***Myxobolus nanokiensis* sp. nov. (Myxozoa: Bivalvulidae), a new pathogenic myxosporean parasite causing haemorrhagic gill disease in cultured Indian major carp fish, *Labeo rohita* (Hamilton 1822) in Punjab, India**

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Abstract The plasmodia of *Myxobolus nanokiensis* sp. nov. were found infecting gills of *Labeo rohita* (Hamilton 1822). The infection rate was found to be 36.67 % (30 fishes were examined and 11 fishes were infected) in the Nanoki pond in Patiala district Punjab. Numerous minute plasmodia each filled with 150–200 spores were detected. Smear of scrapped blood-tinged mucous from gills exhibited millions of spores. Histological sections also indicated numerous plasmodia measuring 38.33–40.33 μm in diameter in the blood vessels of gill filaments. Spores of *M. nanokiensis* sp. nov. were elongate pyriform in shape and morphologically unique in having sharply pointed beak-like anterior end. Spores measured 9.28 μm $\times$ 5.71 μm in size. Polar capsules were equal, pyriform, 5.71 $\times$ 2.73 μm in size, each having polar filament with 7–9 coils. The present species has been proposed as new on the basis of its peculiar shape and morphometrics. This is the first report of any myxobolid infection in the farmland fishes in Punjab (India). The plasmodia in the gill filaments were of intralamellar vascular type (LV) and were present within the entire length of the filament. These plasmodia caused hemorrhage, necrosis of the blood vessels and cellular infiltration.

Keywords Aquaculture · Histopathology · Gills · Indian major carp · *Labeo rohita* · *Myxobolus*

Introduction

In Punjab, aquaculture production per hectare is more than double the national average production of 2,600 kg/ha/year (Dhawan 2006). Establishment of the Fish Farmer Development Agency has helped in the development of the aquaculture in the state. Contribution of fisheries (GSDP) in Punjab was US\$ 7.13 million in 2005–06 as compared to US\$ 1.7 m in 1995–96. Polyculture species consists of many Indian major carps such as Catla (*Catla catla* Ham.), rohu (*Labeo rohita* Ham.) and mrigal (*Cirrhinus cirrhosus* Ham.) and exotic carps such as silver carp (*Hypophthalmichthys molitrix* Valen.), grass carp (*Ctenopharyngodon idellus* Valen.), common carp (*Cyprinus carpio* Linn.) and bighead carp (*Aristichthys nobilis* Rich.).

Development of aquaculture during the last decade have resulted in much greater attention paid to problem posed by parasites and their impact on the fish health leading to constraints in the productivity of aquaculture. Myxosporidians have emerged as one of the most common group of parasites infecting fishes in wetlands of Punjab (Kaur and Singh 2008, 2008/2009, 2009, 2010/2011, 2010a, b, 2011a, b, c, d, e, f, 2012a, b; Singh and Kaur 2012a, b, c). Due to pathogenic potential of some of the species, fish growth and reproduction could be drastically affected and can even cause death of the host. In India, economic losses caused by these parasites in aquaculture have been well documented (Kalavati and Nandi 2007).

Under natural environment or in farmlands the Genus *Myxobolus* has been found to be the largest genus among myxosporeans with approximately 790 valid species worldwide (Lom and Dykova 2006). As many as 131 myxobolid species have been reported from Indian fishes (Kaur and Singh 2012a).

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In the present study, a new species *Myxobolus nanokiensis* sp. nov. was detected infecting gill filaments of the Indian major carp, *Labeo rohita*. The clinical signs in fresh specimens were studied. Histopathological changes due to infection were studied with the help of histological sections.

Materials and methods

Fish specimens were procured from Nanoki pond in district Patiala (Punjab), were brought to the laboratory for further investigation. The fishes were examined with naked eye and dissected under the stereoscopic microscope. The following organs were carefully examined: gills, liver, intestine, stomach, kidneys, gall bladder, scales and fins. Plasmodia were removed, placed on a clean slide and examined in the light microscope under 100× 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. Complete description of the species was prepared according to the guidelines of Lom and Arthur (1989). Identification was made on the basis of various morphological structures of spore such as shape, size, and number of polar capsules, length of polar filaments, number of coils of polar filaments, presence or absence of intercapsular process, presence or absence of any sutural markings, presence of any iodophilous vacuole and number of nuclei in the sporoplasm etc.

