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Molecular identification of a new myxozoan, *Myxobolus dermiscalis* n. sp. (Myxosporea) infecting scales of *Labeo rohita* Hamilton in Harike Wetland, Punjab (India)

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ABSTRACT

In the present study, a new species *Myxobolus dermiscalis* n. sp. infecting scales of *Labeo rohita*, an Indian major carp from Harike Wetland in Punjab, India has been described on the basis of spore morphology and amplification of a part of 18S rDNA gene. The pseudocysts of *M. dermiscalis* n. sp. are milky white with irregular outline, 0.5–3.6 mm in diameter embedded within the dermal scale in the form of a cavity. The spores 5.84–7.98 × 3.98–5.98 μm in size, having two equal polar capsules 3.98–5.98 × 1.85 –3.85 μm in size. The most differentiating feature from closely related species, *Myxobolus saugati* (Kaur and Singh, 2011) is the presence of two parietal folds at the posterior – lateral margins of the shell valves. The present species is regarded as host, organ and tissue specific in nature. The partial sequence of SSU gene of *M. dermiscalis* n. sp. clustered with other *Myxobolus* species infecting cyprinids available in the GenBank. Blast search revealed 98% homogeneity with *Myxobolus* sp (KM401439) infecting scales of *L. rohita* in Myanmar (unpubl. data). The present myxobolid parasite has been recorded to cause serious, highly symptomatic disease of the scales, causing their loosening from the skin of *L. rohita*. It rendered the host fish unsightly giving it cloudy appearance with white patches and mucoid body surface. Scale pseudocyst Index (SPI) has been provided to record the intensity of infection.

1. Introduction

Punjab (India) has 3 main wetlands, i.e. Harike, Kanjli and Ropar wetlands which are included in Ramsar list of International importance. Harike wetland (31° 17′ N latitude and 75° 12′ E longitude) is the largest freshwater wetland (in northern India) of 4100 ha area with Beas and Sutlej as primary inflows. It is a habitat for as many as 26 species of fishes which include *Catla*, *Cirrhinus*, *Channa*, *Mystus*, *Chitala chitala*, *Cyprinus* and *Ambassis ranga*. These wetlands are the major natural fisheries resource for food in whole of the Punjab state. The study indicates that large variety of fishes in these wetlands are infested with myxozoan parasites. Myxozoans include histozoic and coelozoic parasites infecting not only freshwater and marine fishes but have also been detected in molluscs, amphibians, reptiles, waterfowl and mammals (Moncada et al., 2001; Eiras, 2005). As demonstrated firstly by Wolf and Markiw (1984), it has been proven that myxozoan species require an alternate invertebrate host (usually an annelid) to complete the life cycle. Among myxosporeans, the genus *Myxobolus* includes the highest number of species. Eiras et al. (2005) reported 744 valid species, while Lom and Dykova (2006) counted 792 including 7 amphibian species. Several other reports on *Myxobolus* species are available from Punjab and West Bengal, India (Basu & Haldar, 2004-Basu et al., 2009; Bandyopadhyay et al., 2006/2007; Kaur and Singh, 2014). About 131 species of *Myxobolus* have been recorded in India (Kaur and Singh, 2012) and are mainly differentiated by morphological, morphometric characteristics of spores, besides host and organ or tissue specificity. Presently, the molecular analyses have been an important tool in the study of these parasites and this has expanded their taxonomy to the phylogenetic analyses. This has led to correct identification and differentiation of morphologically indistinguishable myxobolid species (Kent et al., 2001; Eszterbauer, 2002; Molnar et al., 2010; Cech et al., 2012; Bartosova et al., 2009). The most revealing aspects of analyses based on SSU rDNA sequences is the incongruence of phylogenetic trees with classification based 63on spore morphology alone (Bartosova et al., 2009). So far, there are 14 sequences i.e *caudatus* KC865607; *Myxobolus*

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cuttacki 465682; *M. orrissae* KF448527; *Myxobolus basuhaldari* KM029974, KM029975, KM029976; *M. kalavatiae* KM029973; *M. meerutensis* KM029977;*M. bhadrensis* KM029968, KM029969, KM029970, KM029971, KM029972 and *M. catlae* KM029967 (Mondal et al., 2014; Szekely et al., 2014; Rajesh et al., 2014; Abraham et al., 2015) from India are available in the Genbank. In this study, we present morphological and molecular characterization of *M. dermiscalis* n. sp. infecting the scales of *L. rohita* collected from Harike wetland, Punjab, India along with 18S rDNA based phylogenetic analysis with *Myxobolus* group and other related texa. In future, the molecular methods can be implied on the diagnostics of the economically important myxozoan parasites in this part of the world.

2. Material and methods

2.1. Collection

Fresh specimens of Indian major carp, rohu, *L. rohita* were collected from the fisher-man at Harike wetland during the period June 2013 to July 2014. The infected scales were removed with the help of forceps in a petridish containing 0.9% saline. The pseudocysts were visible with the naked eye and appeared as creamish white patches on the scale. The plasmodium were teased on a clean slide to liberate spores and were examined under the microscope. Fresh spores were treated with 8% KOH solution for the extrusion of polar filaments. For permanent preparation, air-dried smears were stained with Ziehl–Neelsen and Iron-haematoxylin. Spores were measured with the help of a calibrated ocular micrometre. All measurements were recorded in microns (μm).

2.2. Prevalence

The prevalence (in percentage) of *M. dermiscalis* infecting scales of *L. rohita* was calculated according to Bush et al. (1997). The intensity of infection was determined by following the index proposed by Kaur and Attri (2015) for infection on the scales. 0 = no infection; 1 = one pseudocyst per scale in 10% of total scales (indicating light infection); 2 = two pseudocysts per scale in 15–20% of total scales (moderate infection); 3 = three to four pseudocysts per scale in 50% of total scales (heavy infection); 4 = four to five pseudocysts per scale in 100% of scales (severe infection).

2.3. Molecular analysis

2.3.1. DNA extraction, polymerase chain reaction

The pseudocysts (50 in numbers) present on ethanol fixed scales were ruptured with the help of a sharp needle in a watch glass having double distilled water. The contents in the watch glass containing spores were collected in 1.5 ml micro centrifuge tubes. The DNA was Scientific, Wilmington, USA) spectrophotometer at 175 ng/μl. Polymerase chain reaction (PCR) was carried out according to the Andree et al. (1999) at the final volume of 25 μl using the primers MX5-MX3 which amplified the fragments of 1597 bp respectively of the 18S rDNA gene. The amplification reactions were conducted with 30–75 ng of genomic DNA, 12.5 μl of 1 × reaction buffer (Hi media), 1.0 μl of each primer, 1.0 μl of total DNA and 10.5 μl of double purified water. Amplification was done by initial denaturation at 95 °C for 3 min, followed by 33 cycles of denaturation at 95 °C for 30 s, annealing of primers at 58 °C for 30 s, extension at 72 °C for 1 s. The final extension was at 72 °C for 10 s. The PCRproducts were analysed agarose gel electrophoresis, and size was estimated by comparison with the1Kb Plus DNA ladder (Fig. 1).

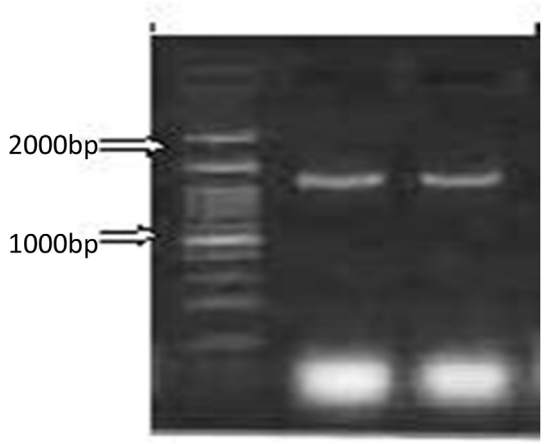

Fig. 1. Agarose gel (1.8%) showing amplified 18S rDNA gene of *M. dermiscalis* n. sp. infecting scales of *Labeo rohita*.
Lane 1: 1kb DNA Ladder
Lane 2, 3: M. dermiscalis n. sp. (1597bp)

2.3.2. DNA sequencing, sequence alignment and phylogenetic analysis

Extracted from spores using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. The product was then quantified in a Nanodrop (Thermocycler). In the present study, the PCR amplified products were sequenced at the Molecular Diagnostics & Research Laboratories (MDRL) Pvt. Ltd. Chandigarh, India. 1597 bp of 18S rDNA sequences of *M. dermiscalis* n. sp. were deposited in GenBank with accession number KM092529. Phylogenetic analysis involving 23 nucleotide sequences was performed using the Bayesian, Maximum likelihood (ML), Maximum parsimony (MP) and Neighbour joining (NJ) methods. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The evolutionary distances were computed using the Kimura 2-parameter method Kimura (1980) and units of the number of base substitutions per site. The rate variation among sites was modelled with a gamma distribution. The best fit model for analysis for the current data was GTR + G with lowest BIS (Bayesian Information Criterion) was estimated by using model test tool in Mega 6.06.

3. Results

3.1. Morphological characteristics of Myxobolus dermiscalis n. sp.

3.1.1. Pseudocyst (Fig. 2)

Round to irregular, white, 4-5pseudocysts per scale, histozoic, present within a cavity and measure 0.5–3.6 mm in diameter. 400–500 of spores were present per pseudocyst.

3.1.2. Spore description (Fig. 3)

(Measurements based on 10 spores in frontal view).

The spores measure 5.84–7.84 × 3.98–5.98 μm, oval to spherical in frontal view having rounded anterior and posterior ends. Both the shell valves are thick, symmetrical and 0.5 μm in thickness. Parietal folds two, present on the posterio-lateral margins of the shell valves. Polar capsules are two, equal, measure 3.98–5.98 × 1.85–3.85 μm and are pyriform with distinct neck at the anterior end. They converge anteriorly and are placed at a distance posteriorly. Polar filaments form 5–6 coils and are arranged obliquely to the polar capsule axis, 7.2 μm in length when extruded. An intercapsular process is absent. Two capsulogenic

Fig. 2. Infected scales of *L. rohita* showing creamish white pseudocysts of *M. dermiscalis* n. sp scale bar = 1 cm.

nuclei are present beneath each of the polar capsule. Sporoplasm agranular, homogenous occupying whole of the extracapsular space behind the polar capsules and contain one nucleus and a large iodinophilous vacuole.

3.1.3. Taxonomic summary of M. dermiscalis n. sp.

Host: *L. rohita* (Ham.) vern. Rohu.

Locality: Harike Wetland, Punjab, India.

Site of infection: Scales.

Type specimen: Paratype are spores stained with Ziehl–Neelsen and Iron-Haematoxylin, deposited in the Parasitology Laboratory, Department of Zoology & Environmental Sciences, Punjabi University, Patiala (India). Slide no. M/ZN/02.11.2014 and M/IH/02.11.2014.

Frequency of parasite species (%): 59.7% (52/87).

Intensity of infection with parasite species (**Index**): **4** (Severe infection).

Clinical Symptomatology: Highly symptomatic; creamish white patches and mucous laden body surface. Loosening of scales.

Etymology: The specific epithet *dermiscalis* has been given on the basis of location of pseudocysts within the layers of dermal scales.

3.2. Molecular data

The edited nucleotide sequence was 1597 bp of *M. dermiscalis* n. sp. which was deposited in Gen-Bank under the accession number of KM092529. BLASTN search (www.ncbi.nlm.gov/BLAST) with 21 *Myxobolus* exhibited 90–93% sequence homology and maximum homology of 98% recorded was recorded with *Myxobolus* sp. (KM401439 unpubl) infecting scales of a cyprinid, *L. rohita* in Myanmar available in the GenBank (Table 1). In the phylogenetic tree based on the final edited alignment with neighbour–joining analysis (Fig. 5), *M. dermiscalis* was placed with the *M.* sp. with highest bootstrap value and confirmed the clustering pattern of maximum likelihood and maximum parsimony analysis (100% in ML, 100% MP).

4. Discussion

4.1. Morphological comparison

The present species has been compared with *M. squamae* (Keysseltz, 1908) from scales of *Abramis brama*; *M. mrigalae* (Chakravarty, 1939) from scales of *Cirrhinus mrigala*; *M. yogendrai* (Tripathi, 1952), *M. rewensis* (Srivastava, 1979) and *M. vanivillasae* (Seenappa and Manohar, 1980) from scales of *Cirrhinus mrigala*; *M. rohitae* (Haldar et al., 1981) from scales of *L. rohita*, *Labeo calbasu*,

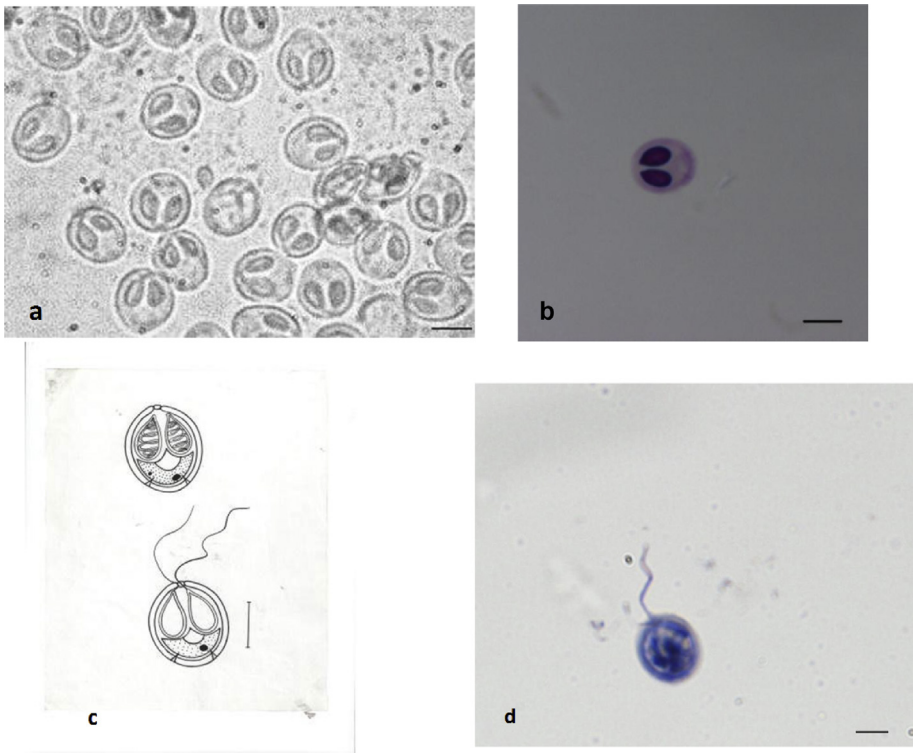

Fig. 3. Photomicrographs of myxospores of *M. dermiscalis* n. sp. a) fresh under phase contrast microscope b) stained with Ziehl–Neelson c) Line drawing d) stained with Iron-haematoxylin Scale bar = 10 μm.

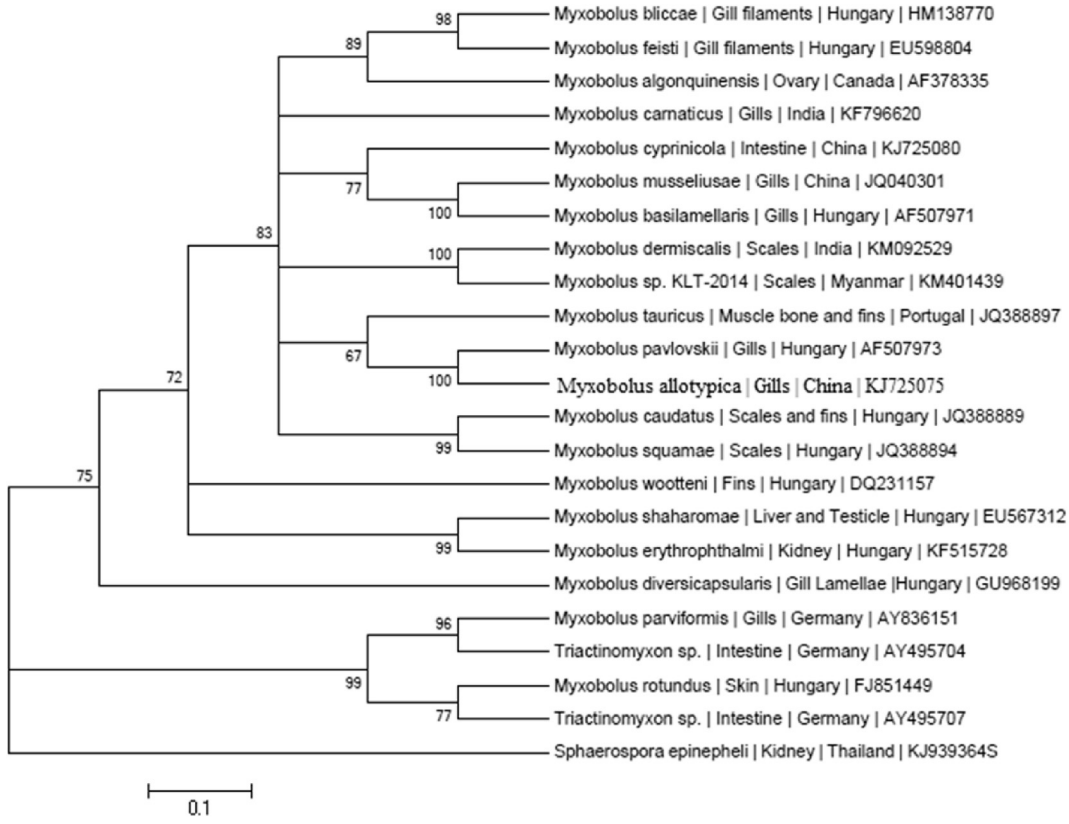

Fig. 4. Neighbour-Joining analysis of small subunit ribosomal DNA sequence of *M. dermiscalis* n.sp.in relation to 23 other sequenced members of the genus.

Labeo bata and *Puntius sarana*; *M. indirae* (Gupta and Khera, 1988) from scale, tail, fin, cartilage and head of *Cirrhinus mrigala M. episquamae* (Egusa et al., 1990) from scales of *Mugil cephalus*; *M. squamaphilus* (Molnar, 1997) from scales of *A. brama*; *M. dermatis* (Haldar et al., 1983) from scales of *L. rohita*; and *M. saugati* (Kaur and Singh, 2011) from scales and gill lamellae of *Catla catla* and *L. rohita*. The present species, *M. dermiscalis* n. sp. was compared with all the above mentioned species and was found different in spore morphology and morphometrics. The spores of the present species are characterized in having oval to spherical shape with rounded anterior end without intercapsular process and posterior end with two parietal folds. Polar capsules are two, equal, pyriform with distinct neck. In this respect, the present species differed from *M. squamae*, *M. yogendrai*, *M. rewensis*, *M. vanivillasae*, *M. rohitae*, *M.*

Table 1
Homogeneity of 18S rRNA gene sequences of *Myxobolus dermiscalis* (Accession number KM092529) and other myxobolids available in NCBI GenBank.

Myxozoan species	Host fish	Site of infection	Accession number	Country	DNA sequence homogeneity to *M. dermiscalis* n. sp (KM029925)
Myxobolus sp	*Labeo rohita*	Scale	KM401439	Myanmar	2771/2771(98)
M. pavlovskii	*Aristichthys nobilis*	Gills	AF507973	Hungry	1796/1796(93)
M. allotypica	*Hypothalmichthys molitrix*	Gills	KJ725075	China	1779/1779(93)
Myxobolus bliccae	*Blicca bjoerkna*	Gills	HM138770	Hungry	1725/1725 (92)
M. mussaliusae	*Cyprinus carpio*	Gills	JQ040301	China	1707/1707(93)
M. diversicapsularis	*Rutilus rutilius*	Gills	GU968199	Hungry	1690/2037(92)
M. caudatus	*Barbus bynni*	Tail fin	JQ388889	Egypt	1679/1679(91)
M. basilamellaris	*Cyprinus carpio*	Gills	AF507971	Hungary	1676/1676(91)
M. wooteni	*Rutilus rutilus*	Fins	DQ231157	Hungary	1670/1670(91)
M. carnaticus	*Cirrhinus mrigala*	Gills	KF796620	India	1663/1663(91)
M. algonquinensis	*Notemigonus crysoleucas*	Ovary	AF378335	Canada	1657/1961(91)
M. cyprinicola	*Leuciscus waleckii*	Intestine	KJ725080	Russia	1635/1635(91)
M. parviformis	*Abramis brama*	Gill lamellae	AY836151	Germany	1609/1609(90)
Triactinomyxon sp.	*Tubifex tubifex*		AY495704	Germany	1609/1609(90)
M. tauricus	*Barbus tauricus*	Gills, fins, muscles	JQ388897	Ukraine	1572/1572(92)
M. rotundus	*Abramis brama*	Gills	FJ851449	Germany	1570/1902(90)
Triactinomyxon sp.	*Tubifex tubifex*		AY495707	Germany	1568/1568(90)
M. shaharomae	*Alburnus alburnus*	Liver, kidney, testis, gut	EU567312	Hungary	1559/1559(91)
M. feisti	*Rutilus rutilus*	Gill filament	EU598804	Hungary	1552/1552(91)
M. squamae	*Barbus barbus*	Scales	JQ388894	Hungary	1504/1504(91)
M. erythrophthalmi	*Scardinius erythrophthalmus*	Liver	KF515728	Hungary	1411/1411(91)
Sphaerospora epinepheli	*Epinephelus coioides*	Kidney	KJ939364	China	Outgroup

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. Myxobolus dermiscalis/Scales/India/KM092529		0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
2. Myxobolus sp./Scales/Myanmar/KM401439	0.00		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
3. Myxobolus pavlovskii/Gills/Hungary/AF507973	0.07	0.07		0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
4. Myxobolus allotypica/Gills/China/KJ725075	0.07	0.07	0.01		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
5. Myxobolus bliccae/Gill filaments/Hungary/HM138770	0.09	0.09	0.07	0.08		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04
6. Myxobolus musseliusae/Gills/China/JQ040301	0.08	0.08	0.07	0.08	0.08		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04
7. Myxobolus diversicapsularis/Gill lamellae/Hungary/GU968199	0.09	0.09	0.08	0.08	0.08	0.09		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04
8. Myxobolus caudatus/Scales and fins/Hungary/JQ388889	0.09	0.09	0.08	0.08	0.10	0.11	0.08		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
9. Myxobolus basilamellaris/Gills/Hungary/AF507971	0.10	0.10	0.08	0.09	0.09	0.03	0.10	0.11		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
10. Myxobolus wootteni/Fins/Hungary/DQ231157	0.09	0.09	0.08	0.08	0.09	0.09	0.07	0.10	0.10		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.04
11. Myxobolus carnaticus/Gills/India/KF796620	0.10	0.10	0.07	0.08	0.09	0.09	0.10	0.11	0.10	0.10		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
12. Myxobolus algonquinensis/Ovary/Canada/AF378335	0.11	0.10	0.08	0.09	0.07	0.09	0.08	0.10	0.09	0.09	0.09		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
13. Myxobolus cyprinicola/Intestine/China/KJ725080	0.10	0.10	0.07	0.07	0.10	0.08	0.10	0.10	0.08	0.10	0.10	0.09		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05
14. Myxobolus parviformis/Gills/Germany AY836151	0.12	0.11	0.10	0.10	0.09	0.10	0.05	0.10	0.11	0.08	0.12	0.10	0.12		0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.04
15. Triactinomyxon sp./Intestine/Germany/AY495704	0.12	0.11	0.10	0.10	0.09	0.10	0.05	0.10	0.11	0.08	0.12	0.10	0.12	0.00		0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.04
16. Myxobolus tauricus/Muscle bone and fins/Portugal/JQ388897	0.08	0.08	0.05	0.05	0.08	0.08	0.08	0.08	0.09	0.08	0.08	0.08	0.08	0.09	0.09		0.01	0.01	0.01	0.01	0.01	0.01	0.04
17. Myxobolus rotundus/Skin/Hungary/FJ851449	0.12	0.12	0.10	0.11	0.09	0.11	0.05	0.10	0.11	0.08	0.12	0.10	0.12	0.01	0.01	0.09		0.00	0.01	0.01	0.01	0.01	0.04
18. Triactinomyxon sp./Intestine/Germany/AY495707	0.12	0.12	0.10	0.11	0.09	0.11	0.05	0.10	0.11	0.08	0.12	0.10	0.12	0.01	0.01	0.09	0.00		0.01	0.01	0.01	0.01	0.04
19. Myxobolus shaharomae/Liver and Testicle/Hungary/EU567312	0.10	0.10	0.08	0.07	0.07	0.09	0.05	0.08	0.10	0.06	0.09	0.08	0.10	0.07	0.07	0.07	0.07	0.07		0.01	0.01	0.01	0.04
20. Myxobolus feisti/Gill filaments/Hungary/EU598804	0.10	0.10	0.08	0.08	0.04	0.08	0.08	0.09	0.08	0.08	0.08	0.06	0.08	0.09	0.09	0.08	0.09	0.09	0.07		0.01	0.01	0.05
21. Myxobolus squamae/Scales/Hungary/JQ388894	0.10	0.10	0.07	0.08	0.10	0.10	0.09	0.07	0.11	0.09	0.11	0.10	0.10	0.12	0.12	0.09	0.12	0.12	0.09	0.10		0.01	0.05
22. Myxobolus erythrophthalmi/Kidney/Hungary/KF515728	0.10	0.10	0.07	0.07	0.07	0.09	0.06	0.08	0.10	0.06	0.09	0.07	0.10	0.07	0.07	0.07	0.07	0.07	0.03	0.07	0.09		0.04
23. Sphaerospora epinepheli/Kidney/Thailand/KJ939364	0.51	0.50	0.49	0.49	0.48	0.47	0.47	0.51	0.50	0.47	0.50	0.49	0.50	0.45	0.45	0.49	0.45	0.45	0.47	0.51	0.50	0.47	

Fig. 5. Estimates of evolutionary divergence between the sequences of *M. dermiscalis* and other Myxosporea available in GenBank.

indirae,*M.episquamae*, *M. squamaphilus*, *M. dermatis*, and *M. saugati* in lacking the intercapsular process. Furthermore, it differed from *M. saugati*, *M. rohitae*, *M. dermatis* and *M. indirae* in having two parietal folds placed posterio-laterally on the shell valves. The present species is also different from *M. mrigalae* in which several triangular markings are present, in addition to unequal polar capsules. In view of the above differences in morphology and morphometrics, the present species under study is proposed as new to the science and named as *M. dermiscalis* n. sp.

4.2. Molecular comparison

The universal primer sets MX5 and MX3 successfully amplified approximately 1600 bp fragments of the 18srDNAgene from the present sample of *Myxobolus* (Fig. 3). The edited nucleotide sequence was 1597 bp of *M. dermiscalis* n. sp. which was deposited in GenBank under the accession number of KM092529. The DNA sequences of *M. dermiscalis* n. sp. was closest to *Myxobolus* sp. infecting scales of a cyprinid, *L. rohita* in Myanmar available in the GenBank (unpubl), which showed 98% homogeneity (Table 1). In the phylogenetic tree based on the final edited alignment with neighbour–joining analysis (Fig. 5), *M. dermiscalis* was placed with the *M.* sp. with highest bootstrap value and confirmed the clustering pattern of maximum likelihood and maximum parsimony analysis (100% in ML, 100% MP). The present study indicated close similarity based on 18S rDNA suggesting sister species, however, the morphological comparisons could not be made due to lack of published record. Minimum evolutionary divergence between the two species was estimated using Bayesian Information Criterion (BIS scores) using best model test tool in Mega 6.06 (Fig. 4). In order to estimate the pattern of nucleotide substitution involving 23 nucleotide sequences using GTR + G model in (Table 2).

Rates of different nucleotide substitution(r) from one base (transitional -in **bold**) and to another base(transversional-in *italics*) were assessed. The rates of transition were more than transversion for all base substitutions. Maximum transition was C→ G having 19.99 probability value followed by T→C 14.38, A→G 13.21, G→A 12.62. As for trasversions A→T, G→T 5.31; T→A, C→A 5.26, T→G, C→G 247 5.51, A→C, G→C 3.82. The nucleotide frequencies were 26.44%(A), 26.69%(T/U), 248(G) 19.19%(C) and 27.68%(G). Furthermore, the information generated confirmed the importance of features such as host, tissue tropism, geographical location of the host and correlation with the results of molecular data based on comparison of 18S rDNA sequence (Kent et al., 2001; Seo et al., 2012; Zhu et al.,2012; Shin et al., 2013). Index of intensity of

Table 2
Showing the transitional and transversion substitutions obtained by maximum likelihood estimate of substitution matrix in Mega 6.0 (Rates of different transitional substitutions are shown in bold and those of transversion substitutions are shown in italics).

	A	T/U	C	G
A	–	*5.31*	*3.82*	**13.21**
T/U	*5.26*	–	**14.38**	*5.51*
C	*5.26*	**19.99**	-	*5.51*
G	**12.62**	*5.31*	*3.82*	–

infection by *M. dermiscalis* n.sp was recorded as 4 indicated by the presence of four to five pseudocysts per scale in 100% of scale. The intensity of infection was recorded as 'severe' as per to the index number 4 of pseudocysts per scale.

The prevalence of *M. dermiscalis* n. sp. infesting scales of *L. rohita* was 59.7%. This study is the first report the molecular and phylogenetic characterization of scale infecting *M. dermiscalis* n. sp. from Indian subcontinent. As the list of Indian species of the Genus *Myxobolus* have increased over the years (Basu et al., 2009; Bandyopadhyay et al., 2006/2007; Singh & Kaur, 2012–2014) thus the correct identification in future studies must focus on molecular and phylogenetic characterization together with morphological characteristics, host, organ and tissue specificity.

5. Conclusions

The present study describe a myxobolid species commonly found infecting scales of an Indian major carp, *L. rohita* in freshwater habitat. The intensity of infection was severe as indicated by the Scale Pseudocyst Index and highly symptomatic to the fish host. Further investigations are being carried out on the presence of gill infecting myxozoan parasites among major Indian carps in polycultured conditions. This is important to have assessment of the impact on these parasites on the carp fishes. Beside morphological features, genetic data has been provided in the present manuscript which can form basis for development of diagnostics of this species.

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