

Description of Three New Myxosporean Species (Myxozoa: Myxosporea: Bivalvulida) of the Genera *Myxobilatus* Davis, 1944 and *Myxobolus* Bütschli, 1882

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Summary. The present communication describes three new species of myxosporeans (Myxozoa: Myxosporea: Bivalvulida), *Myxobilatus odontamblyopusi* sp. n., *Myxobolus catmrigalae* sp. n. and *M. buccoroofus* sp. n. from *Odontamblyopus rubicundus* (Hamilton-Buchanan), Catla-Mrigal hybrid carp [male parent fish *Catla catla* (Hamilton-Buchanan) × female parent fish *Cirrhinus mrigala* (Hamilton-Buchanan)] and *Labeo bata* (Hamilton-Buchanan) of West Bengal, India, respectively. Spores of the new myxobolid species are resolved by LM and SEM.

Key words: *Myxobilatus odontamblyopusi* sp. n., *Myxobolus buccoroofus* sp. n., *M. catmrigalae* sp. n., Myxozoa, India.

Abbreviations: LCP - length of caudal filament, LP - length of polar capsule, LPC - larger polar capsule, LS - length of spore, LSCP - length of spore with caudal prolongation, PC - polar capsule, SP - spore, SPC - smaller polar capsule, WP - width of polar capsule, WS - width of spore.

INTRODUCTION

Myxobilatus odontamblyopusi sp. n., *Myxobolus catmrigalae* sp. n. and *M. buccoroofus* sp. n. from *Odontamblyopus rubicundus* (Hamilton-Buchanan), Catla-Mrigal hybrid carp [male parent fish *Catla catla* (Hamilton-Buchanan) × female parent fish *Cirrhinus mrigala* (Hamilton-Buchanan)] and *Labeo bata* (Hamilton-Buchanan) of West Bengal, India, respec-

tively, are described in the present study. The descriptions of these three myxosporeans are in accordance with the guidelines of Lom and Arthur (1989) and Lom and Dyková (1992).

MATERIALS AND METHODS

Host fishes (total number of host fishes examined are mentioned separately in taxonomic summary) were collected alive from the local fish markets (type locality mentioned separately in taxonomic summary) and brought to the laboratory and necropsied immediately. Sporogonic plasmodia, when found, were carefully removed with sterile forceps, smeared on clean grease-free slides with drops of 0.5% NaCl solution, covered with cover slips and sealed with bee's

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wax 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 mucous 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 determined with aid of a calibrated ocular micrometer. All measurements are presented in μm as mean \pm 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 9.0. Spore surface was visualized by means of SEM.

RESULTS AND DISCUSSION

Myxobilatus odontamblyopusi sp. n. (Figs 1-8)

Plasmodia: Sporogonic plasmodia encased within hosts' cells appearing as 'cysts' are yellowish-white in colour, elongately ellipsoidal in shape and measure 0.77-1.56 mm \times 0.66-1.44 mm. They are attached to almost all the gill filaments in heavily infected fishes.

In the early developmental stages the uninucleate pansporoblasts are scanty. Uninucleate stages (Fig. 1) range from 2.5 to 3.4 in diameter. The nuclei are karyosomatic. With Giemsa the karyosome appears as a small central or eccentric body staining deep red. Binucleate forms (Fig. 2) have a large and a small nuclei and measure 3.7 by 2.9. Tetranucleate ones (Fig. 3), ranging from 5.7 to 6.8 in diameter, possess two large and two small nuclei. Spore forming pansporoblasts (Fig. 4) measure 16 by 12. These have twelve karyosomatic generative nuclei and four somatic residual nuclei which are not, however, always apparent. Developing spores are aligned either posterior end to anterior end or anterior end to anterior end in disporous pansporoblasts.