For histopathological studies infected gills were cut into small pieces and fixed in Bouin's and Carnoy's fixatives, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 8–10 µm and stained with Haematoxylin and eosin (H+E). The histopathological location of plasmodia and terminology followed in the study is according to the Molnar (2002).

Results and discussion

Taxonomy

Phylum Myxozoa Butschli (1882)
 Class Myxosporea Butschli (1881)
 Order Bivalvulida Shulman (1959)
 Family Myxobolidae Thelohan (1892)
 Genus *Myxobolus* Butschli (1882)

M. nanokiensis sp. nov. (Figs. 1, 2)

Fig. 1 Micrographs of spores of *M. nanokiensis* sp. nov.: **a** fresh spore, **b** spores stained in Giemsa, **c** spores stained in Ziehl–Neelsen

Type host	<i>Labeo rohita</i> (Hamilton 1882) vern. rohu
Type locality	Patiala (Punjab)
Material	Paratypes are spore stained by Ziehl–Neelsen and Iron-

Fig. 2 Line drawing (Camera Lucida) of spores of *M. nanokiensis* sp. nov.: **a** spore stained in Ziehl–Neelsen (valvular view), **b** spore in side view (one polar filament extruded), **c** spore stained in Giemsa

haematoxylin, deposited in the museum of Department of Zoology & Environmental Sciences, Punjabi University, Patiala, India. Slide no. M/ZN/02.02.2012 and M/IH/02.02.2012

Site of infection	Gill lamellae
Prevalence of infection	36.67 % (11/30)
Etymology	The specific epithet <i>nanokiensis</i> highlights the name of the pond <i>Nanoki</i> in Patiala district from where the specimens were collected
Pathology	Gills of the infected fish were mucous laden and haemorrhagic. Necrosis and hyperplasia was extensively observed in the gill filaments

Plasmodium

Minute, rounded to ovoid in shape, measure 38.33–40.33 μm in diameter, present in the gills. 150–200 spores present per plasmodium.

Description of spores (Table 1)

(Measurements based on 15–20 spores in frontal view).

Spores are histozoic, measure $9.28 \times 5.71 \mu\text{m}$, pyriform with sharply pointed beak-like anterior end and rounded posterior end. Shell valves are $1.67 \mu\text{m}$, thin and symmetrical. Parietal folds absent. Polar capsules are two, equal, broadly pyriform with pointed anterior end and rounded posterior end, positioned posteriorly from tip and lie parallel to each other inside spore body cavity. Both polar capsules measure $5.71 \times 2.73 \mu\text{m}$ occupying more than 3/4th of the spore cavity. Polar filaments form 7–9 coils in polar capsule and measure $7.26 \mu\text{m}$ in length on extrusion. Intercapsular process (ICP) is absent. Sporoplasm is agranular, homogeneous, hemispherical and contain two sporoplasmic nuclei. An iodophilous vacuole is absent.

Histopathological findings

Gross morphology of infected gills in live specimens of *Labeo rohita* (Ham.) were mucous laden and haemorrhagic. A thick smear of blood-tinged mucous exhibited numerous spores. The classification of plasmodia in histological sections based on its location within the gill filaments was followed as given by Molnar (2002) (Fig. 3).

In the present study, plasmodia in the gill filament were of intralamellar-vascular type (LV) and were present within the entire length of the filament. Several rounded to ovoid plasmodia measuring 38.33–40.33 μm in diameter were present at the centre of the lamellae and therefore designated as LV₁ type. These plasmodia caused hemorrhagic gills, necrosis of the blood vessels and cellular infiltration. Extensive hyperplasia was also observed in the gill filament Fig. 4.

