS | | 2.14 | |
| ICP | | Absent | |
| NC | | 6-7 | |
| Parietal Folds | | Absent | |

Table 2. Comparison of *M. nanokiensis* Kaur et al., 2015 with the original description (measurements in micrometer)

| Species | Host | Site of infection | Locality | Myxospore | Polar capsule |
|--------------------------------------------|--------------------------|-------------------|--------------------------------------|-----------|---------------|
| <i>M. nanokiensis</i> (Present study) | <i>Cirrhinus mrigala</i> | Gill lamellae | Nursery Pond, Fagan Majra, Punjab | 9.91x4.61 | 5.79x1.76 |
| <i>M. nanokiensis</i> Kaur et al., 2015 | <i>Labeo rohita</i> | Gill lamellae | Patiala (Punjab) | 9.28x5.71 | 5.71x2.73 |

Table 3. Measurements (μm) and ratio of *M. slendrii* Kaur and Singh, 2010

| Characters | Range | Mean Values | SD |
|----------------|-----------|-------------|------|
| LS | 11.02- | 12.02 | 0.62 |
| WS | 13.02 | 3.43 | 0.28 |
| LPC | 2.20-4.66 | 6.59 | 0.42 |
| WPC | 5.50-7.68 | 0.97 | 0.22 |
| Ratio: LS/WS | 0.80-1.14 | 3.50 | |
| ICP | | Absent | |
| NC | | 8-9 | |
| Parietal Folds | | Absent | |

Table 4. Comparison of *M. slendrii* Kaur and Singh, 2010 with the original description (measurements in micrometer)

| Species | Host | Site of infection | Locality | Myxospore | Polar capsule |
|--------------------------------------------|--------------------------|-------------------|----------------------------------------------|------------|---------------|
| <i>M. slendrii</i> (Present study) | <i>Labeo rohita</i> | Gill filament | Nursery Pond, Fagan Majra, Punjab (India) | 12.02x3.43 | 6.59x0.97 |
| <i>M. slendrii</i> Kaur and Singh, 2010 | <i>Cirrhinus mrigala</i> | Gill lamellae | Kanjali wetland, Punjab (India) | 9.75x7.15 | 5.25x2.0 |

Polar capsules two equal, pyriform, measure $5.79 \times 1.76 \mu\text{m}$ with sharply pointed anterior end and rounded posterior end and occupying more than half of myxospore body cavity, placed slightly posterior to the anterior tip. Polar filament coils 6-7 in number, arranged perpendicular to the polar capsule axis. Intercapsular Process (ICP) absent. Sporoplasm agranular, homogenous, with two nuclei, $0.25-0.30 \mu\text{m}$ in diameter. Iodinophilous vacuole absent (Fig. 2, 3).

Remarks

The present observations on *M. nanokiensis* Kaur et al., (2015) were in conformity with the original description except for the some minor variations in the size of the myxospore, size and position of the polar capsules. Earlier, the parasite was recorded from the gills of *Labeo rohita* from Patiala (Punjab). During the present study, parasite species was collected from the gill lamellae of *Cirrhinus mrigala*. A new locality- Nursery Pond, Fagan Majra, District Fatehgarh Sahib, Punjab, India has been recorded for this parasite (Table 2). In addition, gill plasmodial index (GPI), type of plasmodia and histopathogenesis for *M. nanokiensis* have been provided in the present study

B. *Myxobolus slendrii* Kaur and Singh, 2010

Plasmodia: Small, minute, microscopic, rounded, white, 0.9-1.4mm in diameter, attached to the gill lamellae, histozoic, 1-4 in number per gill, 60-70 myxospores present per plasmodium. No clinical signs on the gills (Fig. 5).