Spore: Spores are histozoic. Developing spores (Fig. 5) are round to oval in shape, are devoid of caudal filaments, have 3 or 4 filament coils with each polar capsule. Developed spores (Figs 6-8) are 9.0 ± 0.49 (8.1-10.3) \times 5.2 ± 0.41 (4.8-6.3) and are lanceolate in valvular view with pointed anterior and posterior ends (Figs 6, 8). The broadest biconvex point lies at or just anterior to posterior end of polar capsules. Spores are oval with one side convex and other flat as seen in sutural plane (Fig. 7). The spore body is divided into two nearly equal halves by straight sutural ridge (~ 0.4 in width) (Figs 6, 8). Spore valves are moderately thick. Surface of valves are with faint longitudinal striations (Fig. 7). The spore valves extend posteriorly into two

long [length 20.6 ± 1.85 (17.9-24.9)] equal tapering processes (Figs 6, 8) which are separate throughout the entire length, thereby clearly fulfilling the character of the genus *Myxobilatus* as proposed by Davis (1944). These caudal prolongations are transparent in fresh condition and take deep blue colour when stained in giemsa.

Two equal pyriform polar capsules [3.4 ± 0.29 (3.0-3.9) \times 2.1 ± 0.14 (1.9-2.5)] are situated in a plane perpendicular to that of suture (Figs 6, 8). In some cases almost completely extruded polar filaments have been seen (Fig. 8), in others the filaments form 5-6 spiral coils in each polar capsule (Fig. 6).

The granular homogenous mass of sporoplasm occupies the space between and behind the polar capsules but does not extend on the sides of the polar capsules (Figs 6, 8). Iodinophilous vacuole is absent. There are two oblong sporoplasmic nuclei [diameter 1.3 ± 0.12 (1.2-1.7)], lying side by side in the sporoplasm (Figs 6, 8).

Spore index:

LS: WS = 1: 0.578

LSCP: LS = 1: 0.436

LSCP: LCP = 1: 0.558

LP: WP = 1: 0.618

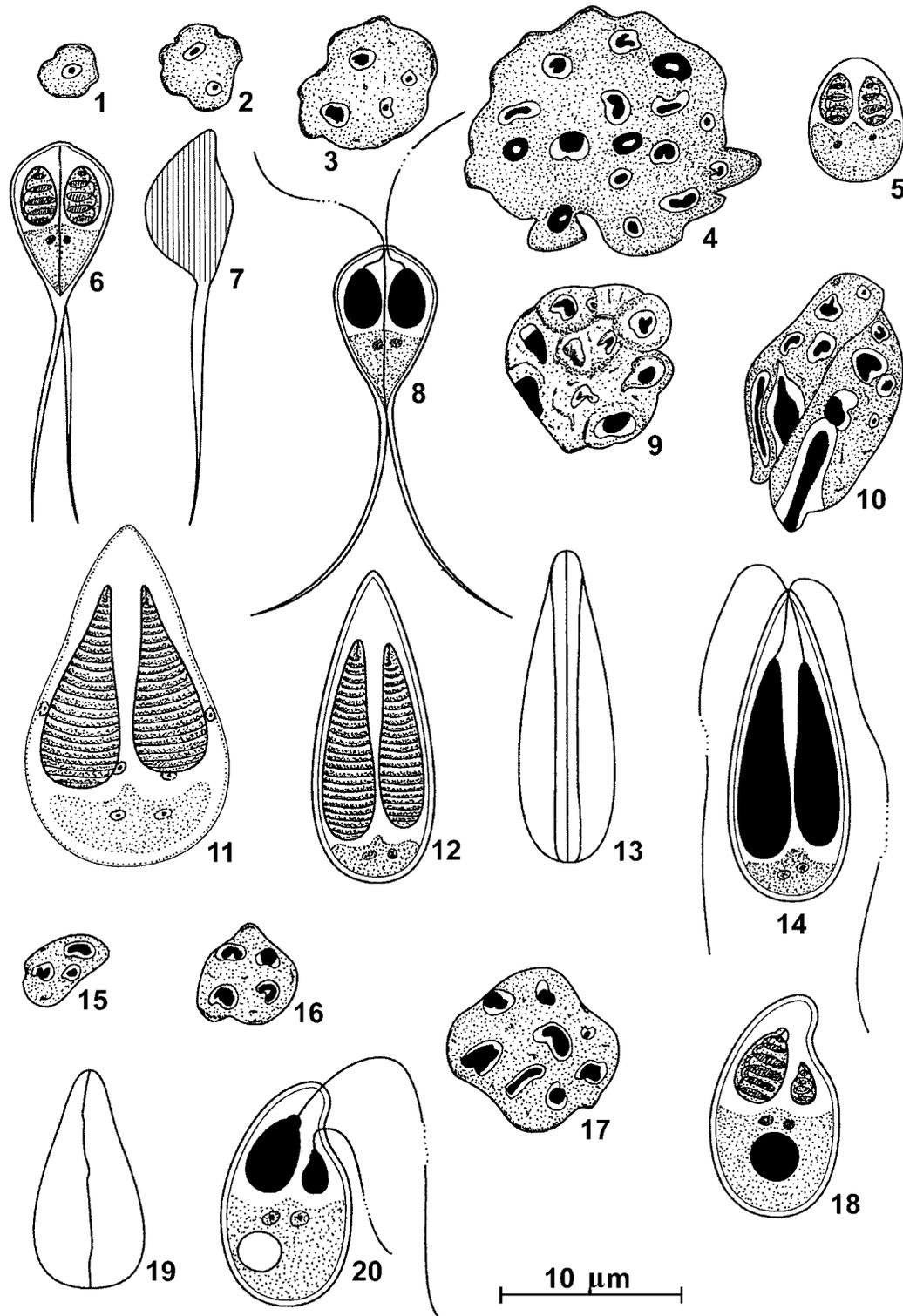
LS: LP = 1: 0.378

WS: WP = 1: 0.404

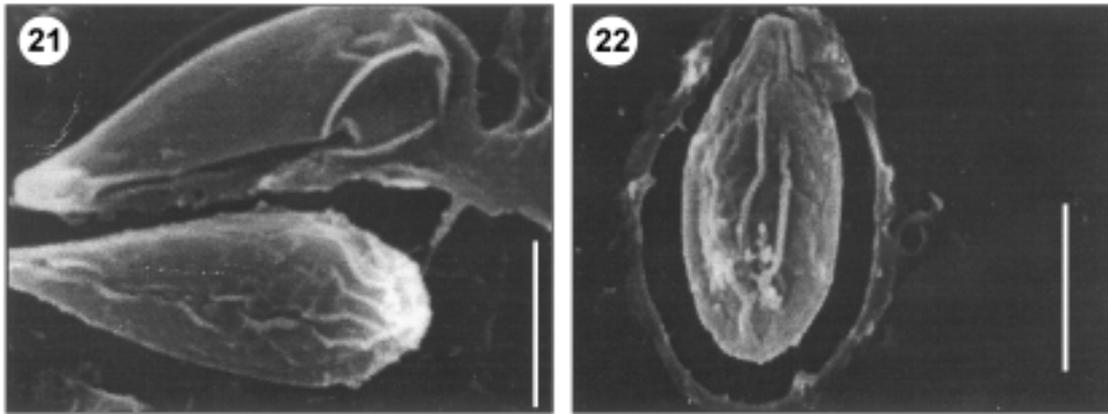
Taxonomic affinities: Davis (1944) has revised the genus *Henneguya* and proposed to divide it into three genera i.e. (1) *Henneguya*, (2) *Unicauda* and (3) *Myxobilatus*. In India, this is the third record of the genus *Myxobilatus* and the first from the gills of any estuarine fish. The other two species are *Myxobilatus mastacembeli* Qadri et Lalitha Kumari, 1965 from the intestine of the fresh water fish, *Mastacembelus armatus* (Lacépède) and *Myxobilatus fossilis* Susha et Janardanan, 1994 from the urinary bladder of *Heteropneustus fossilis* (Bloch).

The present species of *Myxobilatus* resembles, in size and/or in shape, *M. legeri* Cépède, 1905; *M. wisconsinensis* Mavor et Strasser, 1916; *M. sinipercae* Dogiel et Akhmerov, 1960; *M. pseudorasborae* Shulman, 1962; *M. varicorhini* Kandilov, 1963; *M. noturi* Guilford, 1965; *M. cotti* Guilford, 1965; and *M. yukonensis* Arthur et Margolis, 1975.