Discussion

The present species has been compared with other morphologically similar species i.e. *Myxobolus dujardini* Thelohan (1892) from intralamellar in gills of *Rutilus rutilus*, *M. calbasui* Chakravarty (1939) from gall bladder of *L. calbasu*, *L. rohita*, *M. catlae* Chakravarty (1943) from gills of *L. rohita*, *M. bengalensis* Chakravarty and Basu (1948) from gills of *Catla catla*, *M. lomi* Donec and Kulalovskaya in Shulman (1962) from gills of *Phoxinus phoxinus*, *M. hakyi* Ha (1971) from kidneys of *Hypophthalmichthys harmandi*, *M. bhadrensis* Seenappa and Manohar (1981) from muscles of *L. rohita*, *M. mathuri* Jayasri et al. (1981) from gills of *Puntius saranae*, *M. rohita* Haldar et al. (1983) from scales of *L. rohita*, *L. bata*, *L. dyocheilus*, *L. calbasu*, *Puntius sarana* *M. bhadurius*

Table 1 Measurements (μm) and ratio of *M. nanokiensis* sp. nov

Characters	Range	Mean values	SD
LS	8.36–10.2	9.28	0.89
WS	4.62–6.8	5.71	1.31
LPC	5.42–6.0	5.71	0.89
WPC	2.46–3.8	2.73	0.62
Ratio: LS/WS		1.62	
ICP		Absent	
NC		7–9	
Parietal folds		Absent	

(Sarkar 1985) Gupta and Khera (1988) from gall bladder of *Wallago attu*, *M. dermatis* (Haldar et al. 1981) Gupta and Khera (1988) from scales of *L. rohita*, *M. haldari* Gupta and Khera (1989) from fins and gills of *L. rohita*, *M. phoxinaceus* (Bauer 1948) Landsberg and Lom (1991) from gills of *Phoxinus czekanowski*, *M. longisporus* Nie and Li (1992) from gills of *Cyprinus carpio*, *M. kwangsiensis* Hsieh et al. (1993) from gills and kidneys of *L. rohita*, *M. bilongi* Fomena and Bouix (1994) from gills and fins of *Labeo* sp., *M. cuttacki* Haldar et al. (1996) from branchial filaments of *Cyprinus carpio*, *M. rocatlae* Basu and Haldar (2002) from gills and gut wall of *Catla catla* x *Labeo rohita* hybrid, *M. maculatus* Casal et al. (2002) from kidneys of *Metynnis maculatus*, *M. manoramae* Basu and Haldar (2002) from tail fins of *Catla catla* x *L. rohita* hybrid, *M. catmrigale* Basu and Haldar (2004) from gill filaments of *Catla catla* x *C. mrigala* hybrid, *M. bilobus* Cone et al. (2005) from gill filaments of *Notemigonus crysoleucas*, *M. chilensis* (Kalavati et al. 1992) Kalavati and Nandi (2007) from gall bladder of *L. rohita*, *M. punjabii* Kaur and Singh (2010/2011) from caudal fin of *L. rohita*, *M. sushmii* Kaur and Singh (2010/2011) from eye-ball of *L. rohita*, *M. patialensis* Kaur and Singh (2011d) from caudal fin of *L. rohita*, *M. saugati* Kaur and Singh (2011c) from scales of *L. rohita*, *M. myelii* Azevedo et al. (2012) from gall bladder of *Myleus rubripinnis* and *M. potularis* Madhavan et al. (2012) from gill filaments of *L. calbasu*, *L. bata*, *L. gonius* and *C. reba* but differ from all of the above in morphometric characteristics (Table 2).