Taxonomic summary of *M. slendrii* Kaur and Singh, 2010

Family: Myxobolidae

Type host: *Labeo rohita* Hamilton vern. Rohu

Family: Cyprinidae

Age of the fish host: 2-3 months

Length of the fish: 5.8 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/21.09.2015 and HM/IH/21.09.2015

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

Type of Plasmodium: Type A (visible under binocular microscope)

Prevalence of infection (%): 82.5% (33/40)

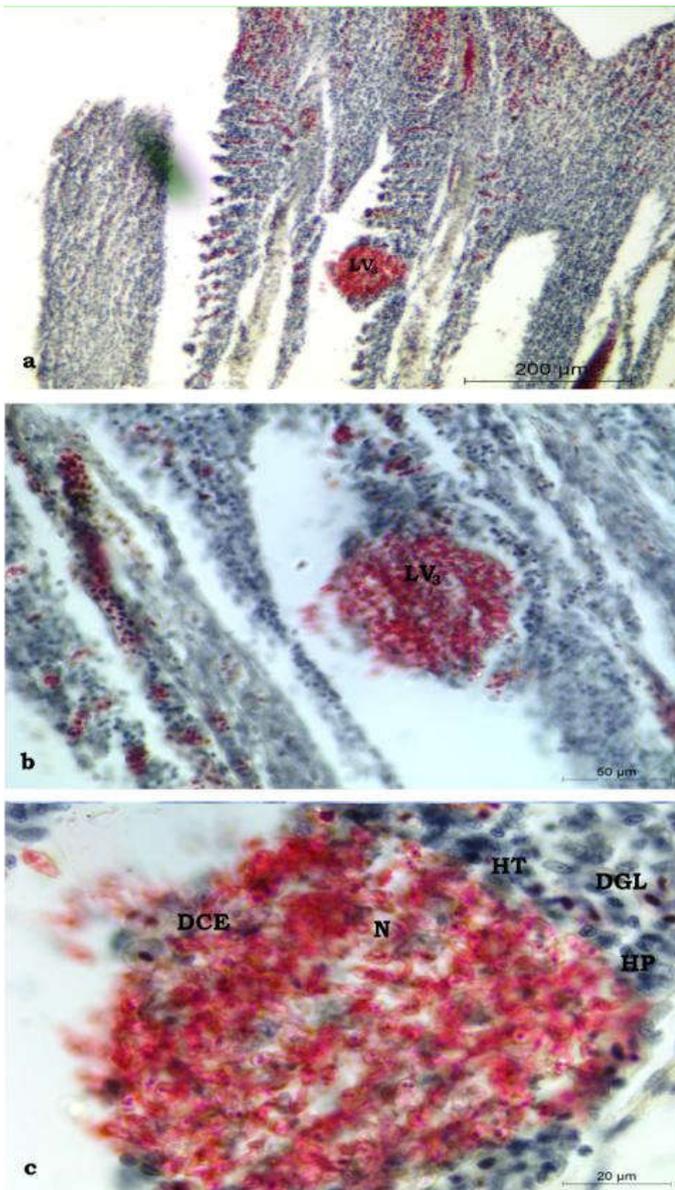


Fig. 4. (a-c) Sagittal sections of infected gills of *M. nanokiensis* showing LV³ type of plasmodia and histopathological effects: LV³: Intralamellar vascular; HT: hypertrophy; N: necrosis; DCE: degeneration of cellular elements; DGL: degeneration of gill lamellae; HP: hyperplasia

Pathogenicity: Vascular hypertrophy, necrosis of cellular elements of gill filament, atrophy of overlying gill lamellae

Gill plasmodial index (GPI): 1 (1-4 plasmodia per gill) indicating light infection

Clinical symptomatology: Mucous laden gills

Myxospore description (Table III)

(Measurements based on 10-12 myxospores in frontal view)

Myxospores measure 12.02x3.43µm, elongately pyriform in frontal view having sharply pointed anterior end and rounded posterior end. Sutural line straight. Both shell valves symmetrical, smooth, thin, 0.30µm thick. Parietal folds absent. Polar capsules two, equal, pyriform, measure 6.59x0.97µm with sharply pointed anterior end and flattened posterior end, occupy more than half of the myxospore body cavity and placed parallel to each other.