Of these *Myxobilatus* species the spores of *M. sinipercae* (LSCP: 45-50); *M. varicorhini* (LSCP: 28-31); *M. cotti* (LSCP: 30.0-98.4); *M. yukonensis* (LSCP: 16.7-36.2) are larger than the present species. Spores of *M. pseudorasborae* closely resemble the spores of present species in the spore length (length



Figs 1-20. Camera lucida drawings of plasmodia and spores. **1-8** - *Myxobilatus odontamblyopusi* sp. n.; **1-4** - pansporoblasts; **5** - fresh developing spore in valvular view; **6** - fresh developed spore in valvular view; **7** - fresh developed spore in sutural view; **8** - fixed developed spore in valvular view with extruded polar filaments. **9-14** - *Myxobolus catmrigalae* sp. n.; **9-10** - late developmental stages; **11** - fresh developing spore in valvular view; **12** - fresh developed spore in valvular view; **13** - fresh developed spore in sutural view; **14** - fixed developed spore in valvular view with extruded polar filaments. **15-20** - *Myxobolus buccoroofus* sp. n.; **15-17** - pansporoblasts; **18** - fresh developed spore in valvular view; **19** - fresh developed spore in sutural view; **20** - fixed developed spore in valvular view with extruded polar filaments. Lugol's iodine: 1-7, 9-13, 15-19; Giemsa: 8, 14, 20. Scale bar 10 μm.



Figs 21, 22. 21 - *Myxobolus catmrigalae* sp. n. spore; 22 - *Myxobolus buccoroofus* sp. n. spore. SEM. Scale bars 10 µm.

range in *M. pseudorasborae* is 9.8-12). But the caudal processes and polar capsule dimensions are much smaller in the latter species (in *M. pseudorasborae* caudal processes and polar capsule dimensions are 19.5-22.5 and 5.0-6.5 × 2.6 respectively). Polar capsule dimensions in *M. wisconsinensis* (3.5 × 2.5) closely resemble to that of present species under discussion. But the shape and dimensions of the spore of former species is different (spore dimension in *M. wisconsinensis* is 11.5 × 7.0). The spores of *M. legeri* (LSCP: 19.5-22.5, SP: 8.5-11 × 6-8, PC: 2.8-3.5 × 2.0) and *M. noturi* (LSCP: 18.6-28.0, SP: 7.2-10.8 × 6.0-7.2, PC: 4.2-5.4 × 2.5) show much similarity with the present species. However, spore width in both of the former two species is distinctly larger than the species under study. Furthermore, the oval spores of *M. legeri* with slightly tapered anterior end lack any valvular striations which are present in the lanceolate anteriorly pointed spores of the present species. Although spores of *M. noturi* have valvular striations, but the shape of the spore (oval with rounded anterior end) is strongly different from the species under discussion. Moreover, the present species is histozoic (all other species referred to above are coelozoic) and its site of infection is not associated with urinary tract which is typical of all species described above.

The present species is also compared with the first Indian described species of *Myxobilatus*, *M. mastacembeli* Qadri et Lalitha Kumari, 1965 described from a fresh water fish *Mastacembelus armatus* (Lacépèdes). The spore dimensions (SP: 8.5-12.5 × 4.6-6.2; LCP: 15-28) and the shape and dimensions of polar capsule

(PC: 3.1-3.9 × 1.5-1.9) of *M. mastacembeli* closely resemble the present species. But the flattened and elongated spores of *M. mastacembeli* with rounded anterior end is distinctly different from the present one (present spores are lanceolate with pointed anterior and posterior extremities). In addition, the valvular striations are lacking in the former species which is a taxonomically important character of *M. odontamblyopusi* sp. n. Furthermore, the host range, site of infection and the geographical location of the host are different.

According to Lom and Dyková (1992), histozoic myxobolus spp. may also be found as in the present instance although majority described species are coelozoic in nature.

After careful comparison with all species described so far, it is proposed to establish a new species for this myxozoan and the name *Myxobilatus odontamblyopusi* sp. n. is assigned to it in this communication.

Taxonomic summary

Type host: *Odontamblyopus rubicundus* (Hamilton-Buchanan). Host family: Gobioididae.

Type locality: Canning (Latitude: 22°20' N, Longitude: 88°40' E), 24 Parganas (South), West Bengal, India.