The shape of the spore under study is pyriform with pointed anterior end and rounded posterior end. In this respect, it is similar to *M. dujardini*, *M. bengalensis*, *M. mathuri*, *M. lomi*, *M. bhadurius*, *M. phoxinaceus*, *M. cuttacki*, *M. rocatlae*, *M. manoramae*, *M. catmrigale*, *M. bilobus*, *M. hakyi*, *M. maculatus* and *M. longisporus* and *M. potularis*. But oval spores in *M. bengalensis* and elliptical spores of *M. hakyi* differentiate them from the spores of species under present study. Polar capsules are in present

Fig. 3 Histopathological changes in gill filament of *Labeo rohita* (Ham): **a** GL damaged gill lamellae, GF gill filament, LV intralamellar vascular type, **b** P plasmodia at the center of gill lamellae, LV1 intralamellar vascular type, **c** MS *Myxobolus* spore P inside the plasmodia, N necrosis around the plasmodia

species are equal, broadly pyriform with pointed anterior end and rounded posterior end, placed posteriorly from tip and lie parallel to each other in the spore body cavity. In

Fig. 4 Infected gill filament of *Labeo rohita* with plasmodia: **a** showing intralamellar vascular type plasmodia (LV1) with spores (MS) occupying the entire gill lamellae; *N* necrosis **b** Plasmodia with *MS* *Myxobolus* spores, *EL* Epithelial layer, *IF* Infiltration of plasmodia with different cells, *H* Hyperplasia, **c** *BV* dilation of blood vessel, *H* Hyperplasia, *N* Necrosis

contrast to this, the polar capsules are equal, convergent and anteriorly placed in *M. cuttacki*, *M. longisporus*, *M. phoxinaceus*, *M. dujardini*, *M. bhadurius*, *M. lomi*, *M. potularis* presence of knob-like end/structure at the anterior end of the polar capsules in *M. maculatus*, unequal polar capsules in *M. rocatlae*, *M. manoramae*, *M. catmriglae*, *M. bilobus* and *M. mathuri* differentiate all of them from the present species. Furthermore, spores of *M. cuttacki* (LS/WS = 2.62), *M. maculatus* (LS/WS = 2.3), *M. longisporus* (LS/WS = 2.4), *M. lomi* (LS/WS = 1.37) and *M. phoxinaceus* (LS/WS = 1.57) are morphometrically different than spores of the present species (LS/WS = 1.62) as indicated by comparing LS/WS ratios (indicated in parentheses). Lastly, absence of intercapsular process in the present species as compared to *M. lomi* having a large one justify the establishment of this myxozoan as a new species and is named as *M. nanokiensis* sp. nov.

Due to the presence of spores within the entire length of the gill filament, the blood vessels were dilated and bounding endothelial cells were highly compressed. Similar observations were made by Molnar and Szekely (1999) in four *Myxobolus* species (*M. macrocapsularis*, *M. impressus*, *M. bramae* and *M. hungaricus*) from the gills of bream infecting capillary network of the gill lamellae and respiratory plate. Also, Rukyani (1990), Azevedo et al. (2010) and Campos et al. (2011) reported the alterations in capillary network, hyperplasia of gill epithelium and structural disorganization of secondary lamellae. According to Adriano et al. (2009) these alterations may partially compromise gill functions and therefore diminish the respiratory capacity and ionic exchange. Furthermore, Awal et al. (2001) reported the pathological changes in the gills of *Cirrhina mrigala* from Bangladesh showing hypertrophy and hyperplasia with the presence of numerous inflammatory cells and accumulation of blood cells at the base of the secondary gill lamellae. Chavda et al. (2010) studied myxozoan parasite in the gills of major aquaculture carp *Catla catla* in central Gujarat region. According to them, infected carps exhibited hemorrhagic condition with necrotic changes in epithelia and in connective tissues of gills. Earlier, similar observation have also been made by McCraren et al. (1975) in gill infections of American catfish with *Henneguya exilis*, Kalavati and Narasimhamurti (1985) in *Channa punctata* with *Henneguya waltirensis* and Rukyani (1990) in carp with *Myxobolus koi*. According to Schulman (1966) and Kalavati and Narasimhamurti (1985) rupture of cysts also leads to haemorrhages, sometimes resulting in considerable loss of blood.

Table 2 Comparative description of *M. nanokiensis* sp. nov. with morphologically similar species (measurements are in micrometer)

Species	Host	Site of infection	Locality	Spore size	Polar capsule size	Polar capsule (= or ≠)	Number of coils	Intercapsular process (ICP)
<i>Myxobolus nanokiensis</i> sp. nov. (Present study)	<i>Labeo rohita</i>	Gills	Nanoki Pond, Punjab (India)	9.28×5.71	5.71×2.73	=	7–9	Absent
<i>M. dujardini</i> Thelohan, (1892)	<i>Rutilus rutilus</i>	Intralamellar in gills	France	11.5×7.4	5.5×2.3	=	5–6	Absent
<i>M. calbasui</i> Chakravarty, (1939)	<i>L. calbasu</i> , <i>L. rohita</i>	Gall bladder	India	$12.4\text{--}15.0 \times 8.2\text{--}10.0$	$6.2\text{--}4.1$	≠	4 & 7	Absent
<i>M. catlae</i> Chakravarty, (1943)	<i>L. rohita</i>	Gills	West Bengal (India)	$14.5\text{--}16.5 \times 6.18$	$10.3\text{--}12.3 \times 2.06\text{--}3.01$	=	13–14	Absent
<i>M. bengalensis</i> Chakravarty and Basu, (1948)	<i>Catla catla</i>	Gills	West Bengal (India)	8.96×6.6	4.84×2.85	=	–	Absent
<i>M. lomi</i> Donec and Kulalovskaya in Shulman, (1962)	<i>Phoxinus phoxinus</i>	Gills	Danube	11×8.0	5.5×2.35	=	–	Present
<i>M. hokyi</i> Ha, (1971)	<i>Hypophih- almitichys harmandi</i>	Skin	Thailand	15.9×6.6	6.3×2.3	=	6–7	Absent
<i>M. bhadrensis</i> Seenappa and Manohar, (1981)	<i>L. rohita</i>	Muscle	Karnataka (India)	9.5×7.14	3.5×2.2 & 2.5×1.75	≠	–	Present
<i>M. mathuri</i> Jayasri et al. (1981)	<i>Puntius Saranae</i>	Gills	West Bengal (India)	16.1×7.6	7.5×3.2 & 5.25×3.2	≠	8–9 & 3–4	Absent
<i>M. rohita</i> Haldar et al. (1983)	<i>L. rohita</i> , <i>L. bata</i> , <i>L. dyocheilus</i> , <i>L. calbasu</i> , <i>Puntius sarana</i>	Scales	West Bengal (India)	10.6×9.0	6.6×3.3	≠/≠	17–19 & 15–18	Absent
<i>M. bhadurius</i> (Sarkar, 1985) Gupta and Khera, (1988)	<i>Wallago attu</i>	Gall bladder	West Bengal (India)	10.59×6.28	5.31×2.78	=	5–6	Absent
<i>M. dermatitis</i> (Haldar et al. 1981) Gupta and Khera, (1988)	<i>L. rohita</i>	Scales	West Bengal Kerala (India)	10.3×9.4	4.4×2.2	=	5	Present
<i>M. haldari</i> Gupta and Khera, (1989)	<i>L. rohita</i>	Fin, Gills	Punjab (India)	9.31×7.95	4.31×2.97 & 2.95×1.98	#	–	Present
<i>M. phoxinaceus</i> (Bauer 1948) Landsberg and Lom, (1991)	<i>Phoxinus czekano-wski</i>	Gills	Siberia	16.5×10.5	$8.5\text{--}9 \times 3$	=	–	Absent
<i>M. longisporus</i> Nie and Li, (1992)	<i>Cyprinus carpio</i>	Gills	China	$16.0\text{--}17.5 \times 6.5\text{--}7.0$	$7.5\text{--}8.2 \times 2.0$	=	–	Absent
<i>M. kwangsiensis</i> Hsieh et al. (1993)	<i>L. rohita</i>	Gills, Kidney	China	10.9×6.9	5.5×2.9	=	–	Absent
<i>M. bilongi</i> Fomena and Bouix (1994)	<i>Labeo</i> sp.	Gills, Fin	Cameroon	15.3×12.2	7.4×4.8	=	–	Absent

Table 2 continued

Species	Host	Site of infection	Locality	Spore size	Polar capsule size	Polar capsule (= or ≠)	Number of coils	Intercapsular process (ICP)
<i>M. cuttacki</i> Halder et al. (1996)	<i>Cyprinus carpio</i>	Branchial filament	Orissa (India)	17.04 × 6.48	8.64 × 2.8	=	5–8	Absent
<i>M. rocatlae</i> Basu and Halder (2002)	<i>Catla catla</i> × <i>L. rohita</i>	Gut, Gills	West Bengal (India)	18.5 × 5.9	12.9 × 2.8 & 11.3 × 2.2	≠	17–19 & 15–18	Absent
<i>M. maculatus</i> Casal et al. (2002)	<i>Metynnis maculatus</i>	Kidney	Brazil	21 × 8.9	12.7 × 3.2	=	14–15	Absent
<i>M. manoramae</i> Basu and Halder (2002)	<i>Catla catla</i> × <i>Labeo rohita</i>	Tail fins	West Bengal (India)	11.8 × 5.6	6.1 × 3.73 & 5.44 × 2	=	5–6 & 4–5	Absent
<i>M. catmrigale</i> Basu and Halder (2004)	<i>C. mrigala</i> × <i>Catla catla</i>	Gill filaments	West Bengal (India)	20.4 × 16.3	11.9 × 2.3 & 11.0 × 2.3	≠	20–25 & 20–23	Absent
<i>M. bilobus</i> Cone et al. (2005)	<i>Notemigonus crysoleucas</i>	Gills	Canada	21.0 × 8.4	10.8 × 2.7 & 10.1 × 2.8	≠	7–9	Absent
<i>M. chilensis</i> (Kalavati et al. 1992) Kalavati and Nandi, (2007)	<i>L. rohita</i>	Gall bladder	Orissa (India)	7.2–8.0 (7.77) × 5.6–6.6 (6.2)	3.2–4.8 (4.2) × 1.8–2.2 (1.98) & 1.0–1.2 (1.08)	≠	6–8 in larger polar capsule	Absent
<i>M. punjabii</i> Kaur and Singh (2010/2011)	<i>L. rohita</i>	Caudal fin	Kanjali Wetland Punjab (India)	8.1–9.7 (8.9) × 6.4–7.5 (6.9)	3.4–5.4 (4.4) × 2.0–3.4 (2.7) & 2.8–3.6 (3.2) × 1.2–2.2 (1.7)	≠	6–7 & 4–5	Absent
<i>M. sushmiti</i> Kaur and Singh (2010/2011)	<i>L. rohita</i>	Eye ball	Harike wetland, Punjab (India)	9.0–10.2 (9.6) × 7.3–9.3 (8.3)	4.1–5.3 (4.70) × 2.9–3.5 (3.20) & 2.9–3.4 (3.10) × 1.0–3.0 (2.0)	≠	4–6 & 3–4	Present
<i>M. patialensis</i> Kaur and Singh, (2011d)	<i>L. rohita</i>	Caudal fin	Ropar wetland, Punjab (India)	11.28 × 6.67	4.8 × 3.1 & 1.70 × 1.51	#	5–6 & 2–3	Absent
<i>M. saugati</i> Kaur and Singh, (2011c)	<i>L. rohita</i>	Scales	Kanjali wetland Punjab (India)	8.3 × 6.6	4.0 × 2.4	=	4–5	Absent
<i>M. myleus</i> Azevedo et al. (2012)	<i>Myleus rubripinnis</i>	Gall bladder	Brazil	19.3 × 8.3	13.2 × 3.0	=	19–21	Absent
<i>M. pontularis</i> Madhavan et al. (2012)	<i>L. calbasu</i> , <i>L. bata</i> , <i>L. gonius</i> , <i>C. reba</i>	Gill filaments	Tripura (India)	9.0 × 6.0	5.4 × 2.2	=	7–8	Absent

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