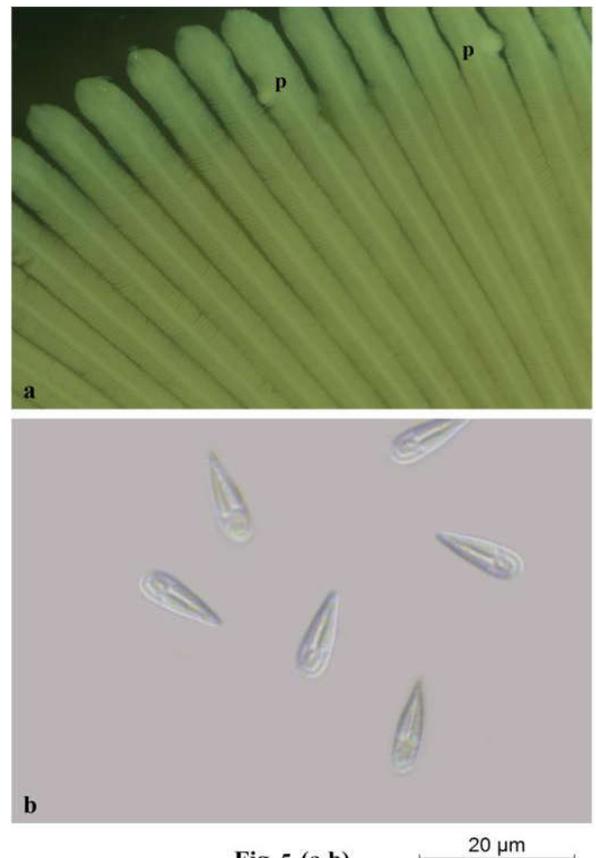


Fig. 5. (a) Gills of *Labeo rohita* infected with myxozoan plasmodia *Myxobolus splendrii* (b) Fresh myxospores of *M. splendrii* (Phase contrast)

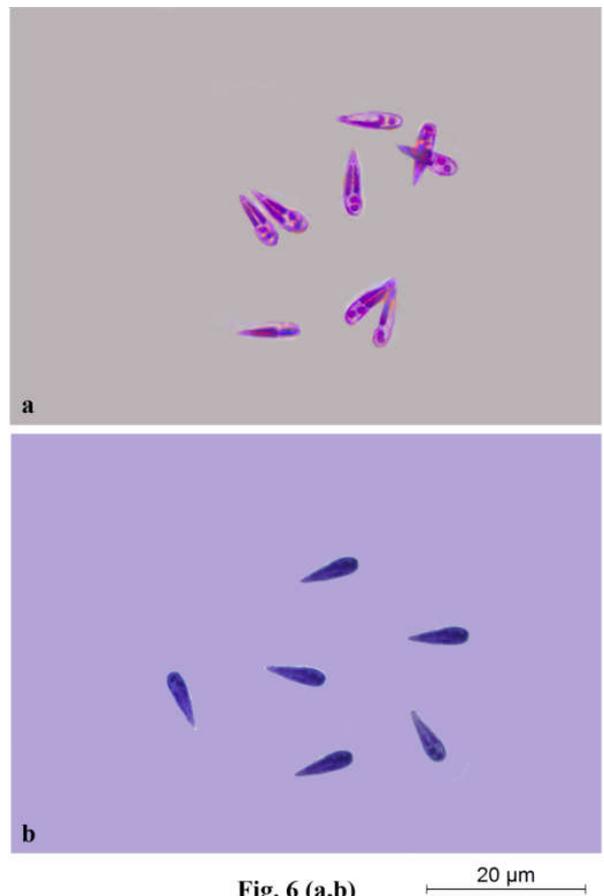


Fig. 6. (a) *Myxobolus splendrii* stained with Ziehl-Neelsen stain (20µm) (b) *M. splendrii* stained with Iron Haematoxylin (20µm)