Type specimens: Paratypes are spores stained in Giemsa, in the collection of Harold W. Manter Laboratory of Parasitology, Nebraska, USA, No. HWML 16704.

Prevalence of infection: 44/254 (17.32 %).

Etymology: The species epithet *odontamblyopusi* is from the name of its type host *Odontamblyopus rubicundus* (Hamilton-Buchanan).

***Myxobolus catmrigalae* sp. n. (Figs 9-14, 21)**

Plasmodia: Fully developed creamy white coloured plasmodia appear on the gill lamellae as spherical 'cyst', contain few late developmental stages (Figs 9-10), developing spores (Fig. 11) and developed spores (Figs 12-14, 21).

Spore: Developing spores (Fig. 11) are elongately pyriform or large tear shaped bodies in valvular view with broadly rounded posterior end. These measure 20.4 ± 0.38 (20.0-21.8) \times 16.3 ± 0.29 (15.7-17.1). Two unequal polar capsules are also elongated and pyriform. The dimension of larger polar capsule is 14.7 ± 0.42 (12.9-15.3) \times 7.1 ± 0.25 (5.2-8.1) and that of smaller polar capsule is 11.8 ± 0.18 (10.2-12.7) \times 5.6 ± 0.31 (4.8-5.9). All the six nuclei (i.e., two each of valvogenic, capsular and sporoplasmic) are seen in Giemsa stain preparation.

Developed spores (Figs 12-14) are 18.8 ± 0.47 (17.8-19.5) \times 5.9 ± 0.16 (5.7-6.2) are elongated and pyriform in valvular view (Figs 12, 14) and lenticular in sutural view (Fig. 13) with a straight thin sutural line. The spore is bluntly pointed anteriorly with a rounded posterior end (Figs 12, 14). The two shell valves are smooth, symmetrical and thin-walled (Fig. 13). Two anteriorly situated pyriform polar capsules run parallel to each other but sometimes converge slightly towards the anterior end (Figs 12-14). One polar capsule is slightly larger [11.9 ± 0.25 (11.6-12.4) \times 2.5 ± 0.13 (2.2-2.7)] than the other [11.0 ± 0.16 (10.7-11.3) \times 2.3 ± 0.13 (2.0-2.5)] and they occupy almost 4/5th part of the spore cavity. Polar filaments make 22-25 and 21-23 coils in the larger and smaller polar capsules respectively (Fig. 12). When extruded, the polar filaments always cross at the tip of the spore (Fig. 14). Granular homogeneous binucleate sporoplasm fills the extracapsular space of the spore cavity. There is no iodophilous vacuole and mucus envelope around the spore is also absent.

The spore surface is smooth and devoid of mucous (Fig. 21). The sutural line, formed by the fused thick edges of each valve, is generally straight or slightly curved. Preparation technique apparently caused shrinkage of the valves along both sides of the sutural line.

Spore index:

LS: WS = 1: 0.314

LLPC: WLPC = 1: 0.210

LSPC: WSPC = 1: 0.209

LLPC: LSPC = 1: 0.924

WLPC: WSPC = 1: 0.92

Taxonomic affinities: The present myxozoan species resembles *Myxobolus* (= *Myxosoma*) *anurus* (Cohn, 1895) Lom *et* Noble, 1984 reported from gills and fins of *Esox* spp. and *Perca fluviatilis*; *M.* (= *M.*) *dujardini* (Thélohan, 1899) Lom *et* Noble, 1984 reported from the gill lamellae, fins, kidneys, gonads and pancreas of *Rutilus* spp., *Leuciscus* spp., *Abramis* spp. etc.; *M.* (= *M.*) *andhrae* (Lalitha Kumari, 1969) Lom *et* Noble, 1984 reported from the intestinal wall of *Ophicephalus punctatus*; *M. punctatus* Roychaudhuri *et* Chakravarty, 1970 reported from the spleen and pharyngeal epithelium of *Ophicephalus punctatus*; *M. bhadrensis* Seenappa *et* Manohar, 1981 from the muscles of *Labeo rohita*; *M. hosadurgensis* Seenappa *et* Manohar, 1981 from the gills and muscles of *Cirrhinus mrigala*; *M. iranicus* Molnár, Masoumian *et* Abasi, 1996 from the spleen of *Barbus luteus* and *M. orissae* Haldar, Samal *et* Mukhopadhyay, 1996 reported from the gills of *Cirrhinus mrigala*.

