Prevalence, morphology and scanning electron microscopy study of myxozoan parasites

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Abstract The present study was conducted from Garia, West Bengal, India to study the Prevalence, Morphology, Severity of infestation and Scanning Electron Microscopy of Myxozoan parasites in Indian Major Carps. A total of 155 fishes were examined, out of which 80 were found infected with myxozoan parasites (51.61 %) and severity of infestation varied from 0.5 to 2. Three known species *Myxobolus orissae*, *M. carnaticus* and *Thelohanellus caudatus* were found infecting various organs such as gills and fins of Indian major carps. Spores of the species, *T. caudatus* measures 12.84 ± 0.77 (11.4–14.2) $\mu\text{m} \times 8.5 \pm 0.71$ (7.6–9.6) μm and was elongated pyriform in shape with rounded posterior and tapering anterior end. Parietal folds were absent. The single polar capsule is rounded to oval shaped with slightly pointed anterior end and broad posterior end with size measuring 6.15 ± 2.09 (4.2–10.4) $\mu\text{m} \times 3.85 \pm 1.18$ (2.3–4.9) μm . *M. orissae*, size of the mature spore measures $15.6\text{--}19.7$ (17.25) $\mu\text{m} \times 5.7\text{--}9.3$ (6.70) μm and was elongated pyriform in shape. Two polar capsules are distinctly unequal. Large one measures $6.8\text{--}13.5$ (8.75) $\times 1.4\text{--}3.1$ (1.90) μm and smaller one $6.9\text{--}11.5$ (7.44) $\times 1.7\text{--}2.4$ (1.57) μm in size. Both are broadly pyriform with pointed anterior end and rounded posterior end. *Myxobolus carnaticus* mature histozoic spores measures $8.1\text{--}12.9$ (9.49) $\times 7.2\text{--}10$ (8.27) μm are creamy white to yellow in colour tear shaped in valvular view with rounded posterior and bluntly pointed anterior ends.

Keywords Myxozoans · PFI (%) · SEM · Severity · West Bengal · India

Introduction

The aquaculture is considered to be one of the important factors of the world economy in recent decades. The biggest challenges that was faced by the fish farmers is to control many biotic and abiotic factors, influencing fish rearing and aquaculture operations (Perveen and Ullah 2013). The state of West Bengal has always attracted attention for being the highest producer of freshwater table fish and fish seed in the country together with the unique distinction of having the maximum water area under traditional shrimp farming. Everyday about 250 million litre of waste water along with solid waste is discharged into the Bheries. Polluted and contaminated water can cause to stress on the fishes, which lead to many parasitic diseases (Ramudu and Dash, 2013). It is important to mention that the parasitic infestations are reportedly playing a major role in disease occurrences (78 %) in Indian freshwater aquaculture (Lakra et al. 2006). (Mohan and Bhatta 2002) reported that ectoparasitic diseases are the main problem in freshwater fish farms of Andhra Pradesh, India which causes an annual loss of US\$ 1 million due to disease-induced mortality and impaired growth. Further, the freshwater fish farmers of Andhra Pradesh, India (Vineetha and Abraham, 2009) and West Bengal, India (Sil et al. 2013) were estimated to produce about 2 and 26 % less, respectively than the expected production due to diseases, poor farm management practices and impaired growth. In order to increase profitability, health care based on the knowledge of organisms, their ecology, and application of the knowledge in the control of diseases is essential

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(Snieszko, 1983; Kaur et al. 2012). Therefore, contribution to the knowledge of fish parasites is a pre-requisite for the rapid and correct diagnosis of the disease. Early diagnosis can lead to preventive measures which is the best way to reduce outbreak of disease (Abdel 2012; Morsy et al. 2012; Kaur et al. 2012). Most of the fishes are vulnerable to various parasitic infections, out of which Myxozoa is emerging as the major group. Presently, there are more than 2,180 species in 62 genera belonging to the Phylum Myxozoa has been described (Lom and Dykova, 2006). Eiras et al. (2005) listed approximately 744 species of *Myxobolus* all over the world. Recently a new genus *Thelohanelloid bengalensis* gen. nov. sp. nov. from gall bladder of *Arius sagor* (a marine fish in Bay of Bengal) has been described by Sarkar (2009). According to Kalavati and Nandi (2007) 104 species of *Myxobolus* spp. are restricted to the Indian subcontinent. The present study also brings about conclusion that the *Labeo rohita* and *Cirrhinus mrigala* were vulnerable to different parasites.

Materials and methods

The fishes pertaining to the present study were collected and examined from July 2013 to September 2013 from Garia, West Bengal, India. Fishes of medium (120–140 g) and small size (60–120 g) (young in age) were highly infected. Myxozoan cysts found attached on the tissues like gills, fins and body surfaces were isolated carefully. Free floating cysts found in gill mucus were isolated using dropper. For detailed study, fresh cysts were first taken on clean glass slides and slightly ruptured on one end with a needle. Smear was made on clean glass slide with a drop of 0.98 % NaCl solution covered with cover slip and was examined for the presence of spores. Some of the smears were immersed in Lugol's Iodine solution to observe iodophilous vacuole and a few others were treated with 8 % KOH for filament extrusion.

Permanent mounting of myxosporean parasites were done using Giemsa stain. Air dried smears on grease free clean slides were treated with acetone free absolute methyl alcohol for about 8 min to fix the parasites and again dried. The stock solution of Giemsa was diluted with water in the ratio of 1:2 and buffered at pH 7.2. The slides were then placed on a staining rack and covered with dilute stain for 40 min. Finally, the slides were washed by pouring neutral distilled water until the colour did not turn to a noticeable extent and the slides were dried in air. Phenotypic characterizations of all Myxozoans (protozoans) parasites were studied as described by (Soulsby 1982). Photomicrographs and all measurements were taken using a Motic BA400 phase contrast microscope with in-built digital camera.

All measurements are in microns (μm) as range values followed by mean $\pm$ SD in parentheses. The abbreviations used in the paper are as follows: LS: length of spore; WS: width of spore; LPC: length of polar capsule; WPC: width of polar capsule; LLPC: length of larger polar capsule; LSPC: length of smaller polar capsule; WLPC: width of larger polar capsule; WSPC: width of smaller polar capsule; ICP: inter-capsular process; TS: thickness of spore valves; NC: number of coils of polar filaments; SD: standard deviation.

The Parasitic Frequency Index (PFI) was calculated by taking the percentage of the number of hosts infected by an individual parasite species against the total number of hosts examined in a particular area under investigation. The prevalence was expressed in percentage and is calculated by Total number of infected fishes/Total number of fish host examined.

The frequency index were further classified as rare (0.1–9.9 %), occasional (10–29.9 %), common (30–69.9 %) and abundant (70–100 %) as per (Srivastava 1980). In order to assign numerical qualitative value to severity grade of infections surface infestation and disease syndrome severity, the generalize scheme by (Lightner 1993) and Ramudu and Dash, (2013) was followed to determine the severity of infection/infestation in fishes.

The myxosporidian spores of the genus *Thelohanellus* and *Myxobolus* were identified up to species level based on their morphometric measurements and the same samples were taken for scanning electron microscopy. For scanning electron microscopy, sporogenic plasmodia enclosed within the host tissue were isolated with the help of a sterile forceps and placed on to thin cover slips pre-coated with poly-L-lysine. These were then ruptured and the envelopes were removed with the tip of a needle. Utmost care was taken to remove entire remnants of the host tissue so as to free the samples from any foreign particles. The spores were fixed with 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for two hours at room temperature. Following dehydration in a standard ethanol gradient, the samples were transferred to a series of absolute alcohol and amyl acetate mixture (3:1, 1:1 and 1:3) for 30 min each before finally reaching to amyl acetate (Adriano et al. 2002). The samples were then dried up to critical point using CO₂ in a HCP:2 Critical Point Dryer (Hitachi), coated with metallic gold in an IB-2 ion coater and examined in a Hitachi S-530 Scanning Electron Microscope at accelerating voltages of 15 and 20 kV.

Results

During the present study, the percentage and severity of infestation of myxozoan parasites of freshwater fishes is shown in Table 1.

Table 1 Prevalence (PFI %) and severity of infection of parasites in West Bengal during the period between July 2013 and September 2013

Months	Fishes	Size of the fishes (g)	Total no. of fish investigated	Observed parasites	No. of infested fishes	PFI (%)	Site of infestation	Severity of infestation
July	<i>Labeo rohita</i>	135	30	<i>Thelohanellus caudatus</i>	2	6.66 ^a	Dorsal fin	0.5
	<i>Cirrhinus mrigala</i>	143	26	<i>Myxobolus orissae</i>	3	11.53 ^b	Gill lamellae	0.5
August	<i>Labeo rohita</i>	127	27	<i>Thelohanellus caudatus</i>	28	74.07 ^d	Caudal fin, Tail fin	1
	<i>Cirrhinus mrigala</i>	62	25	<i>Myxobolus orissae</i>	22	88.00 ^d	Gill	2
				<i>Myxobolus carnicus</i>	9	36.00 ^c	Gill, Anal fin	1
September	<i>Labeo rohita</i>	181	27	<i>Thelohanellus caudatus</i>	2	7.40 ^a	Pelvic fin	0.5
	<i>Cirrhinus mrigala</i>	32	20	<i>Myxobolus carnicus</i>	12	60.00 ^c	Gill, Dorsal fins	0.5
				<i>Myxobolus orissae</i>	2	10 ^b	Gill	0.5

PFI Parasitic frequency index (%)

^a Rare (0.1–9.9 %)

^b Occasional (10–29.9 %)

^c Common (30–69.9 %)

^d Abundant (70–100 %)

Parasitic Frequency Index of *Myxobolus orissae* highest in August, (PFI, 88 %) which were stated as abundant and lowest (PFI, 10 %) in September, stated as occasional in *Cirrhinus mrigala*. *Myxobolus carnicus* were observed highest in the month of September (PFI, 60 %) which were stated as ‘common’ condition. Lowest prevalence were recorded in the month of August (PFI, 36 %) which were stated as ‘common’ in *Cirrhinus mrigala*. Infestations of *Thelohanellus caudatus* were high in August (PFI, 74.07 %), which were stated as ‘abundant’. Lowest infection were observed in July (PFI, 6.66 %) stated as rare. Among all 3 months highest parasitic infestation were observed in the month of August.

Morphometric measurements of myxosporidian spores

Thelohanellus caudatus

Plasmodia

The species was identified from the caudal fin of freshwater carp *Labeo rohita* (Hamilton). The plasmodia appeared as small, white to pale yellow, elongated, 0.5–1.0 mm in length. There were 2–3 plasmodia per caudal fin and each plasmodia contains about 50–70 spores.

Spores

The size of the mature spore measures 12.84 ± 0.77 (11.4 – 14.2) $\mu\text{m} \times 8.5 \pm 0.71$ (7.6 – 9.6) μm and was elongated pyriform in shape with rounded posterior and tapering anterior end. The sutural ridge was distinct and straight. Thin, smooth and symmetrical shell valves of size 0.6 μm

were observed. Parietal folds were absent. The single polar capsule is round to oval shaped with slightly pointed anterior end and broad posterior end. It measures 6.15 ± 2.09 (4.2 – 10.4) $\mu\text{m} \times 3.85 \pm 1.18$ (2.3 – 4.9) μm and situated just beneath the anterior end of the spore. The polar filament forms 5–6 coils within the capsule. When completely extruded, the anterior end of the long thread like polar filament measures 39.30 ± 24.36 (32.6 – 65.4) μm , appeared to be wavy and tapering (Table 3). The sporoplasm contains three large nuclei measuring 0.49 ± 0.88 (1.5 – 2.7) μm in diameter. Comparisons of the *T. caudatus* with other reports, morphometry and spore index were calculated and shown in Tables 2, 3 and 4 respectively.

Remarks

The present observations (LS/WS: 1:0.66) on *T. caudatus* are in conformity with the original description (LS/WS: 1:0.66) of Pagarkar and Das (1993), except for some minor variations in the length of polar capsules. The SEM study reveals that the group of *T. caudatus* from caudal fin collected from *Labeo rohita* with distinct polar capsule (Fig. 1), having distinct polar filament (Fig. 2) and the anterior end of spore is smooth with suture folds and a sutural protrusion forming a rim around the spores.

Myxobolus orissae

Plasmodia

The species was identified from the gills (gill lamellae) of freshwater carp *Cirrhinus mrigala*. The plasmodia appeared as a very small, white to pale color, elongated.

Table 2 Spore comparison of *Thelohanellus caudatus* with closely related species of the same genus

Characters	<i>Thelohanellus caudatus</i>	<i>Thelohanellus caudatus</i>	Present study
Host(s)	<i>Labeo rohita</i>	<i>Labeo rohita</i>	<i>Labeo rohita</i>
Site of infestation	Caudal fin; anal fin	Caudal fin	Caudal fin, Dorsal fin and Pelvic fins
Locality	West Bengal (India)	Kanjali wetland, Punjab (India)	West Bengal (India)
Length of the spore	13.0–14.0 (13.5)	14.8–15.2 (15)	11.4–14.2 (12.84)
Breadth of the spore	8.5–9.5 (9)	6.8–7.2 (7)	7.6–9.6 (8.5)
Length of polar capsule	7.0–7.5 (7.25)	6.0–6.6 (6.3)	4.2–10.4 (6.15)
Breadth of the polar capsule	5.0–5.5 (5.25)	2.9–3.3 (3.1)	2.3–4.9 (3.85)
Number of coils	5–6	5–6	–
PF	–	–	32.6–65.4 (39.30)
PCs equal	–	–	Yes
Reference	Pagarkar and Das (1993)	Singh and Kaur (2012a, b)	Present study

PF polar filament, PCs polar capsule

– Indicate no data observation on the basis of 20 observations

Table 3 Morphometric study of 30 fresh/stained spores of *Thelohanellus caudatus*

Characters	Range	Mean $\pm$ SD	SE	CV %
LS	11.4–14.2	12.84 $\pm$ 0.77	0.13	6.02
BS	7.6–9.6	8.5 $\pm$ 0.71	0.12	8.37
LPC	4.2–10.4	6.15 $\pm$ 2.09	0.37	34.04
BPC	2.3–4.9	3.85 $\pm$ 1.18	0.21	30.78
LPF	32.6–65.4	39.30 $\pm$ 24.36	4.31	61.98
Size of the plasmodia	1.5–2.7	0.49 $\pm$ 0.88	0.15	179.45

LS length of spore, BS breadth/width of spore, LPC length of polar capsule, BPC breadth of polar capsule

Spores

Size of the mature spore of *M. orissae* measures 15.6–19.7 (17.25) $\mu\text{m} \times$ 5.7–9.3 (6.70) μm and was elongated pyriform in shape. The anterior end is flat between the openings of polar capsules and posterior end is elongated and rounded. Two polar capsules are distinctly unequal. Large one measures 6.8–13.5 (8.75) $\times$ 1.4–3.1 (1.90) μm and smaller one 6.9–11.5 (7.44) $\times$ 1.7–2.4 (1.57) μm in size. Both are broadly pyriform with pointed pointed anterior end and rounded posterior end. The capsules open independently. Both polar capsules are situated parallel to each other in spore cavity. Both occupies similar space in spore cavity, extruded polar filaments are unequal in length, larger polar capsule having long filament, 32.9–91.1 (44.66) μm and smaller polar capsule having small polar filament, 24.5–131.1 (23.83) μm . Base of the polar filament are very thick and edges of the filaments are very thin. Comparisons of the *M. orissae* with other reports, morphometry and spore index were calculated in Tables 5, 6 and 7 respectively.

Table 4 Spore Index comparison with previous studies of earlier workers

Spore index	Singh and Kaur (2012a, b)	Pagarkar and Das (1993)	Present study
LS:BS	1:0.46	1:0.66	1:0.66
LPC:BPC	1:0.49	1:0.72	1:0.62
LS:LPC	1:0.42	1:0.53	1:0.47
BS:BPC	1:0.44	1:0.58	1:0.45

LS length of spore, BS Breadth/width of spore, LPC length of polar capsule, BPC breadth of polar capsule

Remarks

The present observations (LS/WS: 1:0.388) on *M. orissae* are in conformity with the original description (LS/WS: 1:0.43) of Halder et al. (1996), except some minor variations in the size of spores (Table 7). The SEM study reveals that the group of *M. orissae* collected from gills

Fig. 1 Scanning electron micrograph (SEM) of group of *Thelohanellus caudatus* from caudal fin of *Labeo rohita* with distinct polar capsule (arrows), $\times 1500$, scale bar = 20 μm

Fig. 2 *Thelohanellus caudatus* collected from the fins of *Labeo rohita* (Giemsa stained, $\times 200$)

(gill lamellae) in *Cirrhinus mrigala* Ham. *M. orissae* were observed in SEM with high magnification of distinct polar capsule (Fig. 3), and also distinct polar filament observed in contrast phase microscope (Fig. 4). The anterior end of spore is smooth along with suture folds and a sutural protrusion form a rim around the spores.

Myxobolus carnaticus

Plasmodia

The species was identified from the gills (gill lamellae) of freshwater carp *Cirrhinus mrigala*. The plasmodia appeared as a very small, white to pale colour, elongated.

Spores

Size of mature histozoic spores range from 8.1 to 12.9 (9.49) $\times$ 7.2–10 (8.27) μm and are creamy white to yellow in colour, tear shaped in valvular view with rounded posterior and bluntly pointed anterior ends. *M. carnaticus* having unequal polar capsules and an intercapsular ridge, the size of the spore, the polar capsules (larger 2.2–4.4 (3.09) $\times$ 1.1–3.4 (2.07) μm , smaller 2.1–4.6 (2.77) $\times$ 1.3–3.1 (1.90) μm) and length of the polar capsule (large) 22.6–39.8 (20.42), small 8.3–45 (12.52). Polar filaments are unequal, one is long, another one is short. Length of the long polar filament is 22.6–39.8 (20.42) μm and short polar filament is 8.3–45 (12.52) μm which is clearly shown in Tables 8, 9.

Table 5 Morphometric comparison of *Myxobolus orissae* with closely related species of the same genus

Characters	<i>Myxobolus orissae</i>	Present study
Host(s)	<i>Cirrhinus mrigala</i> Ham.	<i>Cirrhinus mrigala</i> Ham.
Site of infestation	Gills	Gills
Locality	Orissa	West Bengal
Length of the spore	13–19.5 (15.71)	15.6–19.7 (17.25)
Breadth of the spore	4.9–8.1 (6.8)	5.7–9.3 (6.70)
Length of polar capsule (small)	6.5–11.4 (7.58)	6.9–11.5 (7.44)
Breadth of the polar capsule (small)	1.6–3.4 (2.57)	1.7–2.4 (1.57)
Length of polar capsule (large)	7.3–11.8 (8.8)	6.8–13.5 (8.75)
Breadth of the polar capsule (large)	2.4–3.2 (1.78)	1.4–3.1 (1.90)
ICP	Present	–
PF	–	Present: small: 24.5–131.1 (23.83) Present: large: 32.9–91.1 (44.66)
PCs equal	No	No
Reference	Haldar et al. (1996)	–

PF polar filament, PCs polar capsule

– Indicate no data observation on the basis of 20 observations

Table 6 Morphometry of 30 fresh/stained spores of *Myxobolus orissae*

Characters	LS	BS	LLPC	LBPC	SLPC	SBPC	LLPF	SLPF	LN
Range	15.6–9.7	5.7–9.3	6.8–13.5	1.4–3.1	6.9–11.5	1.7–2.4	32.9–91.1	24.5–131.1	2.5
Mean	17.25	6.70	8.75	1.90	7.44	1.57	44.66	23.83	0.11
SD	0.98	0.78	3.22	0.74	3.85	0.89	31.52	38.39	0.53
SE	0.21	0.16	0.68	0.15	0.82	0.19	6.72	8.186	0.11
CV	5.70	11.66	36.83	39.07	51.86	56.74	70.58	161.09	469.04

LS length of spore, BS breadth of spore, LPC length of polar capsule, BPC breadth of polar capsule

Table 7 Spore index comparison with studies earlier workers

Spore index	Haldar et al. (1996)		Present study	
	Big	Small	Big	Small
LS:BS	1:0.43	–	1:0.388	–
LPC:BPC	1:0.20	1:0.33	1:0.21	1:0.21
LS:LPC	1:0.56	1:0.48	1:0.50	1:0.43
BS:BPC	1:0.26	1:0.37	1:0.28	1:0.23

LS length of Spore, BS breadth of spore, LPC length of polar capsule, BPC breadth of polar capsule

– Indicate no data observation on the basis of 20 observations

Fig. 3 Scanning electron micrograph (SEM) of group (arrow) of *Myxobolus orissae* from gills of *Cirrhinus mrigala*, $\times 1000$, scale bar = 50 μm

Remarks

The present observations (LS/WS: 1:0.87) on *M. carnaticus* are in conformity with the original description (LS/WS: 1:0.79) of Seenappa and Manohar (1980) except some minor variations in the size of spores (Table 10). The SEM study revealed that the Critical Point Drying (CPD) of dorsal fin of *Cirrhinus mrigala* with group of *M. carnaticus* (Fig. 5) and showing distinct long polar filaments in Fig. 6.

Fig. 4 *Myxobolus orissae* with distinct polar filament (arrows) in the gills of *Cirrhinus mrigala* (Giemsa stained, $\times 200$)

Discussion

In present study, *T. caudatus* spore measurements almost similar with previous study as those obtained by Singh and Kaur (2012a, b) and Pagarkar and Das (1993) these results were strongly supported with present morphometric characteristics.

Myxobolus orissae morphometric characteristics corroborated with results of Haldar et al. (1996). Still some slight minor variations in the size of spores (Table 6).

Myxobolus carnaticus looks like with *M. dispar* having polar capsules and an intercapsular ridge which differ with spore size, also resembles *M. indicum* but differs in the site of infection, size and shape of the spore and the size of the smaller polar capsule. *M. curmucae* n.sp. from beneath the scales of *Puntius curmuca* at Bantwal is similar to *M. batae* in the shapes of the spore and polar capsules, in the presence of an intercapsular ridge and in spore size ($8\text{--}11 \times 7\text{--}8 \mu\text{m}$), but differs in the site of infection, absence of thickenings on the sutural ridge and in the thickness of the spore ($5.0\text{--}5.5 \mu\text{m}$). It also resembles *M. vanivilasae* but differs in spore shape and size of the polar capsule. Present study was supported with Seenappa and Manohar, (1980), with morphometric characteristics,

Table 8 Morphometric comparison of *Myxobolus carnaticus* with closely related species of the same genus

Characters	<i>Myxobolus carnaticus</i>	Present study
Host(s)	<i>Cirrhinus mrigala</i> Ham.	<i>Cirrhinus mrigala</i> Ham.
Site of infestation	Inner base of hemibranch	Gills, Dorsal fin, Caudal fin and anal fin
Locality	Karnataka	Garia station, Kolkata, West Bengal,
Length of the spore	8–9 (8.6)	8.1–12.9 (9.49)
Breadth of the spore	6–7 (6.8)	7.2–10 (8.27)
Length of polar capsule (small)	2–3 (2.1)	2.1–4.6 (2.77)
Breadth of the polar capsule (small)	1–2 (1.5)	1.3–3.1 (1.90)
Length of polar capsule (large)	3.5–4 (3.8)	2.2–4.4 (3.09)
Breadth of the polar capsule (large)	2	1.1–3.4 (2.07)
ICP	Present	–
PF	–	Present: small: 8.3–45 (12.52) Present: large: 22.6–39.8 (20.42)
PCs equal	No	No
Reference	Seenappa and Manohar, (1980)	Present study

PF polar filament, PCs polar capsule

– Indicate no data observation on the basis of 20 observations

Table 9 Morphometry of 30 fresh/stained spores of *Myxobolus carnaticus*

Characters	LS	BS	LLPC	LBPC	SLPC	SBPC	LLPF	SLPF	LN
Range	8.1–12.9	7.2–10	2.2–4.4	1.1–3.4	2.1–4.6	1.3–3.1	22.6–39.8	8.3–45	2.2–3.8
Mean	9.49	8.27	3.09	2.07	2.77	1.90	20.42	12.52	0.67
SD	0.98	0.62	0.48	0.48	0.74	0.56	14.14	15.10	1.27
SE	0.17	0.11	0.08	0.08	0.13	0.10	2.54	2.716	0.22
CV	10.35	7.58	15.81	23.63	26.93	29.92	69.24	120.64	189.14

SD standard deviation, SE standard error, CV Coefficient of variation

Table 10 Spore index comparison with studies of earlier workers

Spore index	Seenappa and Manohar, (1980)		Present study	
	Big	Small	Big	Small
LS:BS	1:0.79	–	1:0.87	–
LPC:BPC	1:0.52	1:0.71	1:0.66	1:0.68
LS:LPC	1:0.44	1:0.24	1:0.32	1:0.29
BS:BPC	1:0.29	1:0.22	1:0.25	1:0.23

LS length of spore, BS breadth of spore, LPC length of polar capsule, BPC breadth of polar capsule

except some minor variations in the size of spores (Table 9).

The slight dissimilarities were observed in size and shape does not exceed the limits of natural variations, typical of populations or species. Perhaps the small discrepancies in morphometry of the parasites are influenced by difference in location, ecology and age of the hosts, which supported with the works of (Moshu and Molnar

Fig. 5 Scanning electron micrograph (SEM) of CPD (Critical Point Drying) of dorsal fin of *Cirrhinus mrigala* with group (arrows) of *Myxobolus carnaticus*, $\times 2000$, scale bar = 20 μm

1997). Molnar (1994) considered myxosporeans are host, organ and tissue specific organisms. Organ specificity is determined by tissue specificity.

Fig. 6 *Myxobolus carnaticus* with distinct polar capsule (arrows) in the gills, fins of *Cirrhinus mrigala* (Giemsa stained, ×200)

Conclusion

The present study brings about the conclusion that the *Labeo rohita* and *Cirrhinus mrigala* were vulnerable to different parasites such as *T. caudatus*, *M. orissae* and *M. carnaticus* respectively. All these parasites spore measurements nearly similar with previous studies and confirmed by Scanning Electron Microscopic study for species level identification. Rainy season (July–September) was the most susceptible period to get these parasitic infestations. During this period the water quality get deteriorates and the fishes were in stressed condition which favours the parasites to infest and which is also favours their reproduction due to the availability of their intermediate hosts. These parasites were found mostly on gills, dorsal fin, caudal fin, anal fins and skin of the fishes. The small (32 g) and medium (62 g) size fishes were found to be more susceptible of Myxozoan Parasites due to their poor immunity power which favours more infestation of parasites. There were few morphometric studies on *T. caudatus*, *M. orissae* and *M. carnaticus* but it was the first SEM study on these three myxozoan parasites.

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