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Mxyobolus vascularis N. Sp. (cnidaria: myxozoa: myxosporea), a New Parasite Infecting Fingerlings of Indian Major Carps in Aquaculture in Punjab, India

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Abstract:

During the present study, a new myxosporean parasite, *Myxobolus vascularis* n. sp. has been described from the gill lamellae of fingerlings of *Cirrhinus mrigala* Hamilton vern. mrigal collected from polyculture nursery ponds located in village Fagan majra, District Fategarh Sahib, Punjab (India). The length and age of the fish host was 4.5cm and 1-2 months respectively. According to the size, the plasmodia were of two types: Type B were small sized, rounded, 0.5mm in diameter and Type C were highly elongated, large sized, 3x0.7mm seen as white pustules on the gill lamellae. The myxospores were pyriform in shape and measured 10.73x 4.88µm. Polar capsules were equal, elongately pyriform, measured 6.51x 1.80µm, with 8-9 polar filament coils arranged perpendicular to the polar axis. The gill plasmodium index was recorded to be 3 indicated heavy infection. Histopathological evaluation of the infection recorded dilation and total obstruction of the gill lamella and showed necrosis, hypertrophy, hyperplasia and lifting of the epithelial cells.

Keywords: Myxozoa, Fingerlings, Gills, *Myxobolus vascularis*, Nursery ponds

INTRODUCTION

A large variety of fishes in aquaculture ponds are vulnerable to various parasitic infections, out of which Myxozoa is emerging as a major group. These are microscopic, parasitic cnidarians of vertebrates and invertebrates with extremely reduced body size, structure and possess very complicated life cycles characterized by the formation of myxospores (Morris and Adams, 2007). 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.

In the recent past, numerous species of myxosporean have been recorded in the region from freshwater fishes inhabiting important wetlands of Punjab (Kaur and Singh, 2008; Kaur and Singh, 2009; Kaur and Singh 2010a, 2010b; Kaur and Singh, 2011a, 2011b, 2011c, 2011d; Kaur and Singh, 2012a, 2012b, 2012c; Kaur et al., 2013a,b; Kaur and Attri, 2015a; Kaur and Attri, 2015b; Kaur and Gupta, 2015; and also from aquaculture fish (Kaur and Katoch, 2014; Kaur and Katoch, 2016; Kaur et al., 2014a,b; Kaur et al., 2015). Fagan Majra fish farm is located in the district of Fatehgarh Sahib, Punjab. Presently, 59 cultured ponds are there, out of which 14 are nursery / hatchery ponds in which seeds of different major carps are cultured.

In Punjab, nearly 9,890 hectare is under fish farming as compared to 343 hactre in 1980-81. For the last ten years, the States aquaculture production has been increased and contributed an annual average growth of 6,000 tons per annum.

MATERIAL AND METHODS

Live fingerlings of different species were procured from nursery ponds for parasitological analysis. Fish species examined included only native carps such as *Cirrhinus mrigala* Hamilton vern. mrigal, *Catla catla* Hamilton vern. thail, *Labeo rohita* Hamilton vern. rohu and *Carassius carassius* Linnaues vern. crucian carp. Various organs such as skin, gills, scales, kidney, stomach, heart, air bladder and intestine were examined under stereozoom binocular microscope for the presence of plasmodia. The infection was recorded in the gills 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. 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. Fresh myxospores were treated with Lugol's iodine solution to observe the presence of iodinophilous vacuole.

In order to cause eversion of the polar filament, fresh myxospores were treated with 8% KOH for five minutes. 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 calculations of prevalence studies, the following formula was applied

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

Gill plasmodial index (GPI) was calculated on the basis of number of plasmodia present per gill (one side) visible under the stereozoom binocular microscope as per Kaur and Attri (2015). 0-0 (no infection); 1-5 (light infection-1); 5-10 (moderate infection-2); 10-20 (heavy infection-3); 20-50 (severe infection-4). 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 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

Also type of plasmodia were categorized into three types

Type A: Plasmodia visible under binocular microscope (size range = 40-200µm)
Type B: Plasmodia visible under stereozoom microscope (size range= 0.2-0.9mm)
Type C: Plasmodia visible with naked eye (size range= 0.9-3.0mm)

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 Schaudinn's and Bouin's fixative. The stains such as Heidenhain's Iron haematoxylin, Delafield 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. Iron haematoxylin stain proved useful to show the presence or absence of intercapsular process and number of sporoplasmic and capsulogenic nuclei. Similarly, 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.

For histology, infected organs were cut into small pieces and fixed in Bouin's fixative, 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) as per the protocol by Katoch and Kaur (2016).

RESULTS

***Myxobolus vascularis* n. sp.**
Vegetative stages (Plasmodia)

The plasmodia were of two types: Type B were small sized, rounded, 0.5mm in diameter and Type C were highly elongated, large sized, 3x0.7mm (length x breadth) seen as white pustules on the gill lamellae, histozoic, 10-15 in number per gill, 1000-1200 myxospores present per plasmodium. Gills were pale in colour (**Fig. 1**).

Taxanomic summary of *M. vascularis* n. sp.

Family: Myxobolidae

Type host: *Cirrhinus mrigala* Hamilton vern. Mrigal

Family: Cyprinidae

Age of the fish host: 1-2 months

Length of the fish: 4.5 cm

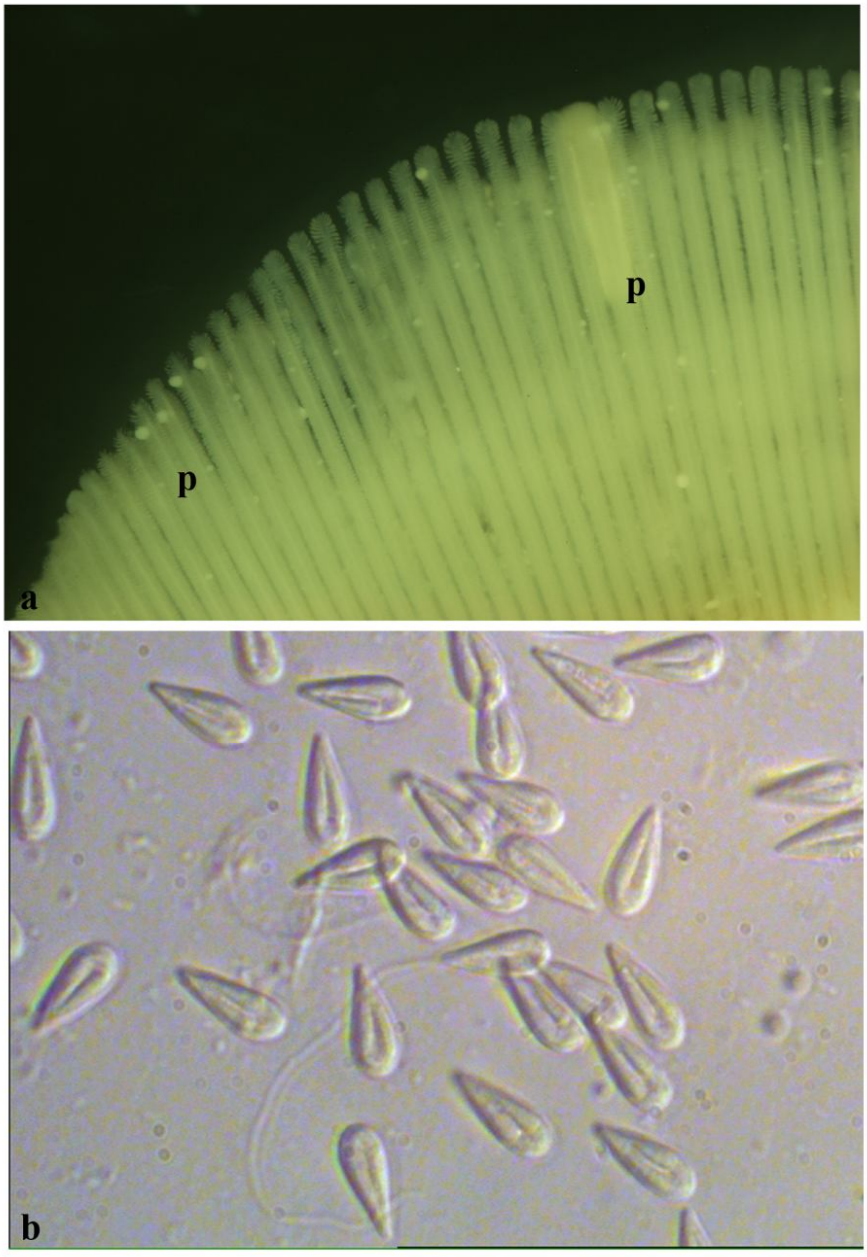

Figure 1.
Gills of *Cirrhinus mrigala* showing (a) Two types of plasmodia of *Myxobolus vascularis* n. sp. (b) Fresh myxospores (frontal view)

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

Type specimen: Paratypes are myxospores stained in Ziehl-Neelsen and Iron-haemotoxylin, deposited in the Parasitology Laboratory, Department of Zoology, Punjabi University, Patiala, India.

Slide no. CM / ZN / 16.11.2015 and CM / IH / 16.11.2015

Site of infection: Gill lamellae (Intralamellar vascular type LV1)

Type of Plasmodia: Type B (visible under stereozoom microscope) and Type C (visible with naked eye)

Prevalence of infection (%): 70% (28 / 40)

Pathogenicity: Hypertrophy and hyperplasia of gill lamellae

Gill plasmodial index (GPI): 3 (10-15 plasmodia per gill) indicating heavy infection

Clinical symptomatology: Mucous laden gills

Etymology: The species epithet '*vascularis*' is based on the tissue location of the parasite i.e blood vessels of gill lamella

Myxospore description (Table 1)

(Measurements based on 10-12 myxospores in frontal view)
Myxospores measure 10.73x4.88µm, pyriform with sharply pointed anterior end and rounded posterior end. Both shell valves thin, smooth, symmetrical, 0.30µm in thickness. Sutural line not visible. Parietal folds absent. Polar capsules two, equal, elongately pyriform, measure 6.51x1.80µm, positioned slightly below from the tip of the myxospore, lie parallel to each other, occupying three fourth of the myxospore body cavity. Polar filament coils 8-9 in number arranged perpendicular to the polar axis. Polar filaments thin and thread-like when extruded. Intercapsular process (ICP) absent. Sporoplasm agranular, homogenous, cup-shaped occupying rest of the myxospore body cavity. Sporoplamic nuclei two, 0.33-0.40µm in diameter. Iodinophilous vacuole absent (**Fig. 2, Fig. 3**).

Table1: Measurements (µm) and ratio of *M. vascularis* n. sp.

Characters	Range	Mean Values	SD
LS	9.73-1173	10.73	1.12
WS	3.88-5.88	4.88	0.28
LPC	5.45-7.57	6.51	0.56
WPC	1.10-2.50	1.80	1.22
Ratio: LS/WS		1.64	
ICP		Absent	
NC		8-9	
Parietal Folds		Absent	

DISCUSSION

The present species is compared with morphologically similar myxospores of the species i.e. *M. andhrae* Lalitha (1969) infecting surface wall of the intestine in *Channa punctatus; M. bengalensis* Chakravarty and Basu (1948) infecting gills of *Catla catla* Hamilton; *M. bhadurius* Sarkar (1985) infecting gall bladder of *Wallago attu*; *M. bilobus* Cone *et al*. (2005) infecting gill filaments of *Notemigonus crysoleucas*; *M. calbasui* Chakravarty (1939) infecting gall bladder of *Labeo calbasu* Hamilton, *Labeo rohita* Hamilton and *C. mrigala* Hamilton; *M. catlae* chakravarty (1943) infecting gills of *Catla catla* Hamilton, *Labeo rohita* Hamilton and *C. mrigala* Hamilton; *M. catmrigalae* Basu and Haldar (2004) infecting gill lamellae of *Catla catla* and *C. mrigala*; *M. chinsurahensis* Basu and Haldar (2003) infecting scales of *Anabas testudineus*; *M. coeli* Haldar *et al*. (1996) infecting gall bladder of *Chanos chanos*; *M. cuttacki* Haldar *et al*. (1996) infecting branchial filaments of *Cyprinus carpio*; *M. dasgupti* Haldar *et al*. (1996) infecting body muscles and gills of *Mugil tade*; *M. eirasi* Kaur and Singh (2009) infecting gills of *C. mrigala*; *M. etsatsaensis* Reed *et al.* (2002) infecting secondary gill filaments of *Barbus thamalakanesis*; *M. harikensis* Kaur and Singh (2011) infecting caudal fin of *C.mrigala*; *M. hyderabadense* Lalitha (1969) infecting gill filaments of *Barbus pinnauratus* and *Puntius filamentosus*; *M. indiae* Lalitha (1969) infecting gill filaments of *Barbus sarana*; *M. kalmani* Kaur and Singh (2011b) infecting gill lamellae of *Cirrhinus reba*; *M. kribiensis* Fomena and Bouix (1994) infecting skin, eyes and sclera of *Brycinus longipinnis*; *M. leptobarbi* Szekely *et al.* (2009) infecting muscle cells of *Leptobarbus hoevenii*; *M. longisporus* Nie *et al.* (1992) infecting gills of *Cyprinus carpio*; *M. maculatus* Casal *et al*. (2002) infecting kidney of *Metynnismaculatus*; *M. maruliensis* Sarkar *et al*. (1985) infecting kidney of *Channa marulius*; *M. mugilli* Haldar *et al*. (1996) infecting gills of *Mugil cephalus*; *M. nanokiensis* Kaur *et al.* (2013) infecting gills of *Labeo rohita* Hamilton; *M. oliveirai* Milanin *et al.* (2010) infecting gill filaments of *B. hilarii*; *M. orissae* Haldar *et al*. (1997) infecting gills of *C. mrigala* Hamilton; *M. procerus* Kudo (1934) infecting muscle cells of *Precopsis omiscomaycus*; *M. rocatlae* Basu and Haldar (2002b) infecting gills and gut of Catla-Rohu hybrid*; M. ropari* Kaur and Singh (2011a) infecting gill lamellae of *C. mrigala; M. scatophagi* Haldar *et al*. (1996) infecting gills of *Scatophagous argus*; *M. slendrii* Kaur and Singh (2010) infecting gills of *C. mrigala* and *M. variformis* Haldar *et al.* (1996) infecting body muscles and gills of *Mystus gulio* Hamilton. The present species *M. vascularis* n. sp. is compared with all the above mentioned species and is found different in myxospore morphology and morphometrics (**Table 2**).

The myxospores of the present species are characterized in having pyriform shape with sharply pointed anterior end and rounded posterior end. In this respect, the present species is compared with *M. bengalensis, M. bhadurius, M. chinsurahensis* and *M. nanokiensis* in having less slender myxospore body. Furthermore, it differed from *M. catlae, M. cuttacki, M. procerus* and *M. slendrii* having extremely slender myxospore body.

The present species is also closely compared with *M. andhrae, M. bilobus, M. calbasui, M. catmrigalae, M. coeli, M. etsatsaensis, M. harikensis, M. indiae, M. kribiensis, M. leptobarbi, M. orisssae, M. rocatlae, M. scatophagi* and *M. variformis* in having unequal polar capsules, in contrast to this, in the present species the polar capsules are equal. In addition, *M. andhrae, M. hyderabadense* and *M. indiae* have parietal folds, however absent in the present species. An intercapsular process is absent in the present species and a prominent one is present in *M. dasgupti, M. kalmani, M. orissae* and *M. ropari*. Also, the present species differs from *M. bhadurius, M. chinsurahensis, M.cuttacki, M. oliveirai* and *M. procerus* in having polar capsules occupying half of the myxospore body cavity and *M. dasgupti* and *M. mugilii* occupy less than half of the myxospore body cavity, but in present species the polar capsules occupy three fourth of the myxospore body cavity.

On the basis of morphometric data, *M. vascularis* n. sp. closely resembled *M. basui* Kaur *et al.* (2013) however it differ in having sharp pointed beak-like anterior end. In view of the above

differences in morphology and morphometrics, the present species has been proposed as new to science and named as *M. vascularis* n.sp.

Table 2: Comparative description of *M. vascularis* n. sp. with morphologically similar species (measurements in micrometer)

Species	Host	Site of infection	Locality	Myxospore	Polar capsule
M. vascularis n.sp.	*Cirrhinus mrigala*	Gills	Nursery Pond, Fagan Majra, Punjab (India)	10.73x4.88	6.51x1.80
M. basui Kaur *et al*., 2013	*C. mrigala*	Gills	Mallumatra Pond, Punjab (India)	13.33x6.04	6.57x1.66
M. procerus Kudo, 1934	*Percopsis omiscomaycus*	Muscle cells	USA	14x4.5	7.2x2.2
M. calbasui Chakravarty, 1939	*Labeo calbasu, Labeo rohita, C. mrigala*	Gall bladder	West Bengal, (India)	13.7x9.1	6.18x4.12(L) 4.12x3.09(S)
M. catlae Chakravarty, 1939	*Catla catla, L. rohita, C. mrigala*	Gills	West Bengal, (India)	15.5x6.1	11.3x2.5
M. bengalensis Chakravarty and Basu , 1948	*Catla catla*	Gills	West Bengal, Andhra Pradesh (India)	8.9x6.61	4.8x2.8
M. kribiensis Fomena and Bouix, 1994	*Brycinus longipinnis*	Skin, Eyes, Sclera	Cameroon	21.2x9.5	16.1x15.4
M. etsatsaensis Reed *et al*., 2002	*Barbus thamalakanesis*	Gill filament	Botswana	13.0x -	7.5x2.3
M. rocatlae Basu and Haldar, 2002	*Catla catla, Rohu rohu*	Gills, Gut	West Bengal (India)	18.3x6.0	12.6x2.85
M. bilobus Cone *et al*., 2005	*Notemigonus crysoleucas*	Gills	Canada	21.0x8.9	12.7x3.2
M. leptobarbi Szekely *et al*., 2009	*Leptobarbus hoevenii*	Mucle cells	Malaysia	16.0x8.9	10.5x3.0(L) 9.9x3.0(S)
M. slendrii Kaur and Singh, 2010	*C. mrigala*	Gills	Ropar wetland, Punjab (India)	14.87x3.4	5.7x1.48
M. oliveirai Milanin *et al*., 2010	*Barbus hilarii*	Gill filament	Brazil	11.2x7.4	5.6x2.3
M. ropari Kaur and Singh, 2011	*C. mrigala*	Gill lamellae	Ropar wetland, Punjab (India)	12.5x4.5	4.96x1.50

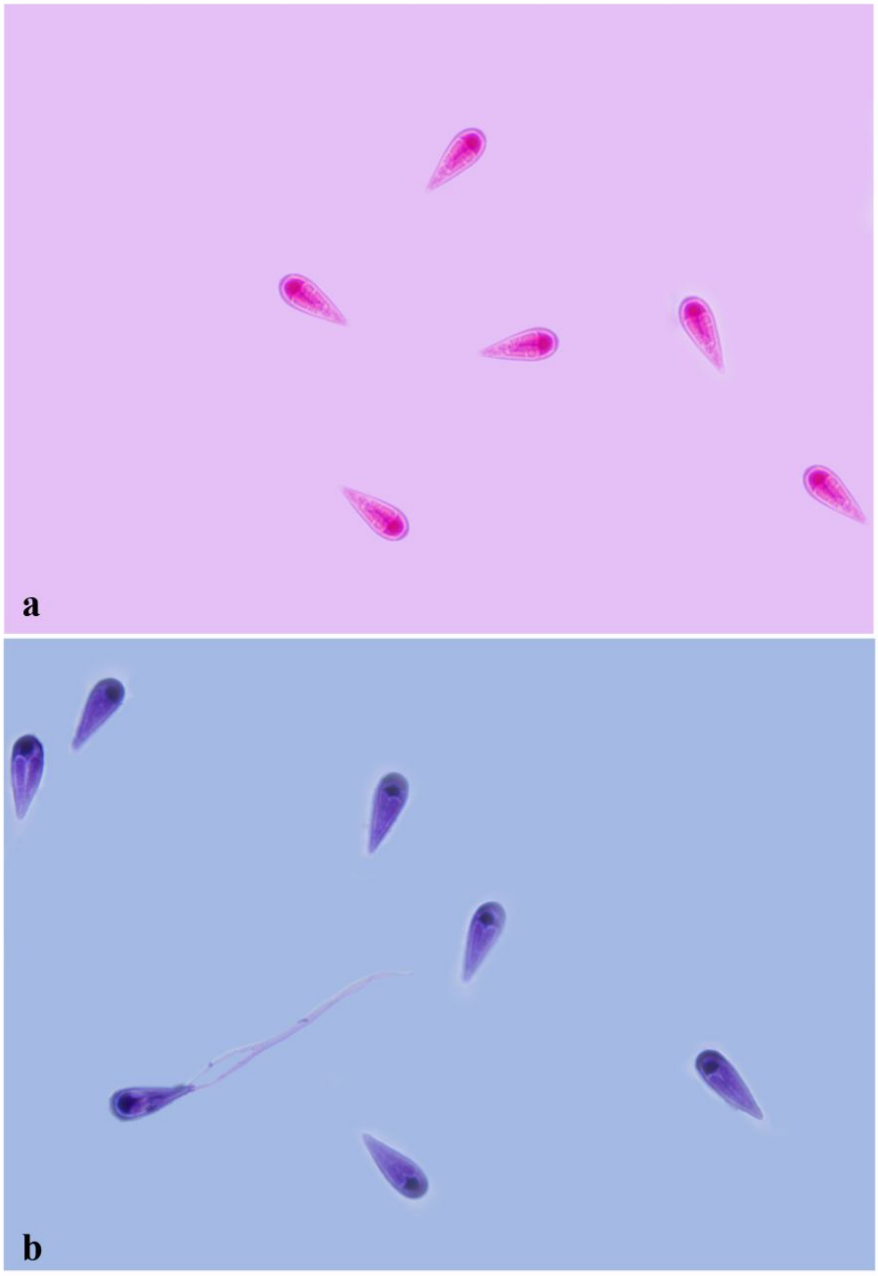

Figure 2.
Myxobolus vascularis n. sp.
(a) Myxospores stained in Ziehl-Neelsen (frontal view)
(b) Myxospores stained in Iron-haematoxylin (frontal view)

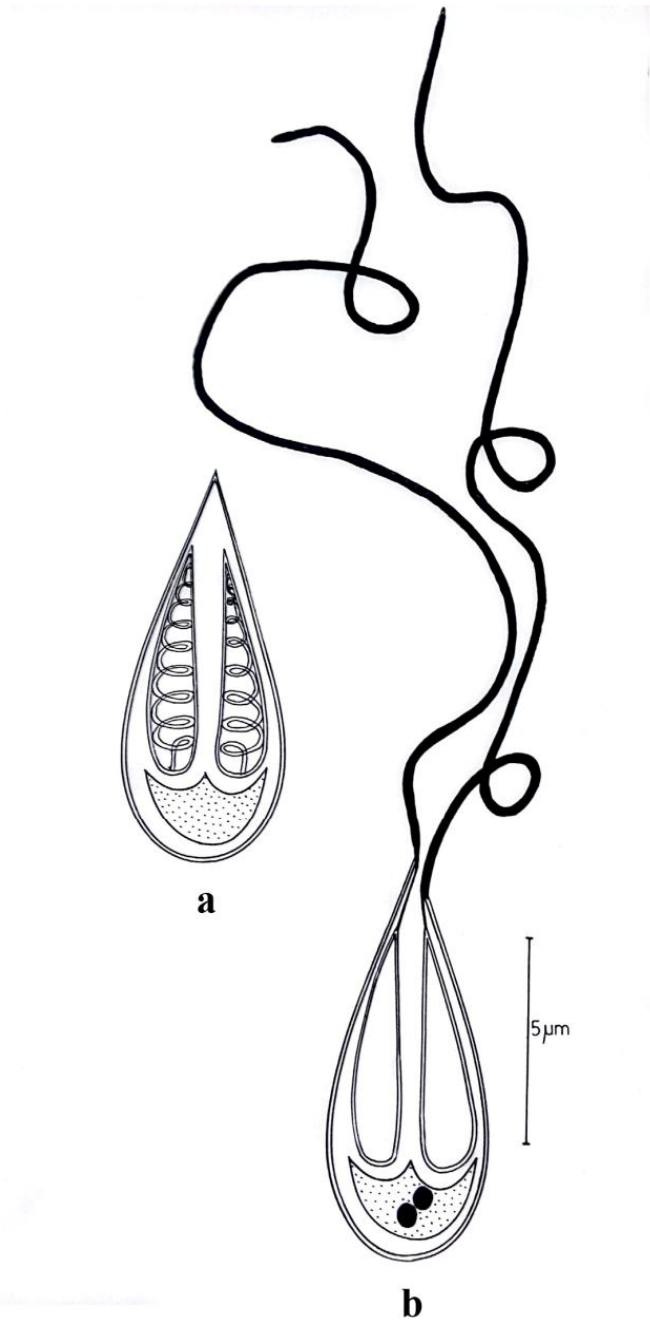

Figure 3.

Line drawings of myxospores of *Myxobolus vascularis* n. sp.
(a) Fresh myxospore (frontal view)
(b) Myxospore stained in Iron-haematoxylin (frontal view)

HISTOPATHOGENESIS

The plasmodium of *M. vascularis* n. sp. is located at the basal position of the gill lamella of fingerlings of *C. mrigala* and is typed as intralamellar vascular type LV1 (**Fig. 4a,b**). The plasmodia of the present species are of two types: Type B (small, rounded) and Type C (big sized, elongated) and size ranging from 0.5mm (in diameter) and 3.0x0.7mm (length x breadth) respectively. The plasmodium cause dilation of the infected gill lamella at the base leaving normal structure at its middle and tip initially and when fully mature occupy whole of the gill lamella. Kaur et al. (2014b) also reported that large-sized plasmodia damaged more than 50% of the gill lamellae and gill filament, causing suffocation and respiratory distress (**Fig. 4c**).

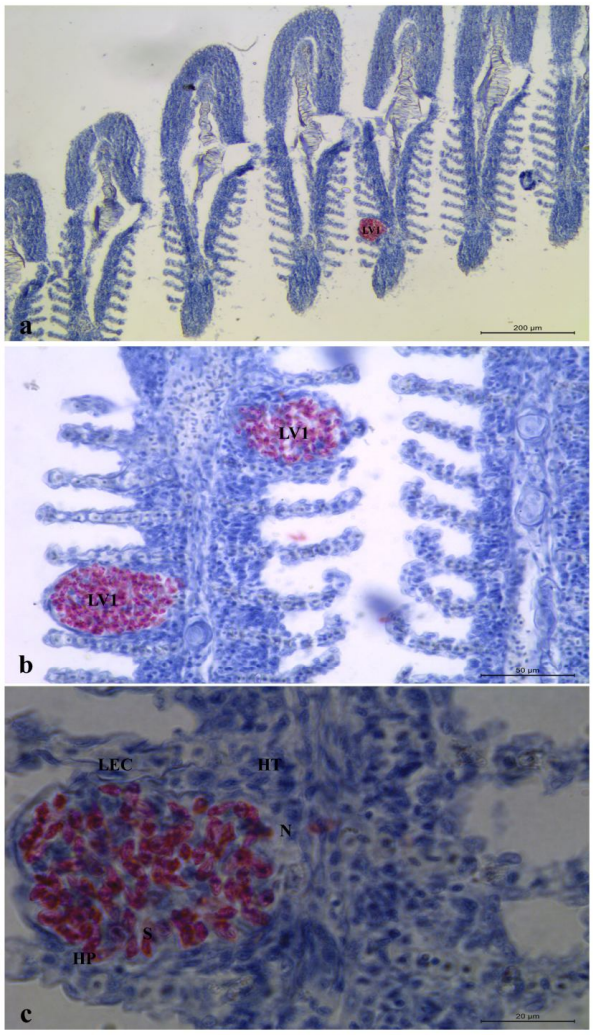

Figure 4.
Sagittal section of gills of *Cirrhinus mrigala* infected with *Myxobolus vascularis* n. sp.
(a) Plasmodia of *M. vascularis* n. sp. located in the gill lamellae (200µm)
(b) LV1 type of plasmodia in the gill lamellae (50µm)
(c) Magnified view of infected gills showing histopathological changes (20µm)

In contrast to other myxozoan species studied in the present survey, the adjacent gill lamellae remain normal and do not show any displacement. Due to the presence of plasmodia within the lamella, the cellular elements show necrosis, hypertrophy, hyperplasia and lifting of the epithelial cells. In the histological sections, it is also clear that plasmodium of *M. vascularis* n. sp. do not cause much displacement of adjacent gill lamellae, therefore cause necrosis of the single gill lamella at its location. Mitchel (1989), Yokoyoma *et al*. (1997) Molnar & Baska (1999), Molnar & Szekely (1999) and Molnar (2000) reported that the intralamellar location to be the most common in the case of *Myxobolus* species. Sanaullah & Ahmad (1980) also followed intralamellar vascular type of development in *M. dujardani, M. koi, M. hungaricus, M. bramae, M. macrocapsularis* and *M. margitae* species. In the present study, plasmodia were recorded in the gill lamellae (LV1 type). Recently, Kaur and Katoch (2016) also recorded majority of the plasmodia in the gill lamellae as LV1 and LV3 type.

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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