Off these myxosporidan species, spores of *M.* (= *M.*) *anurus* (12.0-13.0 \times 7.0-8.0), *M.* (= *M.*) *dujardini* (11.0-13.0 \times 5.0-8.0), *M. bhadrensis* (8.0-11.0 \times 7.0-8.0), *M. hosadurgensis* (9.0-11.0 \times 5.0-8.0) and *M. iranicus* (13.2-14.0 \times 7.5-9.2) have smaller and broader spores and thus these are different from the present species under study. The pyriform spores of *M.* (= *M.*) *andhrae* with 3-6 prominent parietal folds are different from the spores of the present species devoid of parietal folds or markings.

Further, the pyriform spore of *M. punctatus* closely resembles the shape of the present species, but polar capsules are equal in the former species. Moreover, glycogen containing iodophilous vacuole is very much prominent in the former one which is lacking in the latter species. Furthermore, *M. orissae* shows similarity with the present species in having closer dimension of spore and the inequality of polar capsules, but the larger dimensions of two polar capsules, absence of iodophilous vacuole and intercapsular appendix have made the present species distinct from *M. orissae*.

These features justify the establishment of this myxozoan as a new species for which it is designated as *Myxobolus catmrigalae* sp. n. in the paper.

Taxonomic summary

Type host: Catla-Mrigal hybrid carp [male parent fish *Catla catla* (Hamilton-Buchanan) \times female parent fish *Cirrhinus mrigala* (Hamilton-Buchanan)].

Type locality: Kalna (Latitude: 23°13' N, Longitude: 88°22' E), Burdwan, West Bengal, India.

Type specimens: Paratypes are spores stained in Giemsa, in the collection of Harold W. Manter Laboratory of Parasitology, Nebraska, USA, No. HWML 16711.

Prevalence of infection: 182/277 (65.70 %).

Etymology: The species epithet *catmrigalae* is given after its type host Catla-Mrigal hybrid carp [male parent fish *Catla catla* (Hamilton-Buchanan) × female parent fish *Cirrhinus mrigala* (Hamilton-Buchanan)].

Myxobolus buccoroofus sp. n. (Figs 15-20, 22)

Plasmodia: Very minute oval 'cyst' like plasmodia attached to the roof of the buccal cavity (i.e. anterior part of palate). Mechanical rupture of plasmodium shows some trinucleate (Fig. 15), tetranucleate (Fig. 16) and eight nucleated pansporoblasts (Fig. 17), and many developed spores (Figs 18-20).

Spore: Mature histozoic spores [12.1 ± 0.33 (11.6-12.7) × 7.1 ± 0.45 (6.4-8.1)] appear pyriform in valvular view (Figs 18, 20). The anterior end of the spore is narrow and bends on one side, usually towards the smaller polar capsule; while the posterior end becomes broad and rounded (Figs 18, 20). The two shell valves are smooth, uniformly thick and devoid of any parietal folds. In sutural view, the spore is lenticular with slightly undulating median sutural line (Fig. 19). Sutural ridge is indistinct.

Two polar capsules are unequal in shape and size (Figs 18, 20). The larger one is 4.9 ± 0.26 (4.5-5.3) × 2.9 ± 0.12 (2.7-3.0), elongately oval and oblique in position. A very small knob like structure is present at the anterior extremity of the larger polar capsule (Figs 18, 20). In some spores, it bears a short neck or duct through which polar filament extrudes. Usually, the polar filament makes 6-8 spiral coils inside the larger polar capsule (Fig. 18). The smaller polar capsule [2.5 ± 0.24 (2.0-2.9) × 1.5 ± 0.12 (1.3-1.7)] lies a little distance down, near the anteromiddle part of the spore. Hence the smaller polar capsule does not open anteriorly but laterally (Fig. 20). It is mostly pyriform in shape, either with very short duct or pointed anterior end or slightly notched anterior end and its posterior end is broadly rounded (Figs 18, 20). 4-5 loose coils form inside the smaller polar capsule (Fig. 19). The extruded polar filaments are unequal (Fig. 20). The ratio of the length of the two filaments is approximately 1:3. The intercapsular ridge is absent. A thick mucus envelope covers the spore.

