

Three New Species of *Myxobolus* Bütschli, 1882 from Different Food Fishes of West Bengal, India

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Summary. Investigations on the incidence of myxozoan parasites (Myxozoa: Bivalvulida) in fishes have assumed immense importance in view of severe pathogenicity and mortality caused by these organisms on their hosts. The present communication records three new species of myxosporeans, *Myxobolus calcariferum* sp. n.; *Myxobolus chinsurahensis* sp. n. and *Myxobolus mrigalhitae* sp. n. from different food fishes viz., *Lates calcarifer* (Bloch), *Anabas testudineus* (Bloch) and Hybrid between Mrigal [*Cirrhinus mrigala* (Hamilton - Buchanan)] and Rohu [*Labeo rohita* (Hamilton - Buchanan)] of West Bengal, India respectively.

Key words: Bivalvulida, India, *Myxobolus calcariferum* sp. n., *Myxobolus chinsurahensis* sp. n., *Myxobolus mrigalhitae* sp. n., Myxozoa.

INTRODUCTION

Bütschli (1882) established the genus *Myxobolus* with the type species *Myxobolus mulleri*. When the genus was first established, it included myxozoans having spores with or without an iodophilous vacuole and with one or two polar capsules. Thélohan in the year 1892 proposed a new genus *Myxosoma* for those species lacking an iodophilous vacuole. 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 453 species of *Myxobolus* in fishes.

Three new species of *Myxobolus* viz., *Myxobolus calcariferum* sp. n.; *Myxobolus chinsurahensis* sp. n. and *Myxobolus mrigalhitae* sp. n. respectively from *Lates calcarifer* (Bloch), *Anabas testudineus* (Bloch) and Hybrid between Mrigal [*Cirrhinus mrigala* (Hamilton - Buchanan)] and Rohu [*Labeo rohita* (Hamilton - Buchanan)] carp came out from our routine examinations during 1998-99. The description of these three myxozoans has been made in this communication in accordance with the guideline of Lom and Arthur (1989) and Lom and Dyková (1992).

MATERIALS AND METHODS

The host fishes were collected alive from the local fish markets from September, 1998 to December, 1999 and were brought to the

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laboratory and were immediately examined thoroughly for their myxozoan parasites. Sporogonic 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 \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.

RESULTS

Myxobolus calcariferum sp. n. (Figs 1-3)

Plasmodia: round (400 in diameter), creamy white coloured plasmodia are developed within the gill lamellae of infested host fishes. They contain a large number of mature spores only.

Spores: the mature spores, in fresh condition, are light yellowish in colour. Spores are 6.6 ± 0.32 (6.1-7.1) \times 6.2 ± 0.21 (5.7-6.5), almost spherical in front or valvular view (Figs 1, 3) and broadly lenticular in sutural view (Fig. 2) with symmetrical shell valves of uniform thickness. Both the ends of the spores are rounded, but the anterior part of the spore is bulged out to accommodate the polar capsules. Sutural ridge is median and straight (Fig. 2), although sutural line is not observed in fresh as well as in stained preparations.

Two polar capsules are equal [4.2 ± 0.22 (3.8-4.5) \times 2.3 ± 0.21 (2.0-2.7)] in size and pyriform in shape with rounded posterior ends and cover anterior 2/3rd of the spore cavity. Anterior tip of the capsule is pointed in most of the forms. Polar filament is thick and makes 4-5 loose spiral coils inside each capsule (Fig. 1). The polar filament is, however, not extruded although all the conventional methods for the extrusion of polar filament (viz., treatments with various concentrations of KOH, H₂O₂ and urea solution) have been performed.

The intercapsular ridge or appendix is absent.

The posterior 1/3rd of the spore cavity is filled with finely granular, homogenous mass of sporoplasm, which extends slightly into the intercapsular space (Figs 1, 3).

Two very small dot-like compact sporoplasmic nuclei of 0.8 ± 0.11 (0.6-0.9) diameter are situated centrally (Figs 1, 3). These are transversely parallel or oblique in position. Iodinophilous vacuole and mucus envelope around spores are absent.

Spore index:

LS: WS = 1: 0.939

LP: WP = 1: 0.548

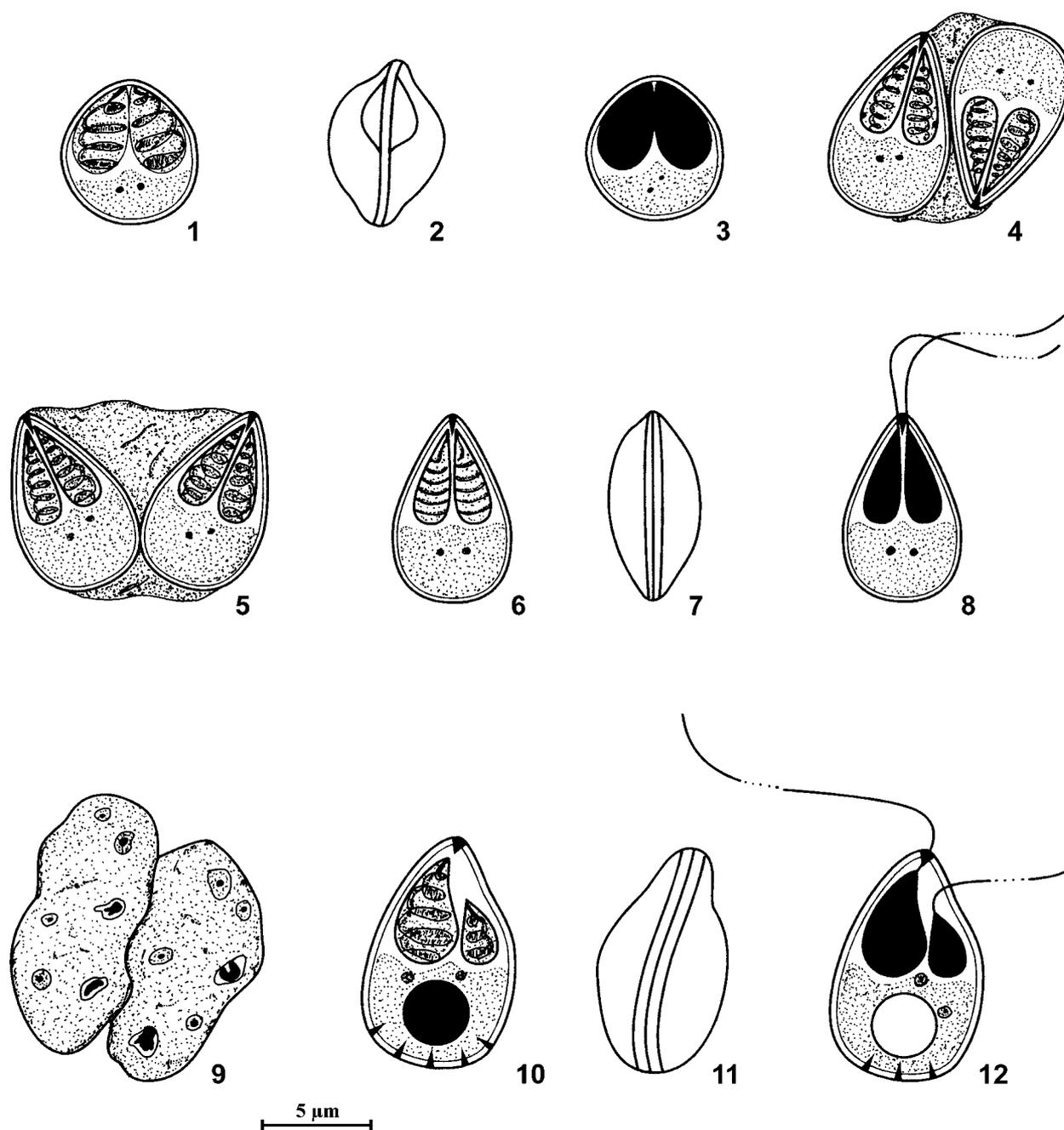
LS: LP = 1: 0.636

WS: WP = 1: 0.371

Intraspecific variability: the present myxozoan obtained from the gill lamellae of *Lates calcarifer* (Bloch) shows little variations among the specimens. The anterior end in a few spores is slightly pointed. Anterior extremity of two polar capsules is almost fused in some forms. A few spores with coarse sporoplasmic granules are also common in some subpopulation.

Taxonomic affinities: the present myxozoan species is assigned to the genus *Myxobolus* Bütschli, 1882 in spite of the absence of the iodophilous vacuole in the sporoplasm of the spore since several authors have been considering the genus *Myxosoma* Thélohan, 1892 a junior synonym of the genus *Myxobolus* (Walliker 1968, Mitchell 1977, Lom and Noble 1984, Landsberg and Lom 1991, Lom and Dyková 1992) as mere presence of an iodophilous vacuole in the sporoplasm of the spore can not be accepted as a distinguishing character for the separation of the genus *Myxosoma* from the genus *Myxobolus*.

The present myxozoan shows similarity with *Myxobolus* (= *Myxosoma*) *funduli* Kudo, 1918; *M. indicum* Tripathi, 1952; *M. barbi* Tripathi, 1952; *M. conspicuus* Kudo, 1966 and *M. ampullaceus*, Lalitha Kumari, 1969 reported from *Fundulus* sp., *Cirrhinus mrigala*, *Barbus ticto*, *Notropis gilberti* and *Barbus kolus* respectively in having close morphometric data of the spores. However, the spherical spores of the present species are apart from the pyriform spores of *M. funduli*. The present myxozoan differs from *M. barbi*, *M. conspicuus* and *M. ampullaceus* by the absence of an iodophilous vacuole in the sporoplasm of the spore while the iodophilous vacuole is present in last three myxozoans. Moreover, the spores of *M. conspicuus* and *M. ampullaceus* are broader than the present myxozoan (breadth of *M. conspicuus* and *M. ampullaceus* are 6.5-8.0 and 6.4-7.9 respectively). The similarity of the present species with *M. indicum* is untenable since the spores of the former species have two equal polar capsules while the two polar capsules in



Figs 1-3. Camera lucida drawings of spores of *Myxobolus calcariferum* sp. n.; **1** - fresh spore in valvular view - Lugol's iodine; **2** - fresh spore in sutural view - Lugol's iodine; **3** - fixed spore in valvular view - Giemsa.

Figs 4-8. Camera lucida drawings of plasmodia and spores of *Myxobolus chinsurahensis* sp. n.; **4, 5** - Disporous pansporoblasts - Lugol's iodine; **6** - fresh spore in valvular view - Lugol's iodine; **7** - fresh spore in sutural view - Lugol's iodine; **8** - fixed spore in valvular view with extruded polar filaments - Giemsa.

Figs 9-12. Camera lucida drawings of plasmodia and spores of *Myxobolus mrigalhitae* sp. n.; **9** - multinucleate pansporoblasts - Lugol's iodine; **10** - fresh spore in valvular view - Lugol's iodine; **11** - fresh spore in sutural view - Lugol's iodine; **12** - fixed spore in valvular view with extruded polar filaments - Giemsa.

the latter species are unequal. It also disagrees with *M. barbi* in the absence of an intercapsular ridge (intercapsular ridge is distinct in *M. barbi*).

Further, the present myxozoan closely resembles in shape with *M. carnaticus* Seenappa et Manohar, 1980, *M. curmucae* Seenappa et Manohar, 1980 and *M. vanivilasae* Seenappa et Manohar, 1980. But the dimensions of spores of *M. carnaticus* (8.6 x 6.8) are larger than in the present species. Moreover, presence of intercapsular ridge and unequal polar capsules distinctly clarify their dissimilarity. The present species differs from *M. curmucae* and *M. vanivilasae* as spores of *M. curmucae* and *M. vanivilasae* with intercapsular ridge (which is absent in the present form) are larger (dimensions of spores of *M. curmucae* and *M. vanivilasae* are 9.8 x 7.6 and 8-10 x 7-9 respectively) and possess both equal and unequal polar capsules. Furthermore, the present species under study resembles *M. anili* Sarkar, 1989 in shape, presence of equal polar capsules and absence of iodophilous vacuole. But the spores of latter species are broadly ellipsoid with slightly acuminate anterior end (in the former, spores are spherical with rounded anterior end); polar capsules have short neck like structure in *M. anili*, which is not encountered in the present species under consideration too.

In view of such differences with the closely related species, the present myxozoan has been considered as a new species for which the name *Myxobolus calcariferum* sp. n. is given.

Type host: *Lates calcarifer* (Bloch).

Type locality: Naihati, 24 Parganas (North), West Bengal, India.

Type specimens: paratypes are spores stained in Giemsa, in the collection of H.W.M. Laboratory of Parasitology, U. S. A., No. HWML 16707.

Prevalence and intensity of infection: 67/385 (17.40 %).

Etymology: the species is named after its type host *Lates calcarifer* (Bloch).

***Myxobolus chinsurahensis* sp. n. (Figs 4-8)**

Plasmodia: numerous yellowish kidney shaped plasmodia are attached with the scales of infested host fishes - *A. testudineus*. These are 59-73 long and 29-41 wide and contain some late developmental stages (Figs 4, 5) as well as mature spores (Figs 6-8).

Spores: mature histozoic spores are 8.4 ± 0.43 (8.0-9.7) x 5.4 ± 0.29 (5.1-6.1), creamy white to yellowish in colour, tear shaped in valvular view with rounded

posterior and bluntly pointed anterior ends (Figs 6,8), although a few spores show a slightly pyriform shape in valvular view. In sutural view the spores are lenticular with prominent sutural ridge and line in fresh as well as in Lugal's iodine preparations (Fig. 7). The shell valves are moderately thick, uniform, without any parietal fold or markings. There lies a prominent but very small intercapsular ridge (Figs 6,8).

Polar capsules are two in number, equal in size and pyriform with greatly rounded posterior and sharply pointed anterior ends converging closely but open side by side. These are 4.4 ± 0.74 (3.9-6.6) long and 2.1 ± 0.16 (1.8-2.5) wide. Five to six tight coils are formed inside each polar capsule (Fig. 6). Polar filaments are thin and extrude through the anterior bluntly pointed end of the spores in extremely rare cases (Fig. 8).

Granular homogenous mass of sporoplasm fills the extracapsular space of the spore cavity and contains two sporoplasmic nuclei of 0.6 ± 0.13 (0.5-0.9) diameters (Figs 6, 8). Spores lack any mucus envelope around as well as the iