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Prevalence, site and tissue preference of myxozoan parasites infecting gills of cultured fish in Punjab (India)

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ABSTRACT: Native carp species cultured in Indian farms in Punjab (catla *Catla catla*, rohu *Labeo rohita*, mrigal *Cirrhinus mrigala*, exotic carps such as silver carp *Hypophthalmichthys molitrix*, grass carp *Ctenopharyngodon idella*, common carp *Cyprinus carpio* and a catfish *Sperata seenghala*) were examined for the presence of myxozoan parasites infecting gills. Firstly, the gills were examined under a zoom-stereomicroscope for the presence of plasmodia. The number of plasmodia per gill was counted to determine the index for the intensity of infection. Infected tissues were processed for histology, and 3–4 µm sections of infected gills were stained with haematoxylin & eosin and Luna's method. A total of 19 species of myxosporean were found infecting various cell types in the gills. Of these, 14 species belonged to the genus *Myxobolus*, 3 species to the genus *Thelohanellus* and 2 species to the genus *Henneguya*. Species belonging to the genus *Myxobolus* formed the interlamellar and intralamellar vascular (LV) type plasmodia, and species belonging to the genus *Thelohanellus* and *Henneguya* formed intrafilamental vascular (FV) type plasmodia. Mixed infections comprising 2, 3 or 4 different myxozoan species were noted in individual fish. The most common type of parasitism was polyparasitism due to 4 myxobolids co-occuring in fish with an infection rate of 23.16%. All species caused mild to severe haemorrhagic gill disease with little clinical symptomatology.

KEY WORDS: Aquaculture · Parasite · Myxozoa · Gills · Freshwater fish

INTRODUCTION

Fishes are commonly infected with myxozoan parasites, and some of these infections may result in fish mortality (Sanaullah & Ahmed, 1980). Myxozoans most commonly infect gills in part due to rich blood supply, gaseous exchange and ready access to the external environment for transmission. They form cream-coloured plasmodia, which vary in size and location depending on the myxozoan species. Adriano et al. (2006) recorded plasmodia of *Myxobolus cuneus* in the adventitia of arterioles in the gill filament, causing deformity of the wall, obstruction of the lumen and macrophage infiltration. Székely et al. (2009) located plasmodia of *Thelohanellus zahrahae* alongside the multilayered epithelium of gill filament. Histopathological analysis of tissues is also an important approach for the detection of gill myxoboliosis. Some species can affect growth, reproduction and cause death of the host (Longshaw et al. 2005), and economic losses caused by these parasites in aquaculture have been well documented (Lom & Dykova 2006). The present study was undertaken to study the myxozoan parasites infecting gills of aquaculture fish in Punjab, India, and to access their tissue location and potential pathogenic effects.

MATERIALS AND METHODS

Fresh specimens of fish were captured in fish farms from different districts of Punjab for parasitological

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analysis. The selected ponds were polyculture having *Labeo rohita* Hamilton (1882), *Catla catla* Hamilton (1882), *Cirrhinus mrigala* Hamilton (1882), *Ctenopharyngodon idella* Valenciennes in Cuvier & Valenciennes (1844), *Cyprinus carpio* Linnaeus (1758), *Hypophthalmichthys molitrix* Valenciennes (1844) and *Sperata seenghala* Sykes (1839). Organs such as gills, gall bladder, liver, heart, gut, eyes, fins, scales and skin were examined and were fixed in Bouin's fixative for histopathological studies. Besides gills, the infection was also recorded in the scales, fins, liver and gall bladder. Each plasmodium was picked under a stereozoom binocular with fine forceps and ruptured in normal saline (0.85%) on a glass slide and examined under a light microscope for the presence of spores. Fresh spores were studied under a phase contrast microscope (Magnus MLX-TR). Polar filaments were extruded by treating the spores with 8% KOH. The number of spores per plasmodium were counted under low magnification on a glass slide. One plasmodium was ruptured on a slide with the help of a fine needle then covered with a coverslip, and the number of spores were counted; 3 to 4 plasmodia were ruptured to obtain the average number of spores.

To make dry preparations, thin smears were air dried, fixed in methanol and stained with Giemsa. In the case of permanent (wet) preparations, smears were fixed in Bouin's fixatives. The 2 stains Heidenhain's iron-haematoxylin and Ziehl-Neelsen were used to study the spore morphology. The former stain elucidated the shape of the spore shell, surface markings if any, presence or absence of intercapsular process and number of sporoplasmic and capsulogenic nuclei. The later stain differentiated the shape of the polar capsule(s) and number of coils of the polar filament.

Infected gills were cut into small pieces and fixed in Bouin's fixative. Tissue samples were dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 3–4 µm thickness and stained with haematoxylin & eosin (H&E) and/or Luna's staining method (Luna 1968). In Luna's method, the cyst stained bright red, and the rest of the gill tissue stained blue and was used to localise cysts within the gills.

For scanning electron microscopy, infected organs were fixed in 3% glutaraldehyde (pH 7.4), post fixed in 1% osmium tetraoxide. The infected gills were washed in buffer overnight and dehydrated in an ascending series of ethyl alcohol. Finally, infected gills were critical point dried and coated with gold.

Prevalence rate was calculated according to Bush et al. (1997). The location of myxosporean plasmodia in different areas of the gills were categorized into types according to Molnár (2002): (1) intralamellar epithelial type (LE); (2) intralamellar vascular (LV) type; (3) intrafilamental vascular (FV) type; (4) plasmodium located in the gill arch (AC). The gill plasmodium index (GPI) was calculated on the basis of number of plasmodia present per gill (one side) visible under the stereozoom binocular microscope and with the naked eye (Kaur & Attri 2015). 0: no infection; 1–5: light infection; 5–10: moderate infection; 10–20: heavy infection; 20–50: severe infection.

RESULTS

The current study examined a total of 1380 fish from ponds in various districts of Punjab. Species examined for myxozoan infection of the gills included catla *Catla catla*, rohu *Labeo rohita*, mrigal *Cirrhinus mrigala* and exotic carps such as silver carp *Hypophthalmichthys molitrix*, grass carp *Ctenopharyngodon idella*, common carp *Cyprinus carpio* and also a catfish *Sperata seenghala*. The mean age of the fish ranged from 9 to 21 mo, and mean length ranged from 20 to 31.5 cm (Table 1). The temperature of the pond water at the time of the collection was 30°C ± 1.41. A total of 418 fish were infected with at

Table 1. Myxozoan parasitism in the gills of aquacultured fish from Punjab, India. Fish length and age are means (±SD)

Host species	Fish length (cm)	Fish age (mo)	No. of fish		Prevalence (%)
			Examined	Infected	
Labeo rohita (rohu)	24 ± 4.6	9 ± 1.03	625	253	40.48
Cirrhinus mrigala (mrigala)	26 ± 2.1	9 ± 1.04	315	98	31.11
Catla catla (thail)	21 ± 1.05	9.5 ± 1.31	240	61	24.42
Hypophthalmichthys molitrix (silver carp)	29 ± 2.16	9 ± 2.1	50	0	0
Cyprinus carpio (mirror carp)	21 ± 2.1	9.5 ± 1.21	50	0	0
Ctenopharngodon idella (grass carp)	20 ± 2.1	9 ± 1.03	50	0	0
Sperata seenghala (seenghara)	31.5 ± 3.6	21 ± 1.05	50	6	12
Total			1380	418	30.29

least one species of myxozoan in the gills (prevalence 30.29%). The infection rate of gill myxozoosis was highest in *L. rohita* (40.48%) followed by 31.11% in *C. mrigala,* 25.42% in *C. catla* and 12% in *S. seenghara*. As many as 19 species of myxozoan parasites belonging to 3 genera, namely, *Myxobolus* (14 species), *Thelohanellus* (3 species) and *Henneguya* (2 species) were recorded. In the present study, *M. potularis* (62%), *M. nanokiensis* (43.75%), *M. longisporus* (40%) and *T. bifurcata* (40%) were most preva-

Table 2. Parasite, host, locality (district), prevalence of infection, gill plasmodium index (GPI) and number of spores in cultured fish from Punjab. GPI score 1: light; 2: moderate, 3: heavy, 4: severe. See Table 1 for host genus names

Parasite species Reference	Host(s)	District(s)	No. of fish per species Examined	Infected	Prevalence (%)	GPI	Spores per plasmodium
Myxobolus venkateshi Seenappa and Manohar, 1981 Seenappa & Manohar (1991)	*L. rohita, C. mrigala*	Patiala	40+40=80	15+10=25	31.25	3	75 ± 26.35
M. slendrii Kaur and Singh, 2010 Kaur & Singh (2010)	*L. rohita, C. catla, C. mrigala*	Mohali	30+20+25=75	10+5+5=20	28.57	4	250 ± 52.70
M. nanokiensis Kaur et al., 2015 Kaur et al. (2015)	*L. rohita*	Patiala	80	35	43.75	4	150 ± 52.70
M. longisporus Nie and Li, 1992 Nie & Li (1992)	*L. rohita, C. catla*	Hoshiarpur, Patiala	40+40=80	16+16=32	40	4	125 ± 26.35
M. stomum Ali et al., 2003 Ali et al. (2003)	*L. rohita*	Patiala	60	12	20	3	45 ± 5.27
M. potularis Madhavan, Bandyopadhyay and Santosh, 2013 Madhavan et al. (2013)	*L. rohita*	Mohali, Pathankot	50	31	62	4	450 ± 52.70
M. naini Kaur and Singh, 2008 Kaur & Singh (2008)	*C. catla*	Patiala	50	15	30	1	450 ± 52.70
M. patialensis Kaur and Singh, 2011 Kaur & Singh (2011)	*C. mrigala*	Mohali	50	18	36	2	150 ± 52.70
M. moli Fomena, Bouix and Birgi, 1985 Fomena et al. (1985)	*C. mrigala*	Patiala	75	18	24	2	55 ± 5.27
M. dossoui Sakiti et al., 1991 Sakiti et al. (1991)	*C. mrigala*	Patiala	60	18	15	2	45 ± 5.27
M. nchoutnounensis Elysée and Fomena, 2011 Elysée & Fomena (2011)	*C. mrigala*	Mohali, Hoshiarpur	60	09	25	2	25 ± 5.27
M. basui Kaur et al., 2013 Kaur et al. (2013)	*C. mrigala*	Patiala, Pathankot, Gurdaspur	60	15	18.75	3	250 ± 52.70
Myxobolus sp. I	*L. rohita*	Mohali	80	20	25	3	325 ± 26.35
Myxobolus sp. II	*L. rohita*	Pathankot, Mohali	60	15	18.75	3	450 ± 52.70
Thelohanellus bifurcata Basu and Haldar, 1999 Basu & Haldar (1999)	*L. rohita*	Mohali	60	24	40	3	650 ± 158.11
T. dykovae Kaur, Dar and Katoch, 2014 Kaur et al. (2014a)	*L. rohita*	Patiala	70	27	38.57	2	350 ± 52.70
T. filli Kaur, Katoch and Gupta, 2014 Kaur et al. (2014b)	*L. rohita*	Pathankot, Mohali	60	20	33.33	3	450 ± 52.70
Henneguya sp. I	*C. mrigala*	Pathankot	70	12	17.14	3	250 ± 52.70
Henneguya sp. II	*S. seenghala*	Patiala	50	06	12	2	35 ± 5.27

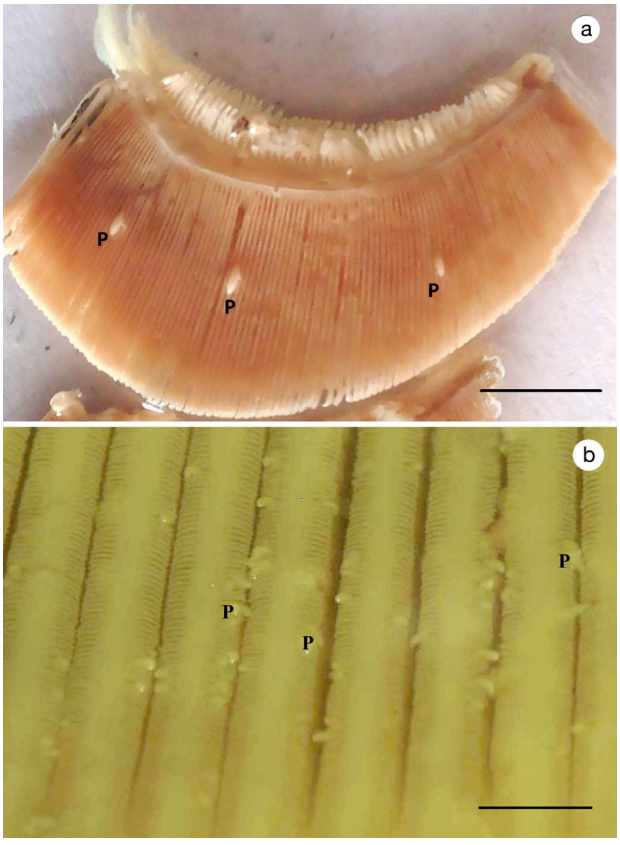

Fig. 1. Gills of aquacultured fish infected with myxozoan plasmodia (P). (a) *Labea rohita* infected with *Thelohanellus bifurcata*; (b) *Catla catla* infected with *M. longisporus*. Scale bars = 10 mm

lent followed by *T. dykovae* (38.57%), *T. filli* (33.33%) and lower prevalence was recorded for *Henneguya* sp. I (17.14%), *M. dossoui* (15%) and *Henneguya* sp. II (12%) (Table 2).

The present study indicated that myxozoan infection in cultured fishes was variable depending upon the species involved from light to heavy to severe as indicated by the GPI. The highest GPI score (4) indicating severe infections having 20–50 or more plasmodia per gill (one side) was detected in *M. nanokiensis*, *M. longisporus*, *M. potularis* and *M. slendrii.*

The size of plasmodia varied with the species of the myxozoan parasite. The plasmodia of 2 species *T. bifurcata* and *T. filli* ranged from 1.5 to 3.0 mm in size and were visible with the naked eye as cream-coloured white cysts on the surface of the gills (Fig. 1). Tissue preferences within gills varied with the myxozoan species as plasmodia were detected in the gill lamellae, gill filament and gill arch (Figs. 2 to 4). The majority of the myxozoans recorded in the gill lamellae were either located centrally within single gill lamella (LV_1) or involved multiple gill lamellae leading to fusion (LV_3) causing hypertrophy of lamellar cells. The plasmodia located in the afferent artery within the gill filament (FV_1) also caused its hypertrophy. *M. slendrii, M. potularis, M. basui and Myxobolus* sp. I were recorded in 2 locations (LV_1, LV_3), *M. moli* in 2 locations (LV_1, LV_2) and *T. bifurcata* was also recorded in 2 locations (FV_1, LV_3). The plasmodia of only one species, *M. naini*, were located in the cartilaginous tissue of the gill arch (AC type) (Table 3, Fig. 2e). A considerable amount of cellular debris was detected intermixed within the spores inside the plasmodia. The intrafilamental location of plasmodium resulted in the complete distortion of the gill cells. The LV or FV type of plasmodia caused hypertrophy, inflammation and also led to adherence of adjacent secondary lamellae, which may lead to respiratory distress and suffocation.

In the present study, 259 (65.66%) out of 395 infected fishes examined showed mixed infection comprising 2 (biparasitism), 3 (triparasitism) and 4 (polyparasitism) myxozoan species in individual fish. The most common combination was polyparasitism comprising *M. potularis* + *M. longisporus* + *M. stomum* + *M. slendrii* (23.16%) in *L. rohita* followed by *M. patialensis* + *M. slendrii* + *M. basui* + *M. venkateshi* (17.7%) in *C. mrigala* (Table 4).

Myxozoan infection was also recorded in the scale, fin, gall bladder and liver of Indian major carps. Furthermore, no myxozoan gill infection was recorded in the exotic carps, i.e. *H. molitrix*, *C. idella* and *C. carpio*; however, scales and fins of *H. molitrix*, and fins of *C. idella* were infected with as many as 8 species of myxozoan parasites.

DISCUSSION

The present study exhibited 28.6% infection in cultured carps whereas Kaur & Singh (2012) reported a higher prevalence of 36% in a number of wild carp species from Punjab wetlands. Furthermore, the infection rate was highest in *Labeo rohita* (40.48%) than other carp fishes. In *L. rohita*, the prevalence of *Myxobolus potularis* was highest (62%) followed by *M. nanokiensis* (43.75%), *M. longisporus* (40%), *Thelohanellus bifurcata* (40%), *T. dykovae* (38.57%) and *M. venkateshi* (31.25%) (Table 2). Kaur & Singh (2012) also recorded 23% prevalence for *M. slendrii*, 63% for *M. patialensis* and 64% for *M. naini.* Abdel-Baki et al. (2015) recorded 51.9% (40/77) prevalence for *M. brachysporus* and 26% (20/77) for *M. israelensis* infecting kidney and spleen of *Oreochromis niloti-*

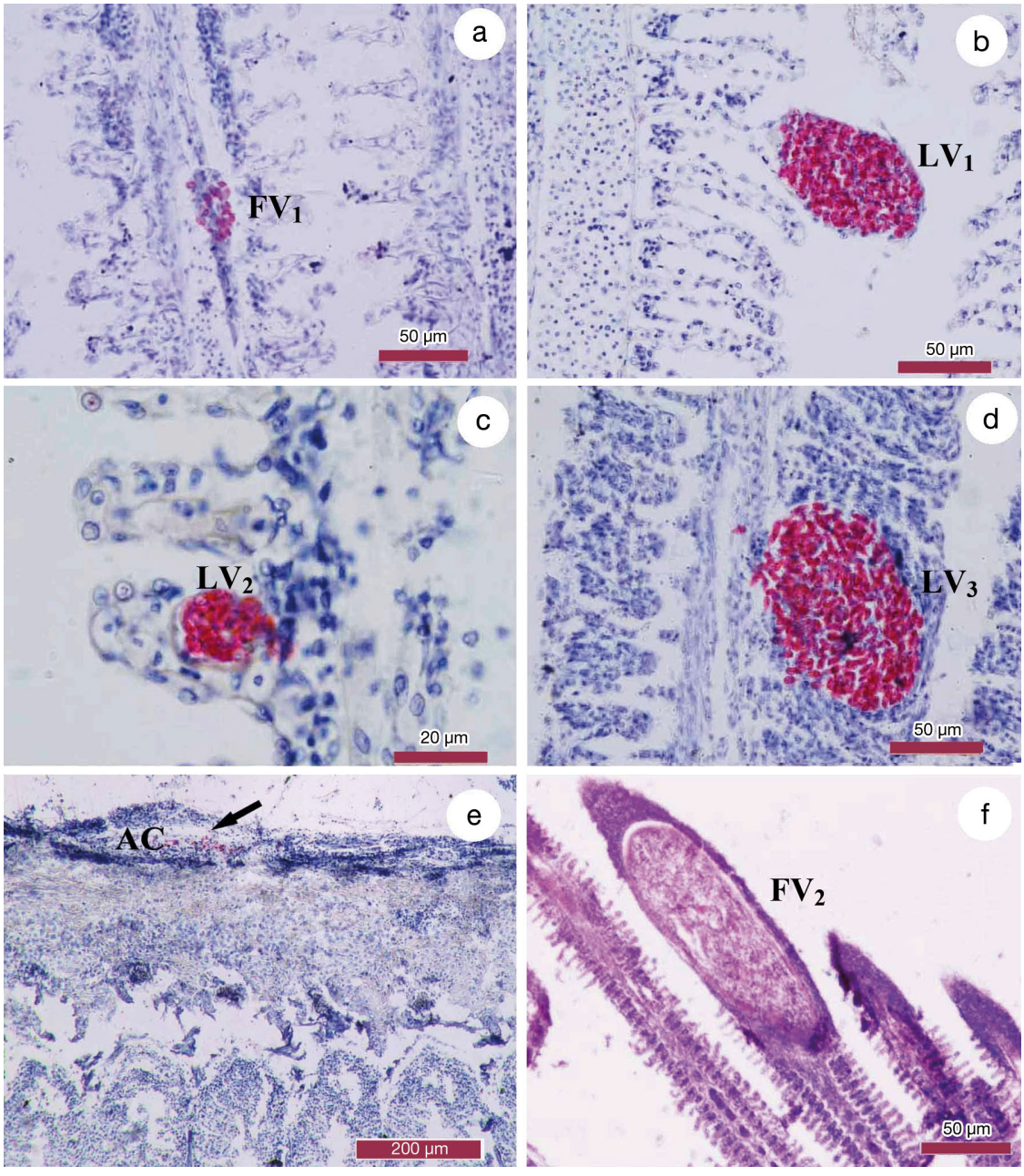

Fig. 2. Histological sections of gills from aquacultured Indian cyprinids, showing plasmodium types by location, stained by (a–e) Luna's method, (f) H&E. (a) FV_1: intrafilamental vascular (FV) plasmodium of *Myxobolus dossoui*. (b) LV_1: intralamellar vascular (LV) plasmodium of *M. potularis*. (c) LV_2: LV plasmodium of *M. moli*. (d) LV_3: LV plasmodium of *M. basui*. (e) AC: plasmodium of *M. naini* in the cartilaginous structure of gill arch. (f) FV_2: intrafilamental vascular plasmodium of *Thelohanellus bifurcata*

cus (tilapia) from Egypt. The GPI ranged from 1 to 4 (light, moderate, heavy, severe) for different species of myxozoan infecting farmed fishes. Kaur & Attri (2015) recorded the GPI of 2 in *Henneguya bicaudi* infecting gills of *Cirrhinus mrigala*.

In the present study, the majority of plasmodia were recorded in gill lamellae (LV_1, LV_3 type). Earlier in India, Kaur & Katoch (2014) reported that hypertrophy of the afferent artery was caused by the plasmodia located in the intrafilamental vascular type (FV_2) of plasmodia of *T. bifurcata*, which occupied 15–20% of each gill filament. Székely et al. (2015) reported the development of small cysts in the gill lamellae formed by *M. kalavatiae*, *M. meerutensis*, *M. catlae* and *M. bhadrensis*. Other records of myxozoan infection were by Das et al. (1988) with brain

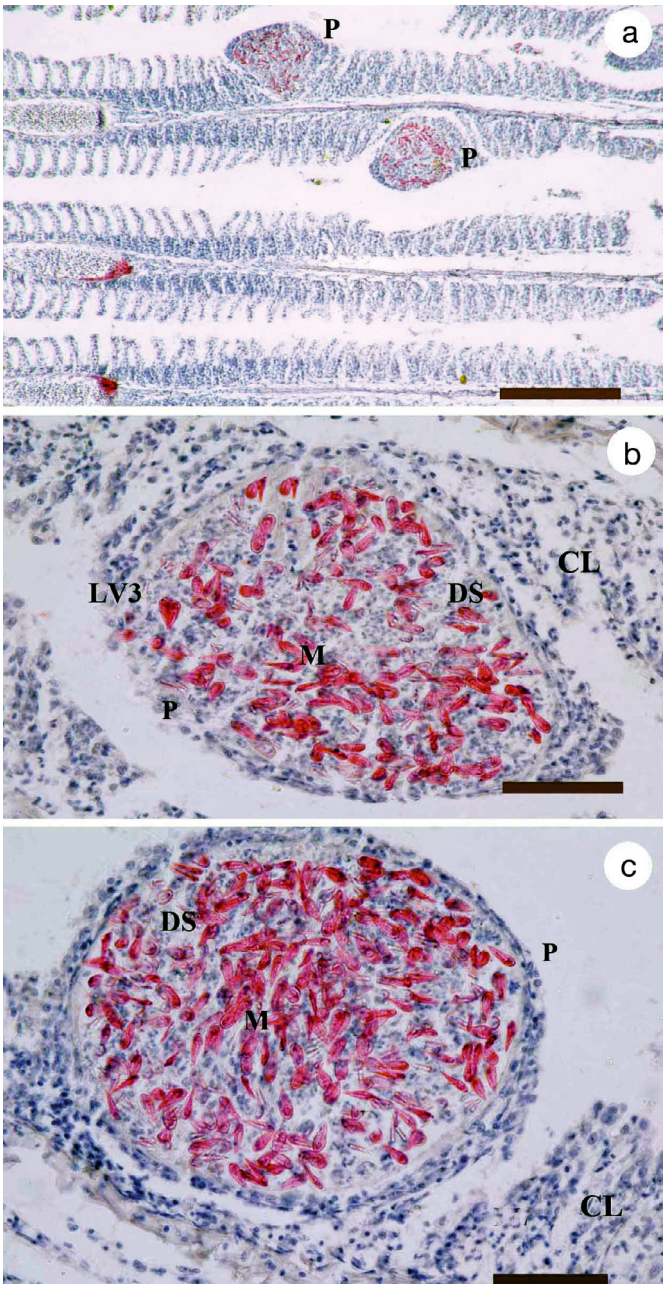

Fig. 3. (a–c) Histological sections of gills from *Labea rohita* infected with *Myxobolus slendri* showing LV_3 type plasmodium (P) containing spores, LV_3 plasmodia containing mature (M) and developmental spore stages (DS), and compressed lamellae (CL). Stained by Luna's method. Scale bars = (a) 200 µm, (b,c) 50 µm

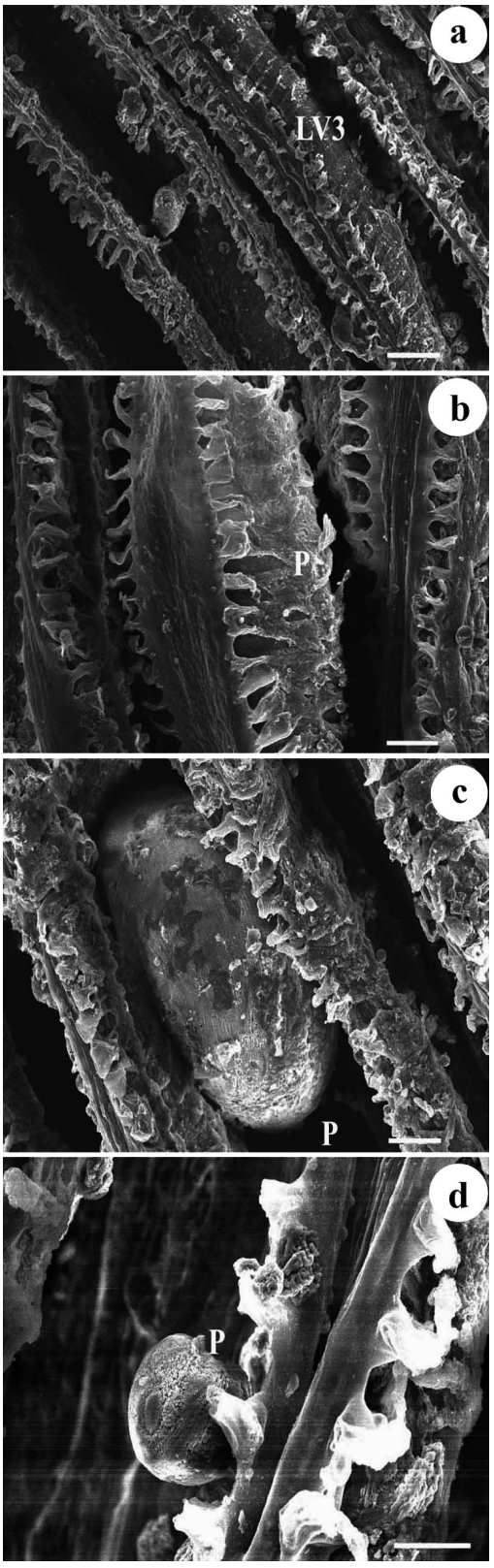

Fig. 4. Scanning electron micrographs of gills from *Labea rohita* infected with *Myxobolus potularis*. (a–c) LV_3 type plasmodia (P), (d) LV_2 type plasmodium. Scale bars = (a) 100 µm, (b,c) 50 µm, (d) 20 µm

myxoboliosis in *L. rohita*; Mohan & Shanker (1995) reported gill and renal myxobolisis in 15 d old fry of *C. catla.* Kalavati & Nandi (2007) reported that studies dealing with pathology of myxozoans parasites were mostly fragmentary. Kaur et al. (2014b) also reported that large-sized plasmodia damaged more than 50% of the gill filament and gill lamellae, causing respiratory distress and suffocation. Similar stud-

Table 3. Parasite, host, clinical presentation, visibility categories (vis. cat.), location, type of plasmodium and lesions caused by myxozoan parasites in the gills of aquacultured fish from Punjab. Vis. cat. A: not visible under stereozoom size range (40–50 µm); B: visible under stereozoom size range (0.2–0.5 mm); C: visible with the naked eye (1.5–3.0 mm). Plasmodium type (per tissue location): FV_1, small and elongated intrafilamental vascular plasmodium in the afferent artery; LV_1, intralamellar vascular plasmodium located centrally in the gill lamella; LV_2, small LV plasmodium protruding from side of the lamella; LV_3, large LV plasmodium formed by fusion of several gill lamellae; AC, plasmodium developing in the cartilaginous structure of the gill arch. See Table 1 for taxonomic and host genus names

Parasite species	Host(s)	Clinical signs	Plasmodium			Lesions
			Vis. cat.	Location	Type	
Myxobolus venkateshi	*L. rohita*, *C. mrigala*	Gill mucus-laden, pale	A	Gill lamellae	LV_1	Hypertrophy of lamellar cells
M. slendrii	*L. rohita*, *C. catla*, *C. mrigala*	Gill mucus-laden, pale	B	Gill lamellae	LV_1, LV_3	Hypertrophy of lamellar cells and fusion of adjacent lamellae
M. nanokiensis	*L. rohita*		B	Gill lamellae	LV_1	Hypertrophy of lamellar cells
M. longisporus	*L. rohita*, *C. catla*		B	Gill lamellae	LV_1	Hypertrophy of lamellar cells
M. stomum	*L. rohita*		A	Gill lamellae	LV_1	Hypertrophy of lamellar cells
M. potularis	*L. rohita*		A	Gill lamellae	LV_1, LV_3	Hypertrophy of lamellar cells and fusion of adjacent lamellae
M. naini	*C. catla*		A	Gill arch	AC	Formation of sac-like protrusion into the lumen of gill arch
M. patialensis	*C. mrigala*	Gill mucus-laden, pale	A	Gill lamellae	LV_1	Hypertrophy of lamellar cells
M. moli	*C. mrigala*		A	Gill filament Gill lamellae	FV_1, LV_2	Hypertrophy of afferent artery, epithelial cell destruction
M. dossoui	*C. mrigala*		A	Gill filament	FV_1	Hypertrophy of afferent artery
M. nchoutnounensis	*C. mrigala*		A	Gill lamellae	LV_1	Hypertrophy of lamellar cells
M. basui	*C. mrigala*	Gill mucus-laden, pale	A	Gill lamellae	LV_1, LV_3	Hypertrophy of lamellar cells and fusion of adjacent lamellae
Myxobolus sp. I	*L. rohita*	Gill mucus-laden, pale	C	Gill lamellae	LV_3	Hypertrophy of lamellar cells and fusion of adjacent lamellae
Myxobolus sp. II	*L. rohita*		A	Gill lamellae	LV_1, LV_3	Hypertrophy of lamellar cells and fusion of adjacent lamellae
Thelohanellus bifurcata	*L. rohita*	Gill mucus-laden, pale, haemorrhagic	C	Gill lamellae Gill filament	LV_3, FV_1	Hypertrophy of lamellar cells and fusion of adjacent lamellae
T. dykovae	*L. rohita*	Gill mucus-laden, pale	B	Gill filament	FV_1	Hypertrophy of afferent artery
T. filli	*L. rohita*	Gill mucus-laden, pale	C	Gill filament	FV_1	Hypertrophy of afferent artery
Henneguya sp. I	*C. mrigala*		B	Gill filament	FV_1	Hypertrophy of afferent artery
Henneguya sp. II	*S. seenghala*		A	Gill lamellae	LV_1	Hypertrophy of afferent artery

ies have been made by Schulman (1957), Current & Janovy (1978), Dykova & Lom (1978), Shariff (1982), Bowser & de Campos (1985), Kalavati & Narasimhamurti (1985), Rukyani (1990), Martins et al. (1997), Adriano et al. (2009), Chavda et al. (2010), de Campos et al. (2011) and Kaur & Katoch (2014). Clinical presentation was not well marked in most of the cases except in some gills which were mucus-laden and pale in appearance. Longshaw et al. (2005) and Kaur & Singh (2012) also recorded that the majority of infection with *Myxobolus* species was in the gills. Kalavati & Nandi (2007) recorded gill myxoboliasis as the most widely distributed disease in cultured carps in many states of India and reported heavy mortality due to the myxozoans.

Mixed infection with more than one species was recorded in 65.15% of fishes examined. Holzer et al. (2010) also detected 3 myxozoan species comprising *Tetracapsuloids bryosalmonae*, *Sphaerospora truttae* and *Chloromyxon schorovi* in the renal tubules.

Table 4. Occurrence of mixed infections (n = 259 fish total) of myxozoan parasites in the gills of aquacultured cyprinids from Punjab. See Table 1 for genus names

Host	Parasites involved	Cases (% total mixed infections)
Biparasitism		
C. catla	*M. naini* + *M. slendrii*	25 (9.65%)
C. mrigala	*M. dossoui* + *M. moli*	23 (8.88%)
L. rohita	*M. nanokiensis* + *T. dykovae*	32 (12.36%)
	M. potularis + *Myxobolus* sp. I	35 (13.51%)
Triparasitism		
C. catla	*M. patialensis* + *M. naini* + *M. slendrii*	38 (14.67%)
Polyparasitism		
C. mrigala	*M. patialensis* + *M. slendrii* + *M. basui* + *M. venkateshi*	46 (17.76%)
L. rohita	*M. potularis* + *M. longisporus* + *M. stomum* + *M. slendrii*	60 (23.17%)

Recently, Laamiri (2014) studied the phenomenon of polyparasitism in sea bream *Sarpa salpa* and recorded biparasitism with parasites *Ceratomyxa herouardi* and *C. pallida* occurring at a frequency of 16.97%. The high incidence of mixed infection in aquaculture may be due to the nutrient enrichment of the ponds practising polyculture in Punjab. Besides this, there can be multiple environmental factors such as temperature and eutrophication affecting disease development. Future studies are needed to evaluate if greater pathogenicity results from infection with multiple spores and other parasites and pathogens.

In the present study, the myxozoan gills infection was recorded in the Indian major carps, and no parasite was recorded in the gills of the 3 exotic carps. According to White & Perkins (2012), invasive populations frequently harbour a reduced parasite community as compared with their native counterparts. Torchin et al. (2003) and Mitchell & Power (2003) reported 53% fewer helminths in non-native host species as compared to native ones. Blossey & Nötzold (1995), suggested that in the absence of parasites, invasive species reallocate energetic resources away from unnecessary defence mechanisms into fitness and growth, potentially leading to a mechanism explaining invader's success and evolution of the increased competitive ability.

It is concluded from the study that in order to identify and describe myxozoan species sufficient attention should be paid to host, tissue and organ specificity since many *Myxobolus* spp. are known for their strict host specificity whereas others may infect several closely related cyprinids.

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