The sporoplasm is granular and homogenous and occupies the whole extracapsular space behind the polar capsules (Figs 18, 20). There is a large [diameter 2.9 ± 0.11 (2.7-3.1)] centrally placed iodophilous vacuole (Fig. 18), but in a few forms it is lateral (Fig. 20). The two karyosomatic sporoplasmic nuclei of 1.2 ± 0.11 (1.0-1.3) diameter are always situated above the iodophilous vacuole (Figs 18, 20).

Spore valves are generally smooth and the thick edges of each valve are fused to form a straight or slightly curved or undulated sutural line (Fig. 22). Two lateral ridges are seen in some spores separated from each other by a gap of 1.07.

Spore Index:

LS: WS = 1: 0.587

LLPC: WLPC = 1: 0.592

LSPC: WSPC = 1: 0.6

LLPC: LSPC = 1: 0.510

WLPC: WSPC = 1: 0.517

Taxonomic affinities: The *Myxobolus* species under consideration shows similarity with the spores of *M. toyami* Kudo, 1915; *M. calbasui* Chakravarty, 1939; *M. catlae* Chakravarty, 1943; *M. barbi* Tripathi, 1952; *M. drjagini* Akhmerov, 1954; *M. chondrostomi* Donec, 1962; *M. infundibulatus* Donec et Kulakowskaja, 1962; *M. anisocapsularis* Shulman, 1962; *M. osmaniae* Lalitha Kumari, 1969; *M. attui* Sarkar, 1984; *M. inaequus* Kent et Hoffman, 1984; and *M. undasuturiae* Sarkar, 1994. Of these *Myxobolus* species spores of *M. barbi* and *M. attui* are much wider (WS in *M. barbi* is 9.0 and that in *M. attui* is 7.5-9.6) than in the present species. Further, the spores of *M. toyami* (LS: 14.0-15.0), *M. catlae* (LS: 14.5-16.5) and *M. anisocapsularis* (LS: 15.0-15.5) are larger than those in the species under study.

Similarly, besides the shape and inequality of polar capsules roughly oval spores of *M. calbasui* (SP: 12.4-15.0 × 8.2-10.0); elongated oval spores of *M. drjagini* (SP: 10-11 × 9-10), ovate spores of *M. chondrostomi* (SP: 13.5-17.0 × 10.0-11.7) and oval spores of *M. infundibulatus* (SP: 13.4-15.4 × 11.0-13.0) are morphometrically different from the pyriform spores of the present myxosporidan species. In addition spores of *M. osmaniae* are larger in dimension (SP: 12.0-15.0 × 8.2-10.0) than the present species. Moreover, the shell valves, in former species, have 8-10 parietal folds which is absent in the latter species under report.

The spore and polar capsules of *M. inaequus* are nearly similar in shape with this *Myxobolus* species.

However, the spores (19.8×8.6) in the former are much larger and possess small, rod-like intercapsular spine. Although the shape of the spore of *M. undasuturæ* is almost identical with that of present species, the former species with smaller spore (SP: $10.0-11.0 \times 5.0-6.0$) and larger polar capsules (LPC: $5.0-6.5 \times 2.0-2.5$; SPC: $3.0-3.5 \times 1.0-1.2$) are distinctly different from the present species under consideration.

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

Taxonomic summary

Type host: *Labeo bata* (Hamilton-Buchanan).

Type locality: Hooghly (Latitude: $22^{\circ}54'$ N, Longitude: $88^{\circ}24'$ E), Hooghly (Dist.), West Bengal, India.

Type specimens: Paratypes are spores stained in Giemsa, in the collection of Harold W. Manter Laboratory of Parasitology, Nebraska, USA, No. HWML 16712.

Prevalence of infection: 59/296 (19.93 %).

Etymology: The species name *buccoroofus* is given to stress the infestation site (roof of the buccal cavity) of spores.

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