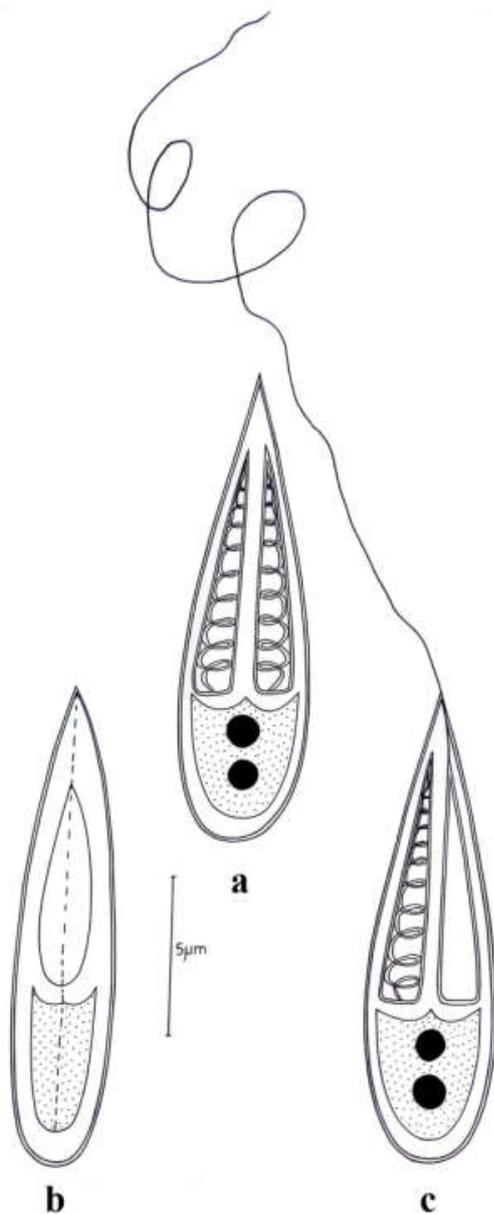


Fig. 7. (a-c) Line drawings of myxospores infected with *M. splendrii*

Polar filament coils 8-9 in number arranged perpendicular to the polar capsule axis. Polar filaments thread-like when extruded, 45.0µm in length. Intercapsular process (ICP) absent. Sporoplasm agranular and homogenous filling the extracapsular space behind the polar capsules. Sporoplasmic nuclei two, 1.70-1.90µm in diameter. Iodinophilous vacuole absent (Fig. 6, 7).

Remarks

The present observations (LS/WS: 3.50) on *M. splendrii* Kaur and Singh (2010) are in conformity with the original description (LS/WS: 4.35) except some variations in the size of the myxospore and polar capsules (as indicated by LS/WS ratio). Earlier, the parasite was recorded from the gill lamellae of *Cirrhinus mrigala* in Kanjali wetland, Punjab (India). In the present study, a new host- *L. rohita*, a new location- gill filament and a new locality- Nursery Pond, Fagan Majra, District Fatehgarh Sahib, Punjab (India) are recorded for this parasite (Table 4). In addition, gill plasmodial index (GPI), type of plasmodium and histopathogenesis of *M. splendrii* Kaur and Singh (2010) are provided in the present study.

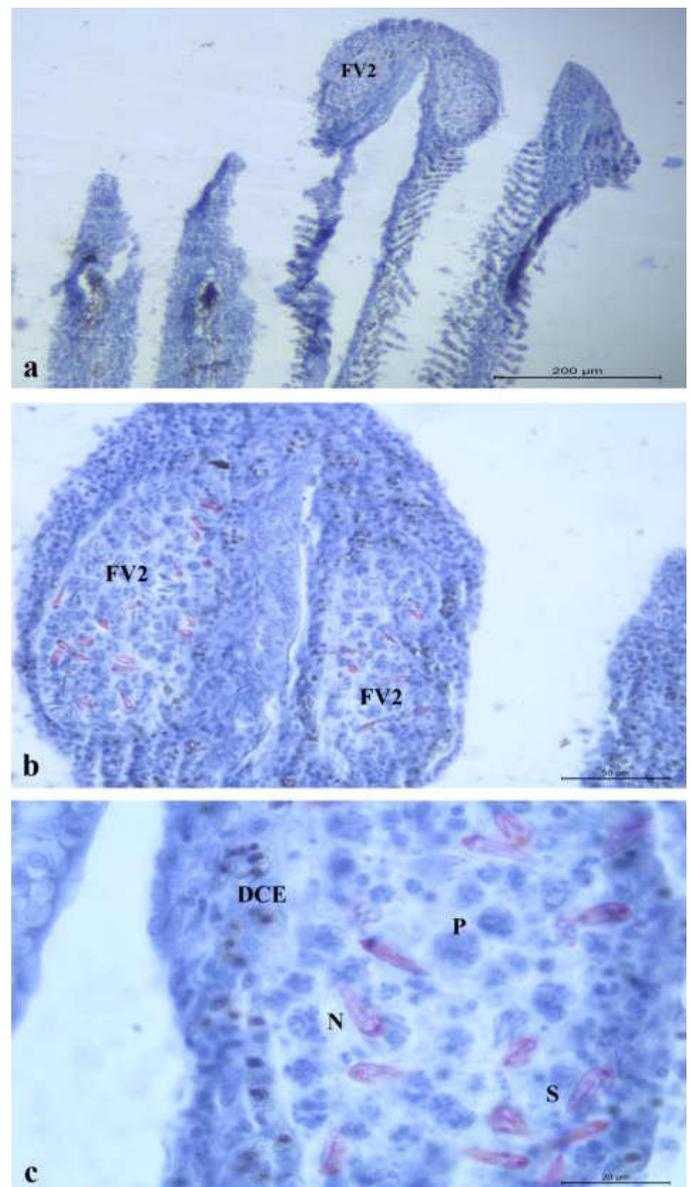


Fig. 8. (a-c) Sagittal sections of infected gills of *M. splendrii* showing FV² type of plasmodia and histopathological effects: FV²: Intrafilamental vascular; N: necrosis; DCE: degeneration of cellular elements; P: pansporoblasts

Histopathogenesis and Discussion

Both species have been already described from the gill lamellae of *Labeo rohita* and *Cirrhinus mrigala* reported from Patiala and Kanjali wetland, Punjab respectively. But in the present study, a new host, new locality were recorded and histopathogenesis were also provided for both the species. The plasmodium of *M. nanokiensis* is located in the fine blood capillaries at the centre of gill lamella of *C. mrigala* and is typed as intralamellar vascular type LV3 (Fig. 4). The plasmodia are rounded in shape and size ranging from 0.2-0.8mm in diameter. The gill lamellae adjoining the infected lamella bend towards the plasmodium in such a way that it tends to cover it on the sides. The adjoining gill lamellae seem to bear young plasmodia developing at their base. The mature plasmodium rupture at the tip releasing myxospores which are seen to attach to the fresh gill lamellae. Young plasmodia also cause necrosis of the vascular elements of the gill lamellae showing haemorrhagia. According to Kaur and Katoch (2014) and Kalavati and Narasimhamurti (1985) rupturing of cysts

can also lead to hemorrhages, resulting sometimes in considerable loss of blood. Histological sections reveal hyperplasia, hypertrophy and destruction of cellular elements such as endothelial of blood vessels, chloride cells and pillar cells etc. These findings are in conformity with Dar *et al.*, (2017) reported for *Myxobolus* species (*M. rocatlae* and *M. kashmiriensis* n. sp.) from the gills of *Labeo rohita* and *Schizothorax esocinus* infecting capillary network of the gill lamellae. Ahmad and Kaur (2017) also recorded such type of changes and total destruction of the cellular elements. Complete necrosis of cellular elements and degeneration of gill lamellae is also observed due to the large sized plasmodium. Eissa (2002) and Sabri *et al.*, (2010) described that such damages make gills and accessory respiratory organs less functional by reducing the respiratory surfaces. 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).

The plasmodia of *M. splendrii* are located within the arteries at the tip of the gill filaments of *L. rohita* and are typed as intrafilamental vascular type FV2 (Fig. 8). In the histological section, the plasmodia are two in number located at the tip of each gill filament. The plasmodia are round to oval and size ranging from 0.9-1.4mm in diameter. Both the plasmodia are fused at the upper margin forming a large abscess. Hypertrophy, lifting of epithelial cells is observed in the histological sections. Due to the fusion of two plasmodia at the tip of the gill filament, necrosis and degeneration of the cellular elements of gill filament is observed. These cellular changes led to the fusion of adjoining lamellae were also reported by Kaur *et al.*, (2013) and Kaur and Katoch (2014). Adriano *et al.*, (2009); Dykova and Lom (1978) and Sanaullah and Ahmed (1980) also reported myxoboliasis caused by myxozoans in the gills of Jau catfish (Zungaro jahu), *Catla catla* and *Esox lucis*. The plasmodia contain mature myxospores along with developing pansporoblasts occluding the afferent arteries of the gill filament. It is also observed that plasmodia of *M. splendrii* not only cause total destruction of the gill filament but also whole of the secondary lamellar surface present along the infected gill filament. 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. During the sampling period, the age of the fingerlings was also 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). For both species same number of plasmodia were recorded 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).

Ethical Approval

Not required as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

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Conflict of interest: There is no conflict of interest to disclose.

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