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Molecular Identification of Seven *Myxobolus* Species (Myxosporea: Myxobolidae) in Cyprinids from India

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Research Article

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Abstract

This communication aims to detect the myxozoan infection in some cyprinid fishes that were mostly cultured and commonly used for food in the Meerut region, Uttar Pradesh, India. Myxozoan were identified morphologically and for molecular analysis we have used previously established PCR assays for genetic marker ssrDNA then phylogenetic analysis was performed. Total seven species of Myxobolus were identified i.e., *Myxobolus bhadrensis, Myxobolus kalavatiae, Myxobolus haldari* from *Labeo rohita, Myxobolus calbasui, Myxobolus catlae,* from *Cirrihinus reba* and *Cirrihinus mrigala, Myxobolus hosadurgensis* from *Cirrihinus mrigala* and *Myxobolus saranae* from *Labeo bata*. An integrated comparative analysis of the ssrDNA gene supported the identification of the collected species and represents the phylogenetic position of all species. This work addresses the problems in the taxonomy of myxozoans in India, where molecular studies are less focused. My species parasitized similar hosts for which genetic data is the only way to make a clear distinction between species and their validity.

Keywords: *Myxobolus*; SSR DNA; Cyprinids; India 18S

Introduction

Myxozoa Grassé, 1970 are widespread cnidarian parasites and about 2596 species of these have been reported that constitute nearly one-fifth of the known Cnidarian species [1,2]. Among them, more than 850 *Myxobolus* Bütschli, 1882 species are identified [3,4] and from India contribution of about 130 *Myxobolus* species has been reported [5]. The number of myxozoan species is increasing in India [6-10]. Genus *Myxobolus* is characterized by ellipsoidal, ovoid or rounded spores in valvular view and biconvex in sutural view with two polar capsules, equal or unequal in size. Shell valves are generally smooth. Sporoplasm is binucleate, often with an iodinophilous vacuole [11].

The identification of myxozoan species is a big challenge in the case where multiple species of the same genus infected the same host, a situation that creates a taxonomic dilemma. Hence, molecular methods are very popular and boomed the myxozoan systematics, providing a more comprehensive resolution to their morphology and identification [6,8,12,13]. Although in India a large number of myxozoan species have

been described from carp hosts, which are mostly based on the spore morphology using traditional taxonomy methods [14-35]. Many of these described species have insufficient data that need to be revalidated or revised morphologically and should be supplemented with molecular characteristics.

Therefore, the aim of this study is to describe species based on morphological data and to evaluate the phylogenetic affinities of *Myxobolus* species infecting Indian carp fishes based on ssrDNA gene in order to resolve the uncertainties in their identification.

Materials and Methods

Parasite Collection and Morphology

Carp fish were sampled from the Parikshitgarh and Kheri Manihar of district Meerut and Bairaj of district Bijnour of Uttar Pradesh, India in January and February 2017 (n = 58; total length 11-16 cm) and June to August 2018 (n =74; total length 12-15 cm) respectively. Information regarding the collections of specimens of myxozoans is presented in Table 1. Fish were brought to the laboratory at the Department of Zoology, Chaudhary Charan Singh University, Meerut, UP, India and kept in an aerated aquarium. To check myxozoan infections they were euthanized with clove oil and dissected to check various organs like gill filaments, kidney, liver, intestine, gall bladder and muscles for myxozoan plasmodia/cysts under a Motic stereomicroscope (SMZ-168 series). Plasmodia were carefully removed from the gills, liver, kidney and intestine, opened with a fine needle on a slide and observed under an Olympus microscope (CH30) for further morphological analysis. Measurements based on 25-30 fresh myxospores were taken according to the guidelines of Lom J [36]. All measurements are shown in micrometres (µm) unless stated otherwise also followed by the range (mean ± SD) shown in Table 2, with a comparison to the same species described previously. Some spores were fixed in 95% alcohol and stored at -20°C for subsequent molecular study.

Sample	Species	Host	Locality	Prevalence and Intensity of Infection	Gills	Liver	Kidney	Intestine	GenBank Accession Nos.
LR1	*Myxobolus bhadrensis* (Mbh)	*Labeo rohita*	Parikshitgarh	52 % (22 infected out of 42), Moderate	–	–	Mbh	–	MN994379, MN994417
LR2	*Myxobolus kalavatiae* (Mka)	*L. rohita*	Parikshitgarh	88 % (37 infected out of 42), High	Mka	–	–	–	MN994420, MN994422
LR3	*Myxobolus haldari* (Mha)	*L. rohita*	Parikshitgarh	78.6 % (33 infected out of 42), High	–	–	Mha	–	MT002391, MT002392
LB1	*Myxobolus saranae* (Msa)	*Labeo bata*	Bijnour	76.6 % (23 infected out of 30), High	Msa	–	-	–	MT002911, MT002914
CM1	*Myxobolus hosadurgensis* (Mho)	*Cirrhinus mrigal*	Parikshitgarh	48.8 % (21 infected out of 43), Low	–	–	Mho	–	MT002915, MT002924
CM2	*Myxobolus catlae* (Mcat)	*C. mrigal*	Parikshitgarh	69.7 % (30 infected out of 43), Moderate	Mcat	–	–	–	MT003664, MT002743
CM4	*Myxobolus calbasui* (Mcal)	*Cirrhinus reba*	Kheri Manihar, Mawana	64.7 % (11 infected out of 17), Moderate	–	Mcal	–	–	MT009485, MT012423
CM5	*Myxobolus catlae* (Mcat)	*C. reba*	Kheri Manihar, Mawana	52.9 % (09 infected out of 17), Moderate	Mcat	–	–	–	MT002747, MT002748
CM6	*Myxobolus calbasui* (Mcal)	*C. mrigal*	Parikshitgarh	60 % (26 infected out of 43), Moderate	–	–	–	Mcal	MT012462, MT012463

Table 1: *Myxobolus* infection present or absent in the organs of carps examined from India.

Species	Host	Infection site	Size of PC	LOS	WOS	TOS	LLPC	LSPC	WLPC	WSPC	NFC	PFL	Reference
M. bhadrensis	*L. rohita*	Kidney	Unequal	11.02-12.48 (11.87±0.44)	7.28- 8.58 (7.95±0.38)	6.45-7.02 (6.74±0.21)	4.16-5.72 (4.86±0.46)	2.86-4.16 (3.54±0.37)	1.82-2.86 (2.39±0.29)	1.56-2.35 (1.94±0.25)	4(L) 3 (S)	na	Present study
M. bhadrensis	*L. rohita*	Muscles	Unequal	9.2-10.4 (10.0±0.41)	6.0-7.2 (6.6±0.37)	4.0-5.3 (4.5±0.56)	4.8-6.0 (5.5±0.37)	3.6-4.8 (4.2±0.41)	2.6-3.3 (3.0±0.34)	1.6-2.6 (2.0±0.29)	4(L) 3 (S)	na	Szekely, et al. [6]
M. bhadrensis	*L. rohita*	Muscles	Unequal	8.0-11.0 -9.5	7.0-8.0 -7.14	na	3.0-4.0 (3.5)	2.0-4.0 (2.5)	1.0- 2.0 -1.75	1.0- 2.0 -1.75	na	na	Seenappa and Manohar [37]
M. calbasui	*C. mrigala*	Intestine	Unequal	13.0-14.56 (13.79±0.49)	8.84-10.66 (9.79±0.61)	6.76-7.8 (7.31±0.28)	4.68-5.98 (5.16±0.39)	2.34-3.02 (2.69±0.21)	3.12-3.9 (3.5±0.22)	1.3-2.34 (1.87±0.34)	5-6 (L) 3-4 (S)	na	Present study
M. calbasui	*C. reba*	Liver	Unequal	14.3-15.23 (14.83±0.29)	9.88-10.92 (10.39±0.27)	6.8-7.7 (7.3±0.26)	5.2-6.76 (5.9±0.49)	2.6-3.48 (3.03±0.27)	2.6-3.9 (3.31±0.41)	1.52-2.20 (1.85±0.24)	6-7 (L) 4-5 (S)	na	Present study
M. calbasui	*C. mrigala*, *L. rohita*, *L. bata*, and *L. dyocheilus*	Gill, Scales, Fins, Gall bladder	Unequal	12 - 15 (13.32)	8 - 10 (8.94)	na	5 - 7 (6)	3 - 4 (3.56)	3 – 5 -4.18	2 - 3 (2.32)	7 (L) Not seen (S)	52-64 (L) 16-24 (S)	Gupta, et al. [38]
M. calbasui	*L. calbasu*, *L. rohita* and *C. mrigala*	Gall bladder	Unequal	12.4-15	8.2-10.0	na	6.18	4.12	4.12	3.09	na	125 (L) 64 (S)	Chakravarty, et al. [39]
M. opthalmu sculata	*C. mrigala*	Eye muscles	Unequal	12.4-13.97 (13.13)	7.45-9.18 (8.04)	na	4.9-6.12 (5.47)	2.35-3.37 (3.03)	2.65-3.37 (3.06)	1.84-2.14 (1.99)	7-9 (L) 2-3 (S)	53.04-114.24 (83.13) (L) 20.4-32.64 (24.94) (S)	Basu, et al.[40]
M. mrigalhitae	*C. mrigala*-*L. rohita* hybrid	Gills	Unequal	10.8-11.3 (10.8)	7.6-8.1 (7.9)	na	4.3-5.2 (4.8)	2.9-3.2 (3.0)	2.7-3.2 (2.1)	2.0-3.2 (2.1)	5-6 (L) 3-4 (S)	na	Basu, et al. [41]
M. buccoroofus	*L. bata*	Roof of buccal cavity	Unequal	11.6-12.7 (12.1)	6.4-8.1 (7.10	na	4.5-5 (4.9)	2.0-.2.9 (2.5)	2.7-3.0 (2.9)	1.3-1.7 (1.5)	6-8 (L) 4-5 (S)	na	Basu, et al. [42]
M. patialensis	*L. rohita*	Caudal fin	Unequal	10.74-11.82 (11.28±0.76)	6.27-7.07 (6.67±0.56)	na	4.6-5.0 (4.8±0.28)	1.6-1.8 (1.7±0.140	2.9-3.3 (3.1±0.28)	1.22-1.8 (1.5±0.41)	5-6 (L) 2-3 (S)	na	Kaur, et al. [43]
M. catlae	*C. mrigala*	Gills	Equal or slightly unequal	17.42-19.24 (18.47±0.58)	7.28-8.32 (7.93±0.32)	6.74-7.28 (7.0±0.18)	10.14-11.4 (10.92±0.34)	10.14-10.92 (10.51±0.26)	2.88-2.92 (2.92±0.15)	2.6-2.86 (2.70±0.10)	15-Nov	na	Present study

M. catlae	*C. reba*	Gills	Equal or slightly unequal	18.2-19.12 (19.12±0.51)	7.28-8.06 (7.70±0.26)	6.24-7.02 (6.61±0.32)	11.7-13.5 (12.75±0.56)	11.4-13.0 (12.10±0.48)	2.60-2.86 (2.70±0.08)	2.34-2.60 (2.48±0.10)	15-Nov	na	Present study
M. catlae	*C. cirrhosus*	Gills	Equal or slightly unequal	16.6-17.6 (17.1±0.45)	6.5-6.65 (6.6±0.05)	4.8-6.0 (5.4±0.47)	8.4–11.2 (10.4±0.47)	8.4–10.6 (9.9±0.62)	2.5–2.6 (2.5±0.05)	2.3–2.4 (2.4±0.05)	15-Nov	na	Szekely et al. [6]
M. catlae	*C. catla*, *L. rohita* and	Gills	Equal	14.5-16.5	6.18	5.15	10.3-12.36		2.06-3.01		na	150	Chakravarty, et al. [44]
M. kalavatiae	*L. rohita*	Gills	Equal	11.4-12.74 (12.12±0.39)	5.84-6.76 (6.34±0.28)	4.94-5.46 (5.22±0.19)	4.94-5.72 (5.45±0.23)		1.56-1.82 (1.67±0.094)		5	na	Present study
M. kalavatiae	*L. rohita*	Gills	Equal	7.3±0.27 (6.8-7.7)	5.3±0.5 (4.8-5.8)	3.7±0.09 (3.5-3.8)	3.1±0.16 (2.9-3.4)		1.9±0.18 (1.6-2.0)		na	na	Szekely et al. [6]
Myxobolus haldari	*L. rohita*	Kidney	Unequal	10.7-11.6 (11.16±0.27)	7.9-8.63 (8.3±0.19)	5.52-5.8 (5.67±0.08)	4.6-4.8 (4.68±0.06)	3.56-3.68 (3.62±0.4)	2.24-2.39 (2.32±0.04)	1.86-1.98 (1.94±0.04)	6-7 (L) 4-5 (S)	na	Present study
M. haldari	*C. mrigala, L. bata, L. dyocheilus, L. rohita,*	Fins and gills	Unequal	9.0-10.0 -9.31	7.0-8.5 -6.25	na	4.0-5.0 -4.31	2.5-3.0 -2.95	2.5-3.0 -2.97	1.5-2.0 -1.98	na	31-70 (L) 18-31 (S)	Gupta, et al. [45]
Myxobolus saranae	*L. bata*	Gills	Unequal	10.6-11.96 (11.29±0.39)	7.0-8.1 (7.52±0.34)	5.2-5.8 (5.51±0.22)	4.94-6.2 (5.52±0.35)	3.12-3.9 (3.52±0.24)	2.6-3.38 (2.92±0.26)	1.56-1.86 (1.7±0.09)	5-6 (L) 3-4 (S)	Na	Present study
M. saranae	*L. calbasu, Puntius sarana*	Gills	Unequal	6.0-9.0 (7.72)	6.0-7.0 (6.2)	na	4.0-5.0 (4.24)	1.5-3.0 -1.98	2.5-4.0 -3.04	1.0-2.0 -1.3	na	23-32 (L) 5.0-9.0 (S)	Gupta, et al. [46]
M. saranae	*L. rohita*	Caudal fin	Unequal	8.0-9.0 (8.5±0.70)	5.5-6.5 (6.0±0.70)	na	3.97-4.57 (4.27±0.42)	1.9-2.5 (2.2±0.42)	2.31-2.91 (2.61±0.42)	1.74-2.14 (1.94±0.28)	5-6 (L) 2-3 (S)	na	Kaur et al. [47]
M. harpreetae	*L. bata*	Gill lamellae	Unequal	11.4–13.8 -13.2	4.9–7.8 -6.51	na	8.7–9.2 -9.149	7.6–8.3 -7.94	2.9–3.4 -3.45	2.7–3.0 -2.9	na	na	Ghosh, et al. [48]
Myxobolus hosadurgensis	*C. mrigal*a	Kidney	Unequal	12.37-13.52 (12.86±0.41)	7.54-8.32 (7.94±0.25)	5.46-5.98 (5.7±0.17)	4.65-5.3 (4.9±0.23)	2.96-3.44 (3.18±0.15)	2.6-3.1 (2.85±0.15)	1.56-1.82 (1.7±0.10)	5-6(L) 3-4(S)	na	Present study
M. hosadurgensis	*C. mrigal*a	Muscles	Unequal	9.0-11.0 (10.5)	5.0-8.0 (6.25)	na	4.0-6.0 (5.37)	2.5-4.0 (3.3)	2.0-3.0 (2.3)	1.0-2.5 (1.43)	na	na	Seenappaand Manohar [37]

Table 2: Comparative measurements shown as range (mean ± SD) of *Myxobolus* species collected in the present study with morphological similar species (PC: Polar capsule, LOS: Length of spore, WOS: Width of spore, TOS: Thickness of spore, LSPC: Length of the smaller polar capsule, LLPC: Length of the larger polar capsule, WLPC: Width of the larger polar capsule, WSPC: Width of the smaller polar capsule, NFC: Number of filament coil, PFL: Polar filament length), na= data not available; Species sequenced during the present study are shown in bold.

DNA Isolation and Amplification

For genomic DNA extraction, ethanol-preserved plasmodia/cysts were processed using a QIAGEN DNeasyTM tissue kit (animal tissue protocol, Qiagen, Hilden, Germany) according to the protocol recommended by the manufacturer. The ssrDNA was amplified using the universal primers ERIB1 and ERIB10 [49] at the first round of PCR. The reaction was carried out according to the protocol followed as PCR was carried out in a 25-µl reaction mixture with the following thermocycling profile: denaturation at 95°C for 3 min; followed by 35 cycles of amplification at 95°C for 45 s, 56°C for 1 min and 72°C for 1 min, completed with terminal extension at 72 °C for 7 min and then stored at 4 °C. This was followed by a second round of PCR with primers Myx1F-SphR [50,51]. The total volume of the reactions was 50 µl with the following PCR amplification profile: 95 °C for 3 min, then 35 cycles at 95 °C for 50 s, 56 °C for 50 s, 72 °C for 1 min, terminated with an extension at 72 °C for 7 min and then resting at 4 °C. The PCR amplification products were electrophoresed in 1% agarose gel (Tris-Acetate-EDTA buffer) stained with 1% ethidium bromide and examined under ultraviolet light. PCR products were purified with the PurelinkTM Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen). Amplicons were sequenced from both strands with the PCR primers mentioned above using the Big Dye Terminator vr. 3.1 cycle sequencing kit in ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Phylogenetic Analysis

Obtained contiguous sequences were manually assembled, edited using BioEdit, version 7.2.5 [52] checked and submitted to GenBank for accession numbers and put forward to NCBI for BLASTn and comparison with other myxozoan sequences. Newly generated sequences of the ssrDNA of myxozoan species were aligned together with published sequences of myxozoan species, available on GenBank. The sequences were aligned by using Clustal W [53] implemented in the MEGA X [54]. The evolutionary history was analyzed by using the Maximum Likelihood method and the General Time Reversible model. The nodal support was estimated by bootstrapping (n=1,000). The best evolutionary model of HYPERLINK "https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/nucleotide"nucleotide substitution using the Akaike Information Criterion (AICs) was determined with the MEGA X. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4686)). Genetic distance estimation was carried out using the p-distance model of substitution in MEGA X. A total of 59 nucleotide sequences with over 91% similarity were used for phylogenetic analysis. There were a total of 2075 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. The Chlorormyxum cristatum (AY604198) was selected as out-group in the final analysis.

Results

Seven *Myxobolus* species were collected from the locally available cyprinids fish and we have not recorded any external symptoms of infection. All *Myxobolus* sp. were collected from gills, liver, gall bladder, kidney and intestine. The collected species were morphologically identified as belonging to the genus *Myxobolus* Bütschli, 1882 (Myxozoa: Myxobolidae) as *Myxobolus bhadrensis*, *Myxobolus calbasui, Myxobolus catlae, Myxobolus kalavatiae, Myxobolus haldari, Myxobolus hosadurgenesis* and *Myxobolus saranae* Figures 1 & 2. Details regarding comparative morphometrical data of the above-mentioned species with other related species are presented in Table 2.

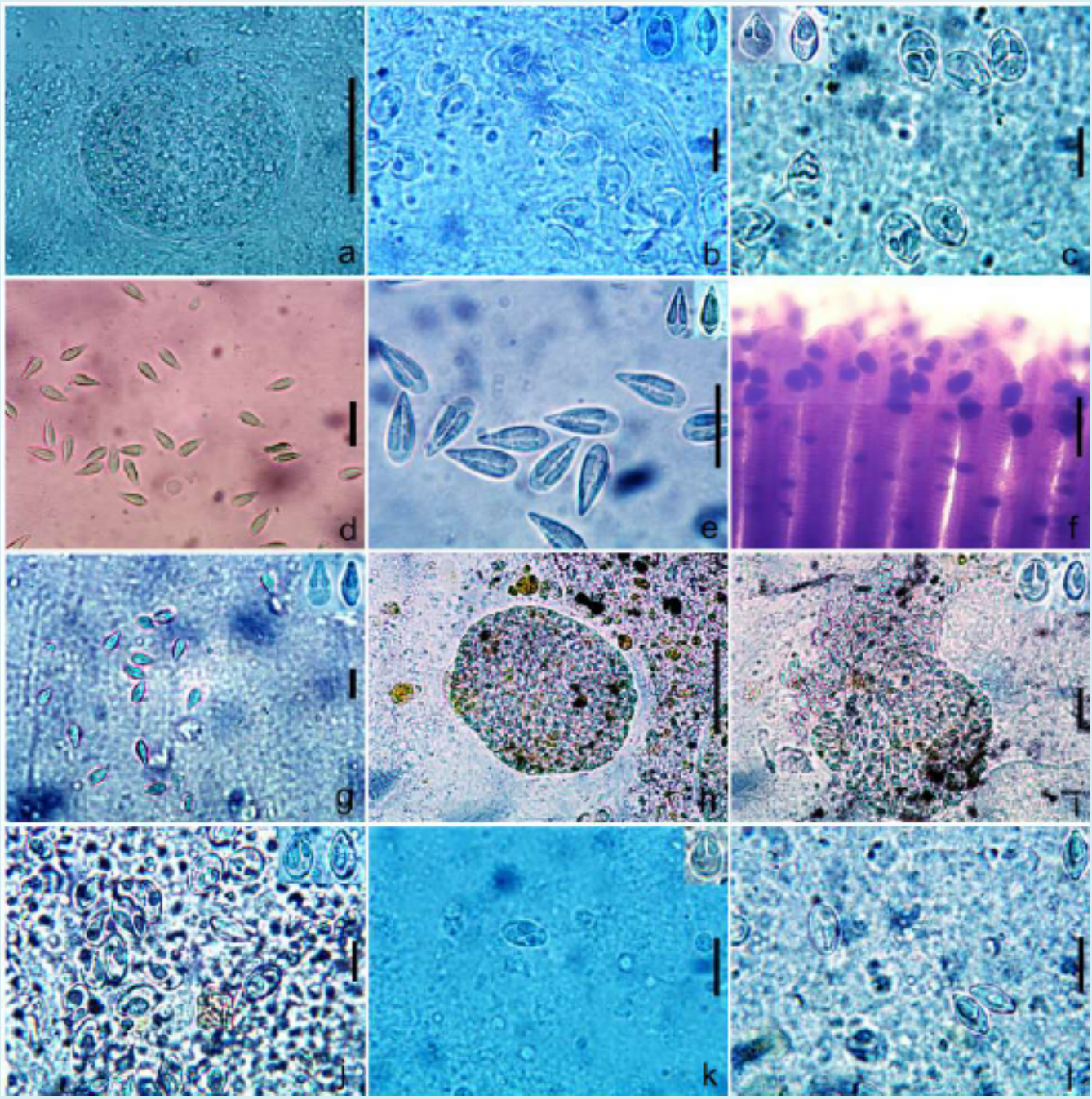

Figure 1: a. Cyst of *Myxobolus bhadrensis*. b. Spores of *Myxobolus bhadrensis*. c. Spores of *Myxobolus calbasui.* d, e. Spores of *Myxobolus catlae*. f. Gills with cyst of *Myxobolus kalavatiae*. g. Spores of *Myxobolus kalavatiae*. h. Cyst of *Myxobolus haldari* i. Spores of *Myxobolus haldari* j. Spores of *Myxobolus saranae*. k, l. Spores of *Myxobolus hosadurgensis*. Scale bars. a,h=50 µm; b=12 µm; c,g=15 µm; d,e=20 µm; f=200 µm; i,j=10 µm; k,l=15 µm.

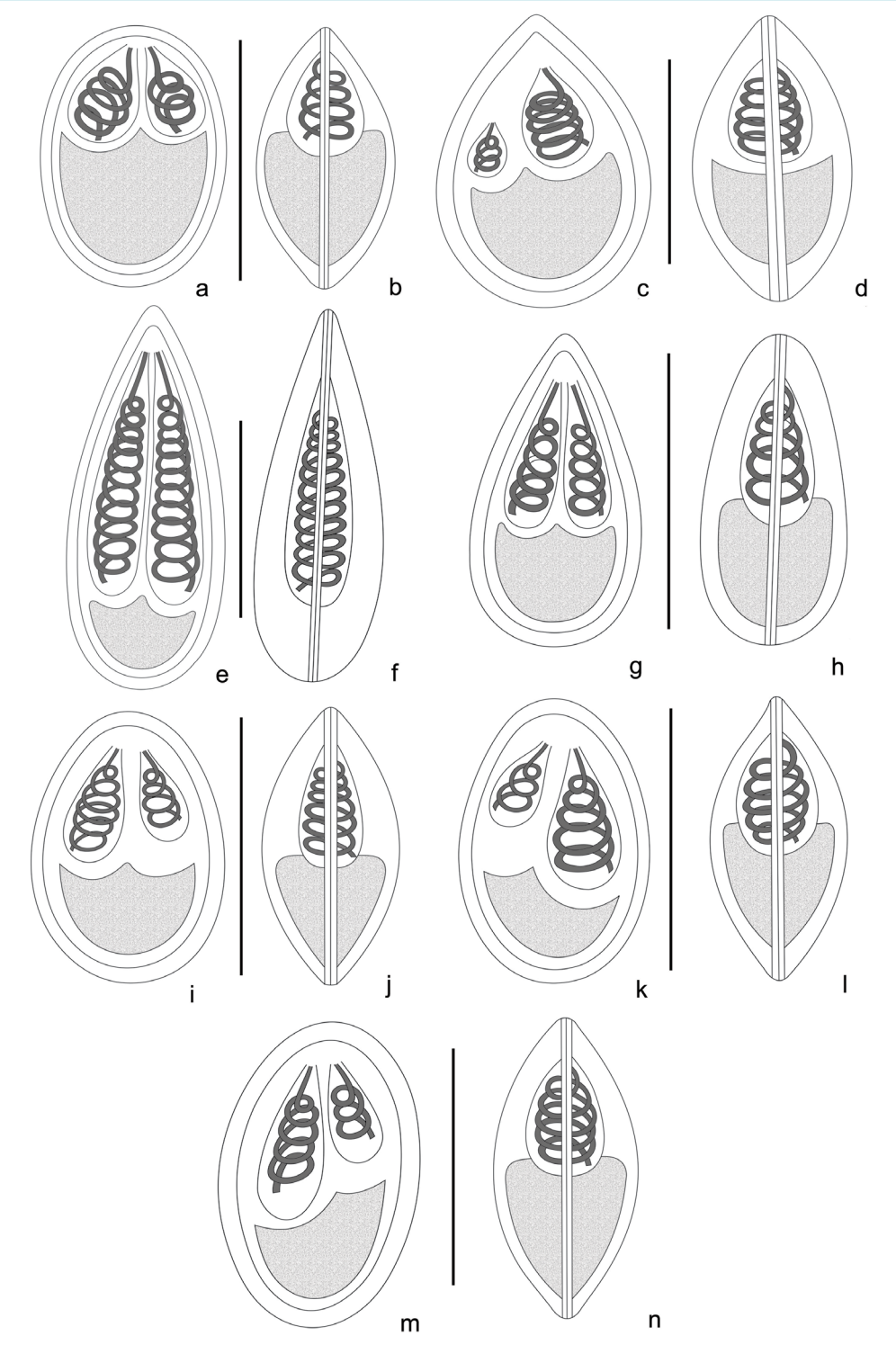

Figure 2: Line drawings of *Myxobolus* parasites collected during the study. a. Frontal view of *Myxobolus bhadrensis*. b. Sutural view of *Myxobolus bhadrensis*. c. Frontal view of *Myxobolus calbasui.* d. Sutural view of *Myxobolus calbasui*. e. Frontal view of *Myxobolus catlae*. f. Sutural view of *Myxobolus catlae*. g. Frontal view of *Myxobolus kalavatiae*. h. Sutural view of *Myxobolus kalavatiae*. i. Frontal view of *Myxobolus haldari*. j. Sutural view of *Myxobolus haldari*. k. Frontal view of *Myxobolus saranae*. l. Sutural view of *Myxobolus saranae*. m. Frontal view of *Myxobolus hosadurgensis*. n. Sutural view of *Myxobolus hosadurgensis*. Scale bars. a-n =10 µm.

Myxobolus bhadrensis Seenappa and Manohar [37], Szekely, et al. [6]

Spores description. Spores oval to ellipsoidal in frontal view with anterior and posterior rounded ends. In sutural view, spores pyriform at both the ends and convex at both sides. Length of spore 11.02-12.48 (11.87±0.44) (N=30); width, 7.28- 8.58 (7.95±0.38) (N=30) and thickness, 6.45-7.02(6.74±0.21) (N=10). Polar capsules pyriform shape and unequal in size. Larger capsule 4.16-5.72 (4.86±0.46) long (N=30) and 1.82-2.86 (2.39±0.29) wide (N=30) while smaller capsule 2.86-4.16 (3.54±0.37) long (N=30) and 1.56-2.35 (1.94±0.25) wide (N=30). 3 polar tubule coils in smaller capsule and 4 coils in larger capsule. Sporoplasm large, binucleated having small iodinophilous vacuole. Intercapsular process not seen (Table 2; Figures 1a,1b & 2a,2b).

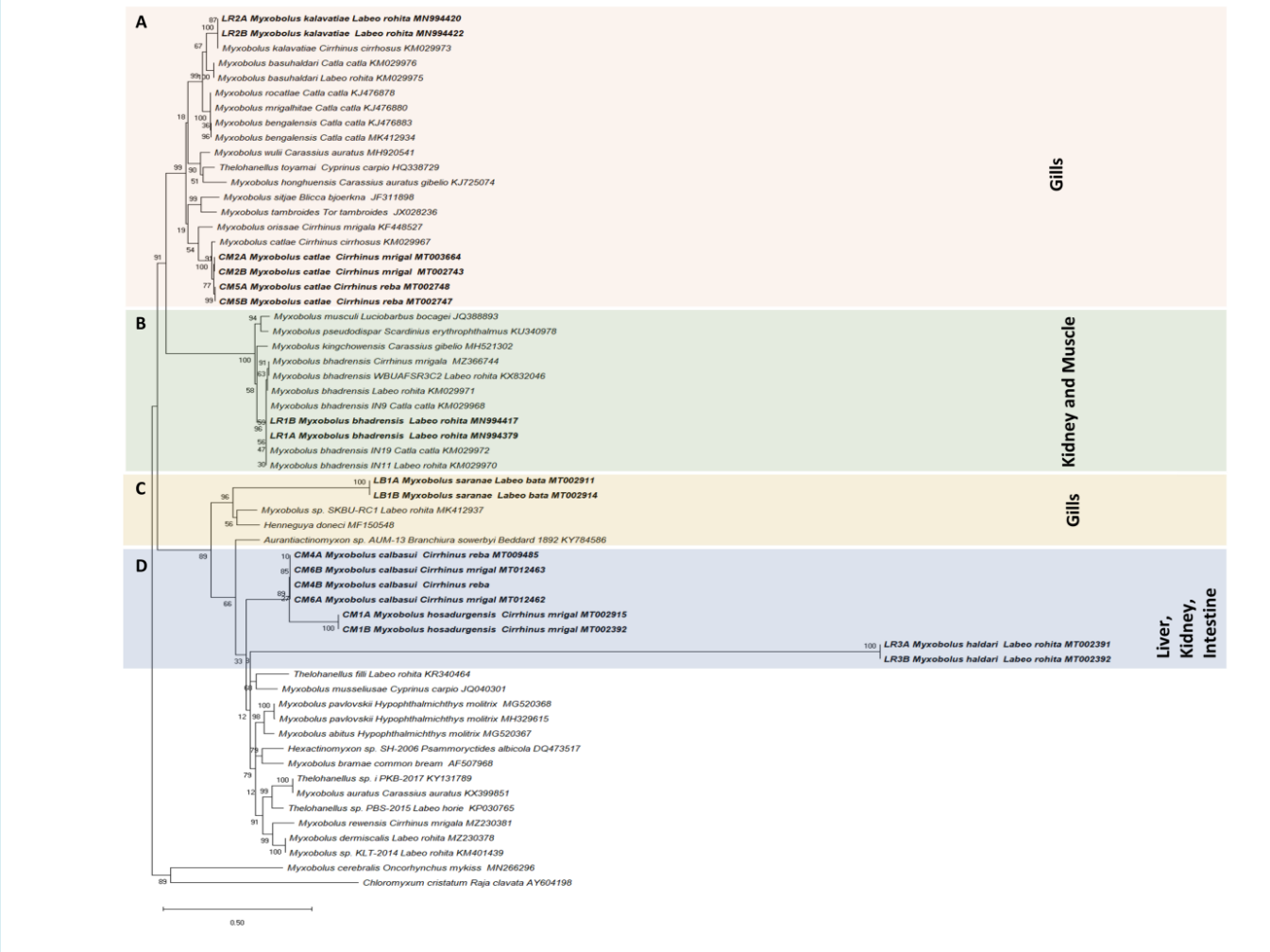

Figure 3: *Myxobolus* species phylogenetic relationships inferred by maximum likelihood analysis based on the ssrDNA sequences. Species in bold were newly sequenced in this study. GenBank accession numbers and their host name are listed along the species names. Numbers at nodes indicate the bootstrap confidence values (ML). *Chloromyxum cristatum* was used as an outgroup.

Present host and locality: *Labeo rohita*, Local name: Rohu; locality: Parikshitgarh Fish Farm (28°59′ N, 77° 56′ E), Meerut (U.P.), India.

Site of infection: Kidney.

Prevalence of infection: 22/42 in the 9-14 cm size group with a prevalence of 52%.

Material deposited: Digitized photographs were deposited in the Museum, Department of Zoology, Chaudhary Charan

Singh University, Meerut, under voucher number HSS/ZOO/MYX/03/19.

Representative DNA sequence: The ssrDNA sequences of *M. bhadrensis* were submitted to NCBI with accession numbers MN994379 and MN994417.

- **Remarks**

Spores of *M. bhadrensis* showed close resemblance in shape and size with spores of other *Myxobolus* species having unequal sized polar capsules. Spores of *M. Mrigalae* Chakravarty [39] showed morphological similarities with spores of *M. bhadrensis* but presence of several triangular marking in the valve of *M. Mrigalae* differentiates these two species. Spores *M. indicum* Tripathi [55] also resemble in size and shape but marked difference is seen in the size of polar capsules of both. Morphologically spores of *M. bhadrensis* shows close resemblance with spores of *M. hosadurgensis* Seenappa and Manohar [37] but spores of M. bhadrensis were oval to ellipsoidal and wider than spores of *M. hosadurgensis* which were elongated ovoidal in shape with anterior narrow end. Spores of *M. vedavatiensis* Seenappa and Manohar [37], M. lalithae Gupta, et al. [56] and *M. haldari* Gupta, et al. [45] also showed resemblance with spores of M. bhadrensis but spores of *M. lalithae* were oval or elliptical in shape while spores of *M. vedavatiensis* and *M. haldari* had an intercapsular process or ridge.

Myxobolus calbasui Chakravarty [21]

Spores description. Spores large size, rounded-oval in shape, anterior end pointed and posterior end much rounded in frontal view. Spores pyriform in sutural view with both sides convex. Length of spores 13.0-14.6 (13.8±0.4), width 8.8-10.7 (9.8±0.61), thickness 6.76-7.8 (7.3±0.28). Polar capsules oval-pyriform in shape, unequal sized. Larger capsule much larger than smaller capsule, showed marked differentiation in size, and reaches up to the anterior end of spore, but smaller capsule lies at some distance below the larger capsule. Larger capsule 4.68-6.0 (5.2±0.39) long and 3.12-3.9 (3.49±0.22) wide. Smaller capsule 2.34-3.0 (2.7±0.21) long and 1.3-2.3 (1.86±0.35) wide. Polar tubule coils seen 5-6 in larger capsule and 3-4 in smaller capsule in host fish *C. mrigala* while in spores obtained from *C. reba* no. of coils seen 6-7 in larger capsule and 4-5 in smaller capsule (Table 2; Figures 1c & 2c,2d).

Present host and locality: *Cirrhinus mrigala* (Ham.1822), Local name: Mrigal; locality: Parikshitgarh Fish Farm (28º59′N, 77°56′E), Meerut and *C. reba* (Ham. 1822), Local name: Raia; locality: Village Kheri Manihar Fish Pond (29º10′N, 77°92′E), Mawana, Meerut (U.P.), India.

Site of infection: *Cirrhinus mrigala* and *C. reba*: intestine and liver respectively.

Prevalence of infection: 26/43 and 11/17 in the 11-16 cm size group of *Cirrhinus mrigala* and *C. reba*, with a prevalence of 60% and 64.7% respectively.

Material deposited: Digitized photographs obtained from both host *C. mrigala* and *C. reba* were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut, under voucher number HSS/ZOO/MYX/11/19 and HSS/ZOO/MYX/10/19 respectively.

Representative DNA sequence: The ssrDNA sequences obtained from both the host *C. mrigala* and *C. reba* were deposited in GenBank with accession numbers MT012462, MT012463 and MT009485, MT012423.

- **Remarks**

The characteristic large, rounded-oval spores of this species, with pointed anterior end and much rounded posterior end shows resemblance with those *Myxobolus* species, having different size polar capsules, infecting the cyprinid fishes. Spores of *Myxobolus calbasui* were identified on the basis of available morphological and morphometric data. Initially, this species was reported from the gall bladder of *L. calbasu*, *L. rohita* and *C. mrigala* at Calcutta district, West Bengal, India by Chakravarty [39]. Earlier, Ray [57] reported this species from liver of *C. mrigala*. Later, Gupta [38] (thesis) redescribed this species from different organs of four species of cyprinid fishes, *L. rohita*, *L. bata*, *L. dyocheilus* and *C. mrigala*. Spores of *M. indicum* Tripathi [55] described from the liver, intestinal wall and muscles of same host *C. mrigala* show close similarity in morphological characteristics but further studies are needed for their identification as a synonymous species due to the poor illustration of the spores. Spores of *M. opthalmusculata* Basu and Haldar [40], *M. mrigalhitae* Basu and Haldar [41], *M. buccoroofus Basu* and *Haldar* [42] obtained from different carp hosts are close in morphology with spores of *M. calbasui*. But the presence of a small depression at the anterior end of spores of *M. opthalmusculata*, the presence of parietal folds in the posterior region of the spores of *M. mrigalhitae* and narrow and bent spores of M. *buccoroofus* at anterior side, differentiate these species from M. calbasui. Spores of *M. patialensis* Kaur and Singh [43] are smaller in size with asymmetrical spore valve, having 4-5 parietal folds in the posterior part of spores as compared to spores of *M. calbasui*.

Myxobolus catlae [45] Szekely, et al. [6]

Spore description. Spores large-sized and elongated in shape. Anterior end of spores pyriform, sharply tapered and blunt in both frontal and sutural view. Posterior ends of spores ovo-rounded in both views. Spores measurement

lies between: length 17.4-19.2 (18.5±0.58), width of 7.28-8.3 (7.9±0.32) and thickness 6.74-7.28 (7.00±0.18). Spore valves thin, uniform and symmetrical. Polar capsules (PC) very long, elongated-pyriform shape and filled most of the spore cavity, leaving a small space for sporoplasm at the posterior part of spore. Size of large PC: length 10.14-11.4 (10.92±0.34) and width 2.7-3.12 (2.92±0.15), size of small PC: length 10.14-10.92 (10.51±0.26) and width 2.6-2.86 (2.70±0.10). Both capsules parallel to the longitudinal axis of body. In most of the spores, polar capsules equal in size but in some spores slightly unequal in size. The number of polar tubule coils ranged from 11 to 15 in each polar capsule. Sporoplasm small, binucleated and lies at the posterior end of the spore cavity. Intercapsular process and mucous envelop were not seen. A small, variable-sized iodinophilous vacuole was also present in the posterior part of the sporoplasm (Table 2; Figures 1d, 1e & 2e, 2f).

Present host and locality: *Cirrhinus mrigala* (Ham.1822). Local name: Mrigal; locality: Parikshitgarh Fish Farm (28º59′N, 77°56′E), Meerut and *C. reba* (Ham. 1822). Local name: Raia; locality: Village Kheri Manihar Fish Pond (29º10′N, 77°92′E), Mawana, Meerut (U.P.), India.

Site of infection: Gills lamellae of *Cirrhinus mrigala* and *C. reba*.

Prevalence of infection: 30/43 and 09/17 in the 11-16 cm size group of *Cirrihinus mrigala* and *C. reba* with a prevalence of 69.7% and 53% respectively.

Material deposited: Digitized photographs obtained from both the host *C. mrigala* and *C. reba* were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut, HSS/ZOO/MYX/07/19 and HSS/ZOO/MYX/08/19 respectively.

Representative DNA sequence: ssrDNA sequences of *M. catlae* obtained from both the host *C. mrigala* and *C. reba* were submitted to NCBI with accession numbers MT002743, MT003664 and MT002747, MT002748 respectively.

- **Remarks**

Spores of *M. catlae* can be differentiated from other known *Myxobolus* species easily because of their large, elongated shaped spores with the large number of polar tubule coils in the polar capsules. Identification was confirmed on the basis of morphological as well as the molecular data also. Spores of *M. bengalensis* Chakravarty, et al. [58] were much smaller in length as compared to spores of *M. catlae*. In *M. cuttaki* Haldar, et al. [59]9 number of coils is less as compared to *M. catlae* while the spores of *M. catmrigalae* Basu, et al. [42] much broader and have more coils in polar capsules. Spores of *M. zoohuri* Majumder, et al. [60] were comparatively smaller in size and have more coils in the polar capsule as compared to the *M. catlae.*

Myxobolus kalavatiae Szekely, et al. [6]

Spore description. Spores medium size, ovoidal, elongated both in frontal and sutural view, with anterior end, tapering and blunt while posterior end broad and rounded. Length of the spore 11.4-12.74 (12.12±0.39) (N=25); width 5.84-6.76 (6.34±0.28) (N=25); and thickness 4.94-5.46 (5.22± 0.19) (N=15). Spore wall thick, uniform and symmetrical. Polar capsules long, pyriform shaped and equal-sized, obliquely located on either side of the midline, filling around more than half of the spore cavity. Capsules 4.94-5.72 (5.45±0.23) long (N=20) and 1.56-1.82 (1.67±0.094) (N=20) wide.Polar tubule coils are 5 in each capsule. Sporoplasm small, binucleate having a small iodinophilous vacuole. Intercapsular process and mucous envelope not seen (Table 2; Figures 1F, 1g & 2g, 2h).

Present host and locality: *Labeo rohita* (Ham. 1822), local name: Rohu; locality: Parikshitgarh Fish Farm (28º59′N, 77°56′E) Meerut (U.P.), India.

Site of infection: Gills.

Prevalence of infection: 37/42 in the 9-14 cm size group with a prevalence of 88%.

Material deposited: Digitized photographs were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University Meerut, under voucher number HSS/ZOO/MYX/04/19.

Representative DNA sequence: The ssrDNA sequences were submitted to NCBI with accession numbers MN994420 and MN994422.

- **Remarks**

Originally Szekely, et al. [6] collected spores of *M. kalavatiae* from gills of the host fish C. cirrhosus but we have collected spores of *M. kalavatiae* from the gills of the host fish L. rohita. As compared to the original description, spores were larger in size, having polar tubule coils, which were not seen by Székely, et al. [6]. Comparison of spores of *M. kalavatiae* was done with other *Myxobolus* sp. having equal polar capsules. Comparison of morphological characters of *M. kalavatiae* showed that it is different from *M. shetti* Seenappa and Manohar [37], M. *venkateshi* Seenappa and Manohar [37], *M. basuhaldari* Szekely, et al. [6], *M. meerutensis* Szekely, et al. [6]. As spores of *M. shetti* were smaller and broader than spores of *M. kalavatiae,* having a small intercapsular process and lacking the polar tubule coils. Spores of *M. venkateshi* were egg-shaped with a well-developed intercapsular

process. Spores of *M. basuhaldari* showed close resemblance with spores of *M. kalavatiae,* as both have two equal size polar capsules, equipped with 5 coils, except spores of *M. basuhaldari* were smaller in size and ovoidal in shape and had a small knob at the anterior end, as compared to the spores of *M. kalavatiae*. In the original description given by Székely, et al. [6] these two spp. showed close resemblance in their size but spores of *M. kalavatiae,* obtained in the present study were longer and elongated than the spores of *M. basuhaldari*. In sutural view spores of *M. meerutensis* are lemon-shaped while spores of *M. kalavatiae* are elongated. No. of polar tubule coils seen 6 in *M. meerutensis* and 5 in *M. kalavatiae*. A small knob-like intercapsular appendix was also present in the spores of *M. meerutensis* but not seen in the spores of *M. kalavatiae.*

Myxobolus haldari Gupta, et al. [45]

Spores description. Spores long, ovoidal or elliptical in frontal view, biconvex in sutural view with round anterior and posterior ends. Length of spore 10.7-11.6 (11.16±0.27) (N=25), width 7.9-8.63 (8.3±0.19) (N=25) and thickness 5.52-5.8 (5.67±0.08) (N=15). Polar capsules (PC) unequal sized and pyriform shaped. Large PC length 4.6-4.8 (4.7±0.06) (N=25); width - 2.24-2.39 (2.32±0.04) (N=25) and small PC length 3.56-3.68 (3.63±0.05) (N=25); width 1.84-2.0 (1.94±0.05) (N=25). Number of polar tubule coils 4-5 in smaller capsule, while 6-7 coils in larger capsule. Intercapsular process was not seen (Table 2; Figures 1h, 1i & 2i, 2j).

Present host and locality: *Labeo rohita* (Ham.1822), local name: Rohu; locality: Parikshitgarh Fish Farm (28º59'N, 77°56'E), Meerut (U.P.), India.

Site of infection: Kidney.

Prevalence of infection: 33/42 in 9-14 cm size group with a prevalence of 78.6%.

Material deposited: Digitized photographs were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut, under voucher number HSS/ZOO/MYX/05/19.

Representative DNA sequence: The ssrDNA sequences of *Myxobolus haldari* were submitted to NCBI with accession numbers MT002391 and MT002392.

- **Remarks**

Spores of *Myxobolus haldari* were found very close to the spores of *M. haldari* in morphometric traits and other *Myxobolus* species with unequal polar capsules. Spores of *Myxobolus haldari* can be differentiated from spores of *M. mrigalae* Chakravarty [39], *M. seshadri* Lalita Kumari [61], *M. carnaticus* Seenappa and Manohar [62] and *M. vedavatiensis* Seenappa and Manohar [37] due to their size differences and by the presences of several triangular markings in shell valve of *M. mrigalae*, presences of inter capsular ridge in spores of *M. seshadri*, *M. carnaticus* and *M. vedavatiensis*. Spores of *Myxobolus haldari* and *M. bhadrensis* Seenappa and Manohar [37] showed differences in the length of their polar capsules.

Myxobolus saranae Gupta, et al. [46]

Spore description. Spores elongated, oval-shaped with blunt- oval anterior and posterior ends in frontal view. In a sutural view, spores lentiform or convexo-convex in shape. Spores 10.6-11.96 (11.3±0.39) long (N=25); 7.0-8.1 (7.52±0.34) wide (N=25) and 5.2-5.8 (5.5±0.22) thick (N=15). Polar capsules unequal sized, longer, pyriform or pear-shaped, converging towards the anterior end of spores. Larger capsule 4.94-6.2 (5.52±0.35) long (N=25) and 2.6-3.38 (2.92±0.26) wide (N=25) whereas length of small polar capsule 3.12-3.9 (3.52±0.24) (N=25) and width 1.56-1.86 (1.7±0.09) (N=25). Polar tubule coils were seen in both polar capsules and no. of coils ranges 5-6 in larger capsule and 3-4 in smaller capsule. Sporoplasm small, cresentric shaped, uni or binucleated. Intercapsular process and mucous envelop not seen. A variable-sized iodinophilous vacuole was seen in the posterior part of sporoplasm (Table 2; Figures 1j & 2k, 2l).

Present host and locality: *Labeo bata* (Ham. 1822), Local name: Bata; locality: Bairaj, Bijnor, (29°23' N, 79°11' E), (U.P.), India.

Site of infection: Gills.

Prevalence of infection: 23/30 in the 15-20 cm size group with a prevalence of 76.6%. Material deposited: Digitized photographs deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut, under voucher number HSS/ZOO/MYX/06/19.

Representative DNA sequence: The ssrDNA sequences of *Myxobolus saranae* are submitted to NCBI with accession numbers MT002911 and MT002914.

- **Remarks**

The morphometric details were similar to the *M. saranae,* which was described by Gupta, et al. [46] from the gills of *Puntius sarana* and *Labeo calbasu*. Later on, Kaur, et al. [47] re-described it from caudal fin of *L. rohita.* ssrDNA sequences generated from *Myxobolus saranae* did not resemble other available myxosporean sequences at the GenBank. Morphologically *M. saranae* shows some

similarities with other *Myxobolus* species as Spores of *M. mrigalae* Chakravarty [39] have several triangular markings on the shell valves as compared to the spores of *M. saranae*. The presence of intercapsular ridge in spores of *M. seshadri* Lalitha Kumari [61] makes a differentiation in both the species. Spores of *M. bhadrensis* Seenappa and Manohar [37] were broader and its polar capsules were smaller than *M. saranae*. Spores of *M. vedavatiensis* Seenappa, et al. [37] larger in size and possesses a large inter capsular ridge. Spores of *M. chakravartyi* Halder, et al. [63] were larger in size with anterior flat end and a triangular thickening at the anterior end. In *M. chakravartyi* number of polar filament coils 8-9 in the larger capsule and 6-7 in the smaller capsule. Spores of *harpreetae* Ghosh, et al. [48] have long and less wide spores than *M. saranae*. Polar capsules of *Myxobolus saranae* are smaller in length as compared to *M. harpreetae*.

Myxobolus hosadurgensis Seenappa and Manohar [37]

Spores description. Spores long, ovoid or ellipsoidal in frontal view and biconvex in sutural view with round anterior and posterior ends. Length of spore 12.37-13.52 (12.86±0.41) (N=25), width 7.54-8.32 (7.94±0.25) (N=25) and thickness 5.46-5.98 (5.70±0.17) (N=10). Polar capsules (PC) unequal sized and pyriform in shape. Large PC length 4.65-5.3 (4.9±0.23) (N=25), width 2.66-3.1 (2.86±0.14) (N=25) and small PC length 2.96-3.43 (3.18±0.15) (N=25), width 1.56-1.82 (1.71±0.09) (N=25). Number of polar tubule coils, 3 in smaller polar capsule and 5 in larger polar capsule. Sporoplasm binucleated, having a small iodinophilous vacuole. Intercapsular process was not seen (Table 2; Figures 1k, 1l & 2m, 2n).

Present host and locality: *Cirrhinus mrigala* (Ham.1822) Local name: Mrigal; locality: Parikshitgarh Fish Farm (28°59' N, 77° 56' E), Meerut (U.P.), India.

Site of infection: Kidney.

Prevalence of infection: 21/43 in the 11-16 cm size group with a prevalence of 48.8%.

Material deposited: Digitized photographs were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut, under voucher no. HSS/ZOO/MYX/09/19.

Representative DNA sequence: ssrDNA sequences of *Myxobolus hosadurgensis* are submitted to NCBI with accession numbers MT002915 and MT002924.

- **Remarks**
  The spores of *Myxobolus hosadurgensis* showed close resemblance in morphometric traits with spores of *Myxobolus hosadurgensis* Seenappa and Manohar, 1981. Because of unequal sized polar capsules *M. hosadurgensis* showed its morphological similarities with *M. mrigalae* Chakravarty [39], *M. pinnaurati* Lalitha Kumari [61], *M. carnaticus* Seenappa and Manohar [37], *M. bhadrensis* Seenappa and Manohar [37] and *M. vedavatiensis* Seenappa and Manohar [37] with some difference in their size, shape and number of polar tubule coils in polar capsules. However, recently generated ssrDNA sequences of this species did not match with any other previous myxozoan sequences available at NCBI.

Phylogenetic Analysis

Molecular analysis of the ssrDNA gene confirmed the phylogenetic position of the studied species (Figure 3). Maximum likelihood (ML) generated a well-resolved tree topology and bootstrap values are presented (Figure 3).

Myxobolus kalavatiae infecting the gills of rohu in the present study shows that ssrDNA sequences of both isolates did not show any intraspecific divergence and shared high identity (99.4%) with other isolates of the same species described by Székely, et al. [6] from gills of mrigal (*Cirrihinus cirrohosus*) (Figure 3). The phylogenetic analyses inferred from ssrDNA data set also show that *M. kalavatiae* is a sister to isolates of *M. basuhaldari* with strong homology (96.4%) reported from the gills of rohu and katla by Székely C, et al. [6]. The present phylogenetic analysis shows that *M. kalavatiae* was positioned within the well-supported clade that belongs to the gill infecting species in a clade A. *Myxobolus catlae* collected in the present study from gills of mrigal and reba was originally described by Chakravarty, 1943 from various hosts katla, rohu and mrigal. Later *M. catlae* was also reported by Székely, et al. [6] from the gills of *Cirrihinus cirrhosus* with a more comprehensive description. In the present study, we have collected *M. catlae* from mrigal and reba and ssrDNA sequences of these *M. catlae* isolates exhibited a similarity of 99.8 % with each other collected from two different hosts (Figure 3). Our isolates showed a 98.2 % similarity with *M. catlae*, which was isolated from the gills of *Cirrihinus cirrhosus.*

In the clade B, *Myxobolus bhadrensis* ssrDNA sequence of two isolates collected from the kidney of rohu were found to be identical and showed a 99.2 to 99.4% similarity with other isolates of same species reported from the kidney and muscles of rohu, katla reported by Székely C, et al. [6] while showing similarity of 99.1% with isolate WBUAFSR3C2 infecting the kidney of rohu from West Bengal, India (Figure 3). The phylogenetic tree also showed that *M. bhadrensis* species formed a sister relationship with *M. kingchowensis* reported from the muscles of *Carassius gibelio* from China

with maximum homology (96.8 %) (Figure 3). According to the Székely, et al. [6] both the *Myxobolus* species (*M. kingchowensis* and *M. bhadrensis*) shared the same subclade and show affinity to tissue tropism towards the muscle and kidney respectively. Regarding *Myxobolus saranae* reported from gills of bata (*Labeo bata*) can be readily differentiated from other *Myxobolus* species studied here in the study based on molecular data of the ssrDNA gene and placed in the clade C (Figure 3). The tree topology strongly supported the independent branch formed by isolates of *Myxobolus hosadurgensis* shows no intraspecific variation among isolates. *Myxobolus saranae* forms a branch close to *Myxobolus sp*. (MK412937) infecting gills lamellae of rohu (*Labeo rohita*) shows only 81.3% similarity with each other and both are members of the same host family Cyprinidae (Figure 3).

Myxobolus haldari was originally described from the fins and gills of mrigal, rohu, bata and kali (*Labeo dyocheilus)* by Gupta, et al. [45]. Nonetheless, the molecular study of this species has not been performed previously. We have collected *M. haldari* from kidney of rohu and the molecular analysis of the ssrDNA gene confirmed its validation. The phylogenetic placement of *M. haldari* isolates clustered them together in an independent branch under subclade D and then clustered with sequences of *Myxobolus hosadurgensis* and *M. calbasui* isolates, parasites of kidney, liver and intestine of cyprinid host mrigal and reba (Figure 3). The retrieved similarity values of *Myxobolus haldari* isolates are 87.6% with isolates of *Myxobolus hosadurgensis* and 85.3% with *M. calbasui* isolates. *Myxobolus hosadurgensis* isolates share a common clade with *M. calbasui* collected in the present work from liver and intestine of reba and mrigal carp respectively in a clade D (Figure 3). Both the species share some morphological similarities as their myxospores have larger and shorter polar capsules. In turn, *Myxobolus haldari* which forms a branch close to *Myxobolus hosadurgensis and M. calbasui* have also significantly large and small polar capsules. The genetic distance estimation revealed the highest similarity of *Myxobolus* spp. to *M. calbasui* isolates (92.3%) (Figure 3). *M. calbasui* collected in the present study from the liver and intestine of reba and mrigal both have unequal polar capsules. Originally, this species was described by Chakravarty MM [39] from gall bladder of calbasu (*Labeo calbasu*), rohu and mrigal with small polar capsules have three to four filament turns while the large ones have five to six turns respectively. In all, *M. calbasui* isolates of reba and mrigal ssrDNA gene sequences revealed 100% similarity with each other (Figure 3). *M. calbasui* isolates share a clade with *Myxobolus hosadurgensis* from the kidney of mrigal and the genetic distance similarity values range 91.8% (Figure 3).

Discussion

The *Myxobolus* Bütschli, 1882 is the largest genus among the myxosporean and the diversity of this genus keeps increasing day by day all over the world. The phylogenetic placement of the seven species of *Myxobolus* using ssrDNA sequences in the present study was consistent as the species in the tree are closely related, all infecting the hosts of the family Cyprinidae. Although, previously many studies also proved that myxozoan species generally lean towards to cluster according to the family of the fish host [64-66] and it can be used as a character to discriminate closely related species. Now a day, it is already accepted that for the identification of myxosporean species morphology alone is not sufficient and the studies should be supplemented with molecular data [6,8,21,28,67,68] as many congener species have morphological similarities with each other and cannot be readily distinguishable. However, the unavailability of molecular data (ssrDNA), restricts the genetic and evolutionary comparison of these species. Although, especially in India focus on molecular aspects is less in comparison to morphology and except for a few, the majority of species were described based on morphology only [14-17].

During the present study, seven *Myxobolus* species were obtained from the carp fish of the Meerut region. Out of these, three species were collected from the renal tissue (*M.bhadrensis, M. haldari, M. hosadurgensis*) three from gills (*M. kalavatiae, M.saranae, M.catlae,)* and one from the liver (*M. calabasui*) and intestine (*M. calabasui*). According to Cech, et al. [24] in general *Myxobolus* species are host specific but may also infect some closely related fishes. Recovery of spores of *M. calbasui* and *M. catlae* from two different hosts, *C. mrigala* and *C. reb*a are supported by the findings of Székely [6] which has mentioned that genetically closely related Indian major carps and their hybrids may share the same Myxobolus species. In the molecular phylogeny of Indian fish species, which also included all the carp genera: Labeo, Catla and Cirrhinus show a close phylogenetic relationship as sister species. In the present study Chakraborty M, et al. [69,70] all the species infected the host of order Cypriniformes and the family *Cyprinidae*. In terms of phylogeny, *M. kalavatiae* and *M. catlae* despite some genetic diversity with other isolates available on Genbank, are placed in the tree with different isolates along with other species in clade A, that are gill infecting myxobolids. However, *M. bhadrensis* collected from the kidney of host fish in the present study and previously reported from muscle and kidney of host fish forms a clade B with other muscle infecting species i.e., *M. terengganuensis*, as kidney and muscle infecting species share a common subclade, also reported in previous studies [28]. According to Molnár K, et al. [71] dispersed spores and large groups of

Myxobolus species are commonly found in the renal tissue; both the coelozoic as well as histozoic species are capable of causing kidney pathology. Regarding *Myxobolus saranae*, it is clustered in a clade C with *Myxobolus* and *Henneguya* species forming a group that is composed of exclusively gill infecting parasites of Cypriniformes. In the clade D, where *Myxobolus haldari*, *Myxobolus hosadurgensis and M. calbasui* is dispersedly grouped in a cluster primarily of liver, kidney and intestine infecting species, while other gills infecting species belong to *Myxobolus and Thelohanellus* occurred in another cluster under clade D.

Thus, the findings of the present study corroborate the idea given in previous studies that the host and inclination for a particular organ/tissue development is an important factor for the identification of species that should be considered for *myxozoan* phylogenetic studies [33,72]. Eszterbauer E [33] suggested that genetic differentiation based on tissue/organ tropism is a more ancient evolutionary characteristic than host specificity. Phylogenetic relationships related to tissue tropism have been reported for myxobolids as stronger evolutionary signals [67,73-75]. Most of the *Myxobolus* species are host specific but as in our study, one species infected two different hosts as also reported earlier that myxosporeans can infect other closely related fish species [33,76-78].

The findings presented above reveal the fact that myxosporean species infected cyprinid hosts are phylogenetically close, so as a side as tissue tropism shows the strongest evolutionary signal, phylogenetic affinity according to the fish hosts also suggests an important factor. Future work of myxosporeans with more molecular data is required to better understand evolutionary relationships and their taxonomy that was previously based purely on morphology for most Indian species.

Conclusion

In this present study, collected *Myxobolus* species data supported by morphological measurement and phylogenetic analysis of ssrDNA sequences data. The *M. saranae*, *M. hosadurgensis, M. haldari* and *M. calabasui* showed different clades from other available species on Genbank, due to a lack of previous data available on these species. This study adds the knowledge of the diversity of *Myxobolus* species in cyprinid fishes. Also, proves that *Myxobolus* species is the most dominant species among other species in cyprinids fishes in India.

- **Conflicts of Interest**

The authors declare no competing interests.

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- **Ethical Approval**

All applicable institutional and national guidelines for the care and use of animals were followed.

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