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Two New Species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Bivalvulida) from Food Fishes of West Bengal, India – a Light and Scanning Electron Microscopy Study

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Summary. Two new myxozoan species – *Myxobolus analfinus* sp. n. and *Myxobolus debsantus* sp. n. are described from *Heteropneustes fossilis* (Bloch) and Catla-Rohu hybrid carp [Male parent fish *Catla catla* (Hamilton-Buchanan) × Female parent fish *Labeo rohita* (Hamilton-Buchanan)], respectively. Spores of *Myxobolus analfinus* are oval with slightly acuminate anterior end and large prominent intercapsular notch. On the other hand, in *Myxobolus debsantus* spores are spherical to oval with intercapsular notch and posterior sutural markings. In both the myxobolid species polar capsules are unequal. The detailed light microscopic and SEM structures and measurements of these two myxozoans are given.

Key words: *Myxobolus analfinus* sp. n., *Myxobolus debsantus* sp. n., Myxozoa, India.

Abbreviations: SP – spore; LS – length of spore; WS – width of spore; LPC – larger polar capsule; SPC – smaller polar capsule; LLPC – length of larger polar capsule; WLPC – width of larger polar capsule; LSPC – length of smaller polar capsule; WSPC – width of smaller polar capsule.

INTRODUCTION

The genus *Myxobolus* Bütschli, 1882 is a member of the Class Myxosporea in the Phylum Myxozoa. Since the establishment of the genus in 1882 different workers from various parts of the world have described several species from freshwater and marine (mostly estuarine) fishes under the genus *Myxobolus*. Landsberg and Lom (1991) listed 444 valid species of *Myxobolus* in fishes. Kalavati and Nandi (2007) reported the existence of 104 myxobolid species from Indian fishes. In a recent communication Eiras, Molnár and Lu (2005) presented a synopsis of 744 nominal species of *Myxobolus* Bütschli, 1882.

This paper records two new species of *Myxobolus* Bütschli, 1882, viz., *Myxobolus analfinus* sp. n. from *Heteropneustes fossilis* (Bloch) and *Myxobolus debsantus* sp. n. from Catla-Rohu hybrid carp [Male parent fish *Catla catla* (Hamilton-Buchanan) × Female parent fish *Labeo rohita* (Hamilton-Buchanan)]. Light Microscope as well as Scanning Electron Microscope resolves the detailed structures of these myxozoans.

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In recent years, considerable interest has been developed in the cultivation of hybrid carps in fresh water ponds for getting better fish yield for human consumption (Basu and Haldar 1998). Bauer *et al*. (1981) were of the view that Polyculture of pure and hybrid fishes has influenced the parasitological situation of fish rearing ponds resulting in eruption of infections and diseases in hybrid carps which have been already known for the pure carps. Basu and Haldar (2003) reported that hybrid carps are more susceptible to myxozoan infestation and in many instances, these myxozoans prove to be highly pathogenic, leading to heavy mortality.

The preparations of species description of the present myxozoans have been done in accordance with the guidelines of Lom and Arthur (1989) and Lom and Dyková (1992).

MATERIALS AND METHODS

A. Light Microscopy

The host fishes, weighing an average of 16.8 g and measuring 10.1 cm, were collected alive from local fish markets in Bandel and Bally in April, 2001. Fishes were returned to the laboratory and were immediately examined thoroughly for their myxozoan parasites. Plasmodia, when found, were carefully removed with the help of a sterile forceps, smeared on clean grease-free slides with drops of 0.5% NaCl solution, covered with thin cover-glasses and properly sealed for examination under the oil immersion lens of Olympus CH-2 phase contrast microscope. Some of the fresh smears were treated with various concentrations (2–10%) of KOH solution for the extrusion of polar filaments. The India ink method of Lom and Vavrá (1963) was employed for observing the mucus envelope of spores. For permanent preparations, air-dried smears were stained with Giemsa after fixation in acetone-free absolute methanol. Measurements (based on twenty fresh spores treated with Lugol's iodine) were taken with the aid of a calibrated ocular micrometer. All measurements are presented in micrometers as mean ± SD followed in parentheses by the range. Drawings were made on fresh/stained materials with the aid of a Camera Lucida (Mirror type) and computer programme Corel Draw 11.0.

