Two new species of *Myxobolus* (Myxozoa: Myxosporaea: Bivalvulida) infecting an Indian major carp and a cat fish in wetlands of Punjab, India

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Abstract A survey of parasites of freshwater fishes in Kanjali and Harike wetlands of Punjab (India) revealed the presence of two new myxosporean species belonging to the genus *Myxobolus* Bütschli (Winter (ed): Protozoa, 1882). Spores of the first species, *M. saugati* sp. nov. parasitizing scales of *Labeo rohita* (Cypriniformis: Cyprinidae) vern. rohu are characterized in having ‘spores measuring $8.3 \times 6.6 \mu\text{m}$ in size, oval to spherical in valvular view having rounded anterior and posterior ends; polar capsules are two, equal, measuring $4.0 \times 2.4 \mu\text{m}$, pyriform with distinct neck at the anterior end; an intercapsular process is absent’. Spores of the second species, *Myxobolus szekeli* sp. nov. parasitizing internal wall of stomach of *Wallago attu* (Cypriniformis: Siluridae) vern. mulle are ‘ $8.7 \times 4.1 \mu\text{m}$ in size, elongately ovoidal in valvular view having tapering, bluntly pointed anterior end and rounded posterior end; polar capsules are two, equal, measuring $4.4 \times 1.5 \mu\text{m}$, tubular with blunt anterior and rounded posterior ends; polar capsules converge anteriorly but are placed at a distance posteriorly and occupies nearly half of the spore body cavity; an intercapsular process is absent’.

Keywords Cypriniformes · Harike wetland · Kanjali wetland · *L. rohita* · *W. attu*

Introduction

Kanjali and Harike are major Wetlands spread over 185 and 4,100 ha area respectively in Punjab, India among other Wetlands (i.e. Ropar, Ranjit Sagar and Nangal wetlands) in the

Ramsar list Wetlands of International importance. Harike wetland harbour 26 species and Kanjali wetland 17 species of freshwater fishes. These large varieties of fishes in these wetlands 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 belong to the Phylum Myxozoa has been described from fishes (Lom and Dykova 2006). Recently a new genus, *Thelohanelloid bengalensis* from gall bladder of *Arius sagor* (a marine fish in Bay of Bengal) has been described by Sarkar (2009). Out of this, 744 species belong to the genus *Myxobolus* and is the most common genus reported worldwide (Eiras et al. 2005). According to Kalavati and Nandi (2007) 104 species of *Myxobolus* spp. are restricted to the Indian sub-continent. Recently, Kaur and Singh (2008, 2009, 2010a, b, 2010/2011, 2011a, b, c, d, e) have contributed 15 new species to the genus *Myxobolus* from freshwater fishes in Wetlands of Punjab.

During the present investigation 62 fishes belonging to *L. rohita* and 25 fishes belonging to *W. attu* were examined to ascertain the prevalence of myxozoan parasites in these wetlands. A variety of other freshwater fishes were also collected and examined which include *Cyprinus carpio*, *Catla catla*, *Amblypharyngodon mola*, *Labeo bata*, *Labeo dero* and *Mystus seenghala*. Two new species *M. saugati* and *M. szekeli* are reported from scales and internal wall of stomach respectively. The description has been prepared according to the guidelines of Lom and Arthur (1989).

Materials and methods

Fishes collected from the Kanjali and Harike wetlands were brought to the laboratory and examined for myxozoan infections. Plasmodia when found were removed and teased on slide and covered with cover slip and examined under the oil

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immersion for the presence of myxospores. 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. Drawings were made from stained material with the aid of camera lucida. Measurement of spores was done with the aid of a calibrated ocular micrometer. All measurements are presented in μm as range values followed by mean $\pm$ SD in parentheses.

Results and discussion

M. saugati sp. nov.

Plasmodia

Large, white to pale yellow, present all over the scales, 10–12 in number and measure 1.0–1.5 mm in diameter. 15–18 Spores are present per plasmodium.

Spore description (Figs. 1a–c, 2a, b, 5a; Table 1)
(measurements based on 8–9 spores in frontal view)

The spores are histozoic, measure $8.3 \times 6.6 \mu\text{m}$, oval to spherical in valvular view having rounded anterior and posterior ends. Two shell valves are thick, smooth, symmetrical and measure $0.6 \mu\text{m}$ in thickness. Parietal folds are absent. Polar capsules are two, equal, measure $4.0 \times 2.4 \mu\text{m}$ and are pyriform with distinct neck at the anterior end. They converge anteriorly and are placed at a distance posteriorly. Polar filaments form 4–5 coils and are arranged obliquely to the polar capsule axis. They are equal when extruded and measure $26.6 \mu\text{m}$ in length. An intercapsular process is absent. Two capsulogenic nuclei are present beneath each polar capsule measuring $1.50 \mu\text{m}$ in diameter. Sporoplasm agranular, homogenous occupying whole of the extracapsular space behind the polar capsules and contain one nucleus and a large iodophilous vacuole measuring 1.6 and $2.5 \mu\text{m}$ in diameter respectively.

Taxonomic summary of M. saugati sp. nov.

Type host	<i>Labeo rohita</i> vern. rohu
Type locality	Kanjali wetland, Punjab, India
Type specimen	Paratypes are spores stained in Ziehl–Neelsen and iron–haematoxylin, deposited in the museum of department of Zoology, Punjabi University, Patiala. Slide no. CM/P/ZN/21.04.2009 and CM/P/IH 11.08.2009.
Site of infection	Scales
Prevalence of infection	56.4% (35/62)
Etymology	The species epithet <i>saugati</i> has been given after the name of the Dr. Saugata Basu, an eminent worker of Protozoology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.

Fig. 1 **a** Spore stained in Ziehl–Neelsen (valvular view). **b** Spore stained in iron–haematoxylin. **c** Spore in side view. Scale bar 0.005 mm

Discussion

The present species under study was compared with *M. pfeifferi*, *M. minutus*, *M. catostomi*, *M. catlae*, *M. sangoricus*, *M. baueri*, *M. lotae*, *M. strelkovi*, *M. bhadrensis*, *M. shetii*, *M. rohita*, *M. gobiorum*, *M. dermatis*, *M. haldari*, *M. cartilaginis*, *M. serrata*, *M. kwangsiensis*, *M. nkolyaensis*, *M. edellae*, *M. shantipuri*, *M. chilensis*, *M. tingrelaensis*, *M. sushmii*, *M. punjabii* and *M. patialensis* but differs from all of the above species in morphometrics (Table 2).

The present species is oval to spherical in shape with rounded anterior and posterior extremities without any parietal folds. They contain equal, pyriform polar capsules with anterior ends terminating into a distinct neck. In this respect, it is comparable with the spores of *M. minutus*, *M. catostomi*, *M. sangoricus*, *M. baueri*, *M. lotae*, *M. tingrelaensis*, *M. shantipuri*, *M. rohita*, *M. shetii*, *M. strelkovi* and

Fig. 2 **a** Spores stained in Ziehl-Neelsen. **b** Spore stained in iron-haematoxylin (extracted polar filaments)

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

Characters	Range	Mean values	SD
LS	7.9–8.7	8.3	0.56
WS	6.4–6.8	6.6	0.28
LPC	3.7–4.3	4.0	0.42
WPC	2.0–2.8	2.4	0.56
Ratio: LS/WS		1.2	
ICP		Absent	
NC		4–5	
Parietal folds		Absent	

LS length of spore, WS width of spore, LPC length of polar capsule, WPC width of polar capsule, ICP intercapsular process, NC number of coils of polar filaments, SD standard deviation

M. pfeifferi. An intercapsular process is absent in the species under study. But the presence of intercapsular process in *M. strelkovi*, *M. pfeifferi*, *M. tingrelaensis*, *M. shetii*, *M. sangoricus*, *M. baueri*, *M. catostomi*, *M. minutus*, *M. lotae*

and a triangular notch in *M. rohitei* differentiates all of the above species from the present species. Ovoidal or tear-shaped spores of *M. shantipuri* with either equal or unequal polar capsules can also be differentiated from the spores of the present species.

In view of the above differences, the present species under study is proposed as new to the science and named as *M. saugati* sp. nov. through this communication.

M. szekeli sp. nov

Plasmodia

White, spherical to rounded, present on the internal wall of the stomach, 1–2 in number and measure 0.6–0.8 mm in diameter. 5–6 spores are present per plasmodium.

Spore description (Figs. 3a, b, 4a, b, 5b; Table 3)
(measurements based on 4–8 spores in frontal view)

The spores are histozoic, measure $8.7 \times 4.1 \mu\text{m}$, elongately ovoidal in valvular view having tapering, bluntly pointed anterior end and rounded posterior end. Two shell valves are thin, smooth, symmetrical and measure $0.3 \mu\text{m}$ in thickness. Parietal folds are absent. Polar capsules are two, equal, measure $4.4 \times 1.5 \mu\text{m}$, tubular with blunt anterior and rounded posterior ends. Polar capsules converge anteriorly but are placed at a distance posteriorly and occupies nearly half of the spore body cavity. Polar filaments form 5–6 coils and are arranged perpendicular to the polar capsule axis. An intercapsular process is absent. One capsulogenic nucleus is present measuring $1.3 \mu\text{m}$ in diameter. Sporoplasm is agranular and homogenous occupying whole of the extracapsular space behind the polar capsules. One sporoplasmic nucleus measuring $1.4 \mu\text{m}$ in diameter is present. An iodophilous vacuole is present.

Taxonomic summary of M. szekeli sp. nov.

Type host	<i>Wallago attu</i> vern. mulle
Type locality	Harike wetland, Punjab, India
Type specimen	Paratypes are spore stained in Ziehl-Neelsen and iron-haematoxylin, deposited in the museum of department of Zoology, Punjabi University, Patiala. Slide no. MH/ZN/01.03.2010 and MH/IH/01.03.2010.
Site of infection	Internal wall of the stomach
Prevalence of infection	4% (1/25)
Etymology	The species epithet <i>szekeli</i> has been given after the name of Dr. Csaba Szekely of Veterinary Medical Research Institute, Hungarian Academy of Sciences, Hungary.

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

Species	Host	Site of infection	Locality	Spore	Polar capsule
<i>M. saugati</i> sp. nov. (present study)	<i>Labeo rohita</i>	Scales	Kanjali wetland Punjab (India)	8.3×6.6	4.0×2.4
<i>M. pfeifferi</i> Thelohan 1895	<i>Barbus barbus</i>	Kidney, spleen, muscles, gills	–	$10.0\text{--}13.0 \times 9.0\text{--}12.2$	$5.0\text{--}5.7$
<i>M. minutus</i> Nemecek 1911	<i>Leuscus leuscus</i> , <i>L. cephalus</i>	Gills	Germany	$6.0 \times 4.2\text{--}5.0$	3.0×2.0
<i>M. catostomi</i> Kudo 1923	<i>Catostomus commersonii</i>	Muscles	Canada	$10.0\text{--}12.7 \times 7.6\text{--}10.0$	$3.6\text{--}5.5 \times 1.3\text{--}2.7$
<i>M. catlae</i> Chakravarty 1943	<i>L. rohita</i>	Gills	West Bengal (India)	$14.5\text{--}16.5 \times 6.18$	$10.3\text{--}12.3 \times 2.06\text{--}3.01$
<i>M. sangoricus</i> Gogebashvili 1966	<i>Varicorhynchus capoeta</i>	Fin, gills, kidney	Caucasus	$9.1\text{--}11.0 \times 7.8\text{--}9.2$	$3.1\text{--}3.9 \times 1.8\text{--}3.0$
<i>M. baueri</i> Chernova 1970	<i>Tinca tinca</i>	Gills, heart, kidney, liver	Russia	$14.0\text{--}16.0 \times 9.3\text{--}12.0$	$5.3\text{--}6.0 \times 2.7\text{--}3.3$
<i>M. lotae</i> Mitenev 1971	<i>Lota lota</i>	Gills	Russia	$8.4\text{--}10.4 \times 6.2\text{--}6.5$	$3.4\text{--}4.4 \times 2.3\text{--}3.4$
<i>M. strelkovi</i> Kostarev and Kulemina 1971	<i>Phoxinus phoxinus</i> , <i>Leuciscus idus</i>	Gills, liver	Russia	$8.0\text{--}12.2 \times 6.0\text{--}11.0$	$3.3 \times 5.4 \times 2.0\text{--}4.1$
<i>M. bhadrensis</i> Seenappa and Manohar 1981	<i>L. rohita</i>	Muscle	Karnataka (India)	9.5×7.14	3.5×2.2 and 2.5×1.75
<i>M. shetti</i> Seenappa and Manohar 1981	<i>Cirrhina mrigala</i>	Gills	Karnataka (India)	8.8×7.4	3.4×2.3
<i>M. rohita</i> Halder et al. 1983	<i>L. rohita</i> , <i>L. bata</i> , <i>L. dyocheilus</i> , <i>L. calbasu</i> , <i>Puntius sarana</i>	Scales	West Bengal (India)	10.6×9.0	6.6×3.3
<i>M. gobiorum</i> Donec 1984 in Shulman	<i>Gobio gobio</i>	Fin	Ukraine	$11.0\text{--}13.0 \times 9.0\text{--}10.0$	$5.2\text{--}6.5 \times 2.2\text{--}3.0$
<i>M. dermatis</i> (Halder et al. 1981) Gupta and Khera 1988	<i>L. rohita</i>	Scales	West Bengal Kerala (India)	10.3×9.4	4.4×2.2
<i>M. haldari</i> Gupta and Khera 1989	<i>L. rohita</i>	Fin, gills	Punjab (India)	9.31×7.95	4.31×2.97 and 2.95×1.98
<i>M. cartilaginis</i> (Hoffman et al. 1965) Landsberg and Lom 1991	<i>Lepomis macrochirus</i>	Head cartilage	USA	10.2×8.9	5.3×3.3
<i>M. serrata</i> Pagarkar and Das 1993	<i>Cyprinus carpio</i>	Gill arch	West Bengal (India)	9.07×8.5	4.47×2.6
<i>M. kwangsiensis</i> Hsieh et al. 1993	<i>L. rohita</i>	Gills, kidney	China	10.9×6.9	5.5×2.9
<i>M. nkolyaensis</i> Fomena and Bouix 1994	<i>Barbus jae</i>	Caudal muscles	Cameroon	9.0×8.3	4.4×3.0
<i>M. edellae</i> Sarkar 1999	<i>Ctenopharyngodon idella</i>	Kidney	West Bengal (India)	10.7×9.4	6.14×3.12
<i>M. shantipuri</i> Basu and Halder 2002	<i>Catla catla</i> $\times$ <i>L. rohita</i>	Gill lamellae	West Bengal (India)	7.3×5.8	4.0×2.4
<i>M. tingrelaensis</i> Bounou et al. 2006	<i>Sarotherodon galilaeus</i>	Among the rays of fin	West Africa	11.6×9.37	4.80×2.40
<i>M. chilkenis</i> (Kalavati et al. 1992) Kalavati and Nandi 2007	<i>L. rohita</i>	Gall bladder	Orissa (India)	$7.2\text{--}8.0$ (7.77) $\times$ $5.6\text{--}6.6$ (6.2)	$3.2\text{--}4.8$ (4.2) $\times$ $1.8\text{--}2.2$ (1.98) and $1.0\text{--}1.2$ (1.08)
<i>M. sushmii</i> Kaur and Singh 2010/2011	<i>L. rohita</i>	Eye ball	Hariki wetland, Punjab (India)	$9.0\text{--}10.2$ (9.6) $\times$ $7.3\text{--}9.3$ (8.3)	$4.1\text{--}5.3$ (4.70) $\times$ $2.9\text{--}3.5$ (3.20) and $2.9\text{--}3.4$ (3.10) $\times$ $1.0\text{--}3.0$ (2.0)

Table 2 continued

Species	Host	Site of infection	Locality	Spore	Polar capsule
<i>M. punjabii</i> Kaur and Singh 2010/2011	<i>L. rohita</i>	Caudal fin	Kanjali Wetland Punjab (India)	8.1–9.7 (8.9) × 6.4–7.5 (6.9)	3.4–5.4 (4.4) × 2–3.4 (2.7) and 2.8–3.6 (3.2) × 1.2–2.2 (1.7)
<i>M. patialensis</i> Kaur and Singh 2011e	<i>L. rohita</i>	Caudal fin	Ropar wetland, Punjab (India)	11.28 × 6.67	4.8 × 3.1 and 1.70 × 1.51

Fig. 3 **a** Spore stained in Ziehl-Neelsen (valvular view). **b** Spore stained in iron-haematoxylin. Scale bar 0.005 mm **Fig. 4** **a** Spore stained in Ziehl-Neelsen. **b** Spore stained in iron-haematoxylin

Discussion

The present species was compared with *M. aligarhensis*, *M. mathuri*, *M. hosadurgensis*, *M. amieti*, *M. attui*, *M. bhadurius*, *M. trichogasteri*, *M. scatophagi*, *M. variiformis*, *M. orissae* and *M. dhanachandi* but differ from all

Fig. 5 **a** Fresh spore of *M. saugati* sp. nov. **b** Fresh spores of *M. szekeli* sp. nov.

Table 3 Measurements (in μm) and ratio of *M. szekeli* sp. nov.

Characters	Range	Mean values	SD
LS	8.4–9.0	8.7	0.42
WS	3.9–4.3	4.1	0.28
LPC	3.9–4.9	4.4	0.70
WPC	1.0–2.0	1.5	0.70
Ratio: LS/WS		2.1	
ICP		Absent	
NC		5–6	
Parietal folds		Absent	

of the above species in morphometric characteristics (Table 4).

The shape of the present species is elongately ovoidal with tapering, blunt anterior end and rounded posterior end. In this respect, the present species can be compared

Table 4 Comparative description of *M. szekeli* sp. nov. with morphologically similar species (measurements in micrometer)

Species	Host	Site of infection	Locality	Spore	Polar capsule
<i>M. szekeli</i> sp. nov. (present study)	<i>Wallago attu</i>	Internal wall of the stomach	Harike wetland, Punjab (India)	8.7×4.1	4.4×1.5
<i>M. aligarhensis</i> Bhatt and Siddiqui 1964	<i>Channa punctatus</i>	Accessory respiratory membrane, pharyngeal epithelium, fin	West Bengal (India)	$12.0\text{--}14.0 \times 6.0\text{--}7.5$	$6.5\text{--}8.0 \times 2.0\text{--}2.5$ and $6.0\text{--}7.0 \times 2.5$
<i>M. hosadurgensis</i> Seenappa and Manohar 1981	<i>Cirrhitina mirigala</i>	Gill, muscles	Karnataka (India)	10.5×6.25	5.37×2.3 and 3.3×1.43
<i>M. mathuri</i> Jayasri et al. 1981	<i>Puntius sarana</i>	Gills	Rajasthan (India)	$8.7\text{--}23.5 \times 5.1\text{--}10.1$	$2.7\text{--}11.9 \times 1.8\text{--}4.6$ and $2.7\text{--}7.8 \times 1.8\text{--}4.6$
<i>M. anietii</i> Fomena et al. 1985	<i>Ctenopoma nanum</i>	Spleen, eye	Cameroon	14.0×7.4	8.4×1.9
<i>M. attui</i> Sarkar 1985a	<i>W. attu</i>	Gut	West Bengal (India)	13.89×8.53	5.92×3.0
<i>M. bhadurius</i> (Sarkar 1985a) Gupta and Khara 1988	<i>W. attu</i>	Gall bladder	West Bengal (India)	10.59×6.28	5.31×2.78
<i>M. trichogasteri</i> (Sarkar 1985b) Gupta and Khara 1988	<i>Trichogaster fasciatus</i>	Gall bladder	West Bengal (India)	15.55×9.35	10.1×3.32
<i>M. scatophagi</i> Haldar et al. 1996	<i>Scatophagus argus</i>	Gills	Orissa (India)	14.0×5.3	7.13×2.36
<i>M. variformis</i> Haldar et al. 1996	<i>Mystus gulio</i>	Body muscles, gills	Orissa (India)	15.2×5.65	9.67×2.84 and 8.6×2.28
<i>M. orissae</i> Haldar et al. 1996	<i>C. mirigala</i>	Gills	Orissa (India)	15.71×6.8	8.8×1.78 and 7.58×2.57
<i>M. dhanachandi</i> Bandyopadhyay et al. 2007	<i>Channa orientalis</i>	Dorsal, ventral and caudal fins	Manipur (India)	18.4×6.05	8.26×1.43

with *M. aligarhensis*, *M. hosadurgensis* and *M. amieti* in having spores with blunt anterior end as spores in rest of other species are pointed anteriorly. The species under study was also closely compared with *M. dhanachandi* on the basis of morphological similarities. But spores in *M. dhanachandi* are dumb-bell shaped, broader at the middle and tapering at both ends. Polar capsules are two, equal, tubular with blunt anterior but rounded posterior end and occupies nearly half of the spore body cavity in species under study. Unequal polar capsule in *M. aligarhensis* and *M. hosadurgensis*, very elongate polar capsules reaching two-third of the spore length in *M. amieti* and tear shaped polar capsules with anteriorly sharply pointed ends in *M. dhanachandi* differentiate them from the present species under consideration. In addition, both polar capsules in the present species converge anteriorly but are placed at a distance posteriorly in contrast to almost parallel polar capsules in *M. dhanachandi*.

In view of the above differences, the present species under study is proposed as new to the science and named as *M. szekeli* sp. nov. through this communication.

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