B. Scanning Electron Microscopy [SEM]

Plasmodia enclosed within the host tissues were isolated with the help of a sterile forceps and placed on thin cover glass with drops of 0.5% normal saline solution. These were then ruptured and their membranes removed with the tip of a needle. Utmost care was taken to remove the host tissue.

Spores were fixed in cold (0–5°C temperature) 4% 0.1 M phosphate buffered Glutaraldehyde (pH 7.4) for 4 hours, and post fixed in 1% buffered Osmium tetroxide (OsO_4) for 2 hours at room temperature. Following dehydration in a standard Ethanol gradient the samples were transferred to a graded 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.

The samples were then dried at critical point using CO_2 in a HCP: 2 Critical Point Dryer (Hitachi), mounted on aluminum stubs and finally sputter coated with 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 AND DISCUSSION

Myxobolus analfinus (Figs 1–3, 7–10)

Plasmodia

Round (diameter 225) to oval (250 × 200) white 'cyst' like plasmodia, developed in the anal fin of infected host fishes, contain very few late developmental stages and many mature spores (Figs 1–3).

Spore

Light Microscopy

Mature spores are 12.3 ± 0.77 (11.1–13.4) × 8.6 ± 0.42 (7.8–9.3) × 6.2 ± 0.53 (6.0–6.5), oval with slightly acuminated anterior end in valvular view (Figs 1, 3). The posterior end is broadly rounded. Two thick shell valves form prominent sutural ridge (Fig. 2). Sutural line is indistinct. Spores are lenticular to pyriform in sutural view (Fig. 4). At the anterior extremity an intercapsular notch is visible (Figs 1, 3).

Polar capsules are unequal in shape and size. Larger polar capsule is 4.1 ± 0.44 (3.2–4.9) × 2.2 ± 0.13 (2.0–2.4), oval or ovoidal in shape with 5–7 loose coils of polar filament (Figs 1, 3). A knob like structure is present at the anterior-most part of the larger polar capsule (Figs 1, 3). The smaller polar capsule, measuring 2.5 ± 0.34 (2.0–3.1) × 1.8 ± 0.14 (1.6–2.0), is oval or in some cases pyriform in shape with a very short neck (Figs 1, 3). Polar filament makes 3–4 spiral coils inside smaller polar capsule.

The extracapsular space is filled with granular homogenous mass of sporoplasm, which contains a large, spherical iodinophilous vacuole of 2.2 ± 0.13 (2.0–2.4) diameter (Fig. 1) and two sporoplasmic nuclei measuring 1.2 ± 0.16 (1.0–1.5) in diameters (Fig. 3). A thin mucus envelope surrounds each spore as revealed by India ink technique of Lom and Vavrá (1963).

SEM

Some features of the spores were more clearly resolved by SEM. Sutural line is slightly wavy (Fig. 8).

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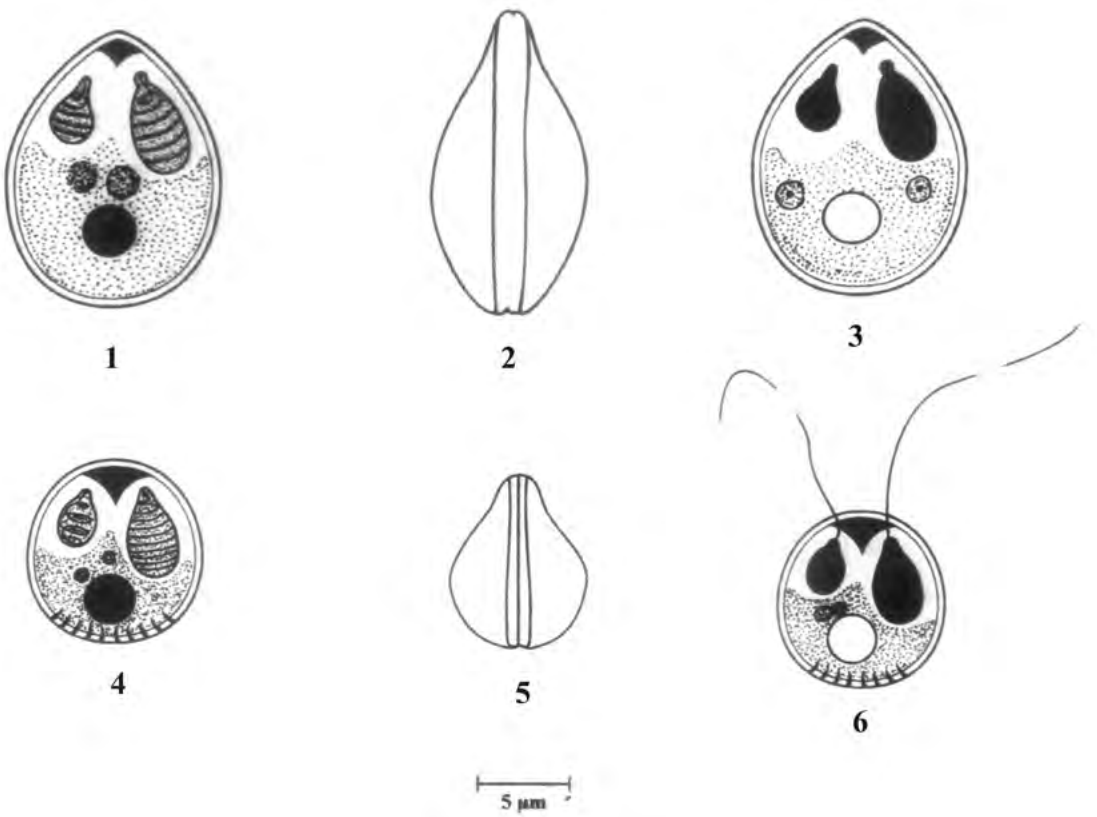

Figs 1–6. Camera lucida drawings of spores. **1–3** – *Myxobolus analfinus* sp. n.; **1** – fresh spore in valvular view; **2** – fresh spore in sutural view; **3** – fixed spore in valvular view, **4–6** – *Myxobolus debsantus* sp. n.; **4** – fresh spore in valvular view; **5** – fresh spore in sutural view; **6** – fixed spore in valvular view with extruded polar filaments. Lugol's iodine: 1–2, 4–5, Giemsa – 3, 6. Scale bar: 5 µm.

Mucus envelope around each spore is very much thick (Fig. 7). Anteriorly, the spore body shows a slit-like opening in the discharge channel of the polar filaments, located in a triangular thickening of the sutural ridge (Figs 8–9). In some cases fixation procedure for SEM may cause spores to break open and the polar capsules with extruded polar filaments are seen (Fig. 10).

Spore index

LS: WS = 1:0.699
LLPC: WLPC = 1:0.537
LSPC: WSPC = 1:0.72
LLPC: LSPC = 1:0.610
WLPC: WSPC = 1:0.81

Taxonomic affinities

The present myxozoan species resembles *M. chakravartyi* Haldar *et al.*, 1983 reported from internal eye musculature of *Catla catla* (SP: 12.3 × 7.7–10.5); *M. mahendrae* Sarkar, 1986 reported from gills of *Catla catla* (SP: 12.7 × 9.2); *M. anomaliformis* Chen in Chen and Ma, 1998 reported from gills of *Abbottina rivularis* (SP: 11.7 × 8.3); *M. chuantungensis* Ma, 1998 reported from kidney, urinary bladder, ureter of *Varicorhinus simus* (SP: 12.3 × 8.0); *M. chuchowensis* Chen in Chen and Ma, 1998 reported from urinary bladder, gills, kidney of *Aristichthys nobilis* (SP: 12.0 × 8.7) in the morphometry of spores. However, larger polar capsule (7.1 × 2.0) of *M. chuantungensis* is different in morphometry from that of the present one. Further, polar capsules of *M. anomaliformis* (LPC: 5.5 × 3.3, SPC: 4.8 × 2.8) and *M. chuchowensis* (LPC: 5.5 × 3.4, SPC: 4.9 × 2.9) are larger than the species under consideration. Moreover, the ovoidal spore of *M. chakravartyi* and cylindrobiconical spore of *M. mahendrae* possess a pair of unequal polar capsules but the dimensions of which (in *M. chakravartyi* LPC: 5.5–6.6 × 3.3–5.0, SPC: 4.4–5.0 × 2.2–4.4 and

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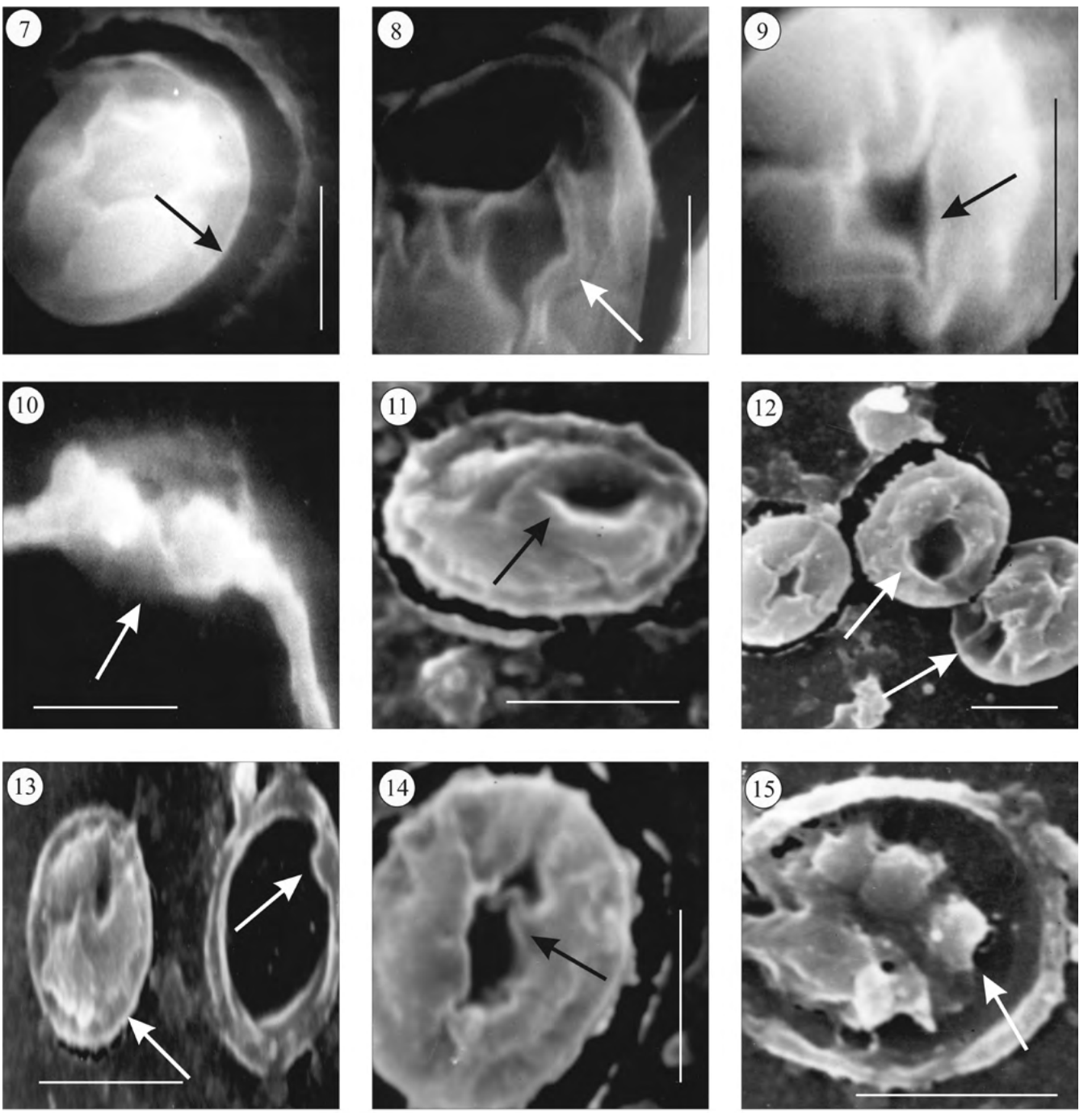

Figs 7–15. Scanning Electron Micrographs of spores. **7–10** – *Myxobolus analfinus* sp. n.; **7** – spore in valvular view; **8** – spore showing wavy sutural line; **9** – spore showing polar filament discharge channel located in a triangular thickening of the sutural ridge; **10** – polar capsules with extruded polar filaments. **11–15** – *Myxobolus debsantus* sp. n.; **11** – spore; **12** – spores showing polar filament discharge channels; **13** – spore showing triangular knotch/thickenings on the inner valve surface and intercapsular spines; **14** – single spore showing polar filament discharge channels; **15** – frontoapical view of spore showing mushroom-like buttons protruding from shell surface. Respective characters are marked by arrow. Scale bar: 5 µm.

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in *M. mahendrae* LPC: 6.9 × 3.7, SPC: 5.4 × 3.4) are much larger and wider than that of present species with slightly pyriform spore.

Spores of *M. aligarhensis* Bhatt and Siddiqui, 1964 reported from accessory respiratory membrane, pharyngeal epithelium, fins of *Channa punctatus* and *M. acinosus* Nie and Li, 1992 reported from gills of *Cyprinus carpio haematopterus* show similarity with the present species by identical length range (LS in *M. aligarhensis* and *M. acinosus* are 12.0–14.0 and 12.6, respectively) and inequality of polar capsules. Although both the spores are shorter (WS in *M. aligarhensis* and *M. acinosus* are 6.0–7.5 and 6.4, respectively) and the morphometry of polar capsules are also different (in *M. aligarhensis* LPC: 6.5–8.0 × 2.0–2.5, SPC: 6.0–7.0 × 2.5; in *M. acinosus* LPC: 5.3 × 3.8, SPC: 2.6 × 1.2).

Dimensions of unequal polar capsules of *M. montanus* Azhurova and Pugachev, 1988 reported from gills of *Schizopygopsis stolitzkai* (LPC: 5.2–6.6 × 2.9–3.9, SPC: 3.3–4.1 × 1.7–2.2) and *M. wuhanensis* Chen in Chen and Ma, 1998 reported from kidneys, gall bladder of *Carassius auratus auratus* (LPC: 6.5 × 3.6, SPC: 5.5 × 2.7) are different from the present species under study.

Furthermore, width of spore of *M. ophthalmusculata* Basu and Haldar, 2002 reported from eye muscle of *Cirrhinus mrigala* (WS: 8.0); shows closer dimensions with that of the present species. But unequal polar capsules of *M. ophthalmusculata* with larger dimensions (LPC: 5.5 × 3.1, SPC: 3.0 × 1.9) and undulated sutural ridge make sufficient differences with the present species.

The oval spore with intercapsular notch of *M. bhadrensis* Seenappa and Manohar, 1981 reported from muscle of *Labeo rohita*, although, shows identical morphometry in the dimensions of polar capsules (LPC: 3.5 × 2.2, SPC: 2.5 × 1.8) but is smaller (SP: 9.5 × 7.1) than that of the pyriform spore of the present species under discussion.

M. mrigalhitae Basu and Haldar, 2003 reported from gills of *Cirrhinus mrigala* × *Labeo rohita* carp shows resemblance in the morphometry of polar capsules (LPC: 4.8 × 2.1, SPC: 3.0 × 2.1). However, larger dimension of spore (spore dimension in *M. mrigalhitae* 10.8 × 7.9), absence of 2–5 parietal folds and broad sutural ridge make clear-cut differences for the species under consideration.

Finally, *M. buccoroofus* Basu and Haldar, 2004 reported from roof of the buccal cavity of *Labeo bata* is very much similar to the present species in overall morphometry of spore (12.1 × 7.1) and polar capsules (LPC: 4.9 × 2.9, SPC: 2.5 × 1.5). But in the former one anterior end of the spore is narrow and bends on one side, sutural line undulating and sutural ridge is indistinct in comparison to straight anterior end, indistinct sutural line but distinct sutural ridge of the present species. Moreover, intercapsular notch is absent in *M. buccoroofus,* which is large and prominent in the species under study.

Considering the differences with the related species, the present myxozoan is regarded as a new species and we designate this as ***Myxobolus analfinus*** sp. n. in this communication.

Taxonomic summary

Family: Myxobolidae Thélohan, 1892.

Genus: *Myxobolu*s Bütschli, 1882.

Type-host: *Heteropneustes fossilis* (Bloch).

Type-locality: Bandel (Latitude: 22º54´N, Longitude: 88º24´E), Hooghly, West Bengal, India.

Type-specimens: Paratypes are spores stained in Giemsa, in the collection of Harold W. Manter Laboratory of Parasitology, University of Nebraska, USA, No. HWML 16709.

Site of infection: Anal fin.

Prevalence and intensity of infection: 26/68 (38.23%).

Etymology: The species epithet *analfinus* is proposed after its site of infection.

***Myxobolus debsantus* (Figs 4–6, 11–15)**

Plasmodia

Yellowish, elongately oval plasmodia are attached to the tail fin and contain mainly the mature spores. Although in a very few cases it shows the presence of late developmental stages.

Spore

Light microscopy

The spores are small, 9.0 ± 0.29 (8.5–9.6) × 8.4 ± 0.24 (8.1–8.9) × 6.3 ± 0.38 (6.2–6.7) in measurement, spherical to oval in valvular view (Figs 4, 6) but broadly lenticular in sutural view (Fig. 5). The suture is broad and straight (Fig. 5). The two shell-valves are symmetrical and thick-walled with sutural markings at the posterior end (Figs 4, 6).

Two polar capsules are distinctly unequal and situated almost parallel to each other (Figs 4, 6). A nipple or knob like structure is present at the anterior most part of each polar capsule (Figs 4, 6). The larger polar cap-

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sule is elongately oval with a measurement of 4.3 ± 0.17 (4.0–4.6) × 2.3 ± 0.18 (2.0–2.6) and 9–10 tightly coiled spiral polar filament (Fig. 4). The smaller polar capsule is 2.8 ± 0.09 (2.6–2.9) × 1.8 ± 0.09 (1.6–1.9), spherical to oval in shape with 4–5 loose spirally coiled polar filament (Fig. 4). Polar filaments from both the polar capsules are extruded through the anterior end of the spore (Fig. 6). A distinct, pointed intercapsular appendix is observed which usually extends not beyond the anterior 1/3rd of the two polar capsules (Figs 4, 6).

The finely granular sporoplasm fills the posterior part of the spore cavity. The sporoplasm is crescentic with a median intercapsular projection (Figs 4, 6). The iodinophilous vacuole, 2.5 ± 0.11 (2.3–2.7) in diameter, is large and spherical (Fig. 4); sporoplasmic nuclei are round to oval in shape, having a diameter of 1.1 ± 0.08 (1.0–1.2) (Fig. 6), sometimes the nuclei are invisible. The mucus envelope is absent.

SEM

SEM study shows triangular notches and/or thickenings (typically 8 to 10) occurring on the inner valve surface throughout the spore body (Fig. 13). A few open valves with intercapsular spines are noticed by SEM (Fig. 13). Two slit like openings – one large (approx. 2.1 × 1.3) and other small (approx. 0.8 long) of the polar capsule's discharge channel are present in a triangular thickening in two spore valves (Fig. 14). Some spores in fronto-apical view show mushroom-like buttons protruding from shell surface (Fig. 15).

Spore index

LS: WS = 1:0.933
LLPC: WLPC = 1:0.535
LSPC: WSPC = 1:0.643
LLPC: LSPC = 1:0.651
WLPC: WSPC = 1:0.782

Taxonomic affinities

The present myxozoan species belonging to the genus *Myxobolus* Bütschli, 1882 resembles either in shape or in size with *M. curmucae* Seenappa and Manohar, 1980 reported from below the scales of *Puntius curmuca*; *M. dossoui* Sakiti *et al.*,1991 reported from gill arch cartilages of *Tilapia zillii*; *M. haldari* Gupta and Khera, 1989 reported from fins, gills of *Cirrhinus mrigala*; *M. indicus* Tripathi, 1952 reported from muscles, liver, intestinal wall of *Cirrhinus mrigala*; *M. labeosus* Sarkar, 1995 reported from mesentery of *Labeo fimbriatus*; *M. lalithae* Gupta and Khera, 1988 reported from gills of *Labeo calbasu*; *M. liangshanensis* Ma and Jhao, 1993 reported from kidney, liver of *Garra pingi pingi*; *M. magaddi* (Bajpai *et al.* 1981) Landsberg and Lom, 1991 reported from branchial filaments of *Trichogaster fasciatus*; *M. mrigalae* Chakravarty, 1939 reported from scale of *Cirrhinus mrigala*; *M. niei* Shulman, 1962 reported from skin of *Percottus glehni*; *M. oloi* Fomena and Bouix, 1994 reported from gill arch epithelium, gullet of *Barbus aspilus*; *M. pinnarauti* Lalitha Kumari, 1969 reported from gill filaments of *Barbus pinnauratus*; *M. polymorphum* Ma and Jhao, 1993 reported from gills of *Schizothorax prenanti*; *M. psilorhynchi* Lalitha Kumari, 1969 reported from branchial filaments of *Psilorhynchus balitora*; and *M. seshadri* Lalitha Kumari, 1969 reported from gills of *Labeo fimbriatus.*

Among these myxobolid species, spore dimensions are either larger and wider/narrower in *M. curmucae* (9.8 × 7.6), *M. dossoui* (9.9 × 9.2), *M. indicus* (9.5–10.8 × 7.5–8.2), *M. lalithae* (10.0 × 8.4), *M. magaddi* (11.2 × 9.2), *M. niei* (9.8 × 9.5), *M. pinnarauti* (8.0–11.4 × 6.5–7.9), *M. psilorhynchi* (10.0 × 9.4), *M. seshadri* (12.2 × 9.0) or smaller and narrower in *M. mrigalae* (7.2–8.2 × 6.1).

Further spores of *M. labeosus*, *M. liangshanensis*, *M. oloi* and *M. polymorphum* closely resemble the species under study in the spore morphometry. However, larger dimensions of polar capsules in the former species (dimensions of LPC and SPC in *M. labeosus*, *M. liangshanensis*, *M. oloi* and *M. polymorphum* are 6.1 × 2.7 and 4.0 × 2.3; 5.0 × 2.8 and 4.0 × 2.2; 5.7 × 3.1 and 3.9 × 2.0; 6.1 × 2.8, respectively) make sufficient differences from those of the present species under consideration.

Lastly, the spore of *M. haldari* agrees well in detail morphology and morphometry with the spore of presently discussed species. However, the oblong to oval spores (elliptical in sutural view) having broad sutural ridge, no sutural markings, and small and distinct neck in polar capsules are not found in the spherical to oval spores (broadly lenticular in sutural view) of the present species. Moreover, presence of a nipple or knob like structure at the anterior most part of each polar capsule, sutural markings at the posterior end of spore, crescentic sporoplasm with median intercapsular projection and mushroom-like buttons protruding from shell surface make the present species distinct from the former one.

In view of the differences with the closely related species it is proposed to establish a new species for the

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present myxozoan and the name ***Myxobolus debsantus*** sp. n. is assigned to it in this communication.

Taxonomic summary

Family: Myxobolidae Thélohan, 1892.

Genus: *Myxobolus* Bütschli, 1882.

Type-host: Catla-Rohu hybrid carp [Male parent fish *Catla catla* (Hamilton-Buchanan) × Female parent fish *Labeo rohita* (Hamilton-Buchanan)].

Type-locality: Bally (Latitude: 22º39´N, Longitude: 88º23´E), Howrah, West Bengal, India.

Type-specimens: Paratypes are spores stained in Giemsa, in the collection of Harold W. Manter Laboratory of Parasitology, University of Nebraska, USA**,** No. HWML 16710.

Site of infection: Tail fin.

Prevalence and intensity of infection: 14/55 (25.45%).

Etymology: The species epithet *debsantus* is proposed after Sri Deb Kumar Basu and Smt Santi Lata Basu, father and mother of the senior author, who are constant source of encouragement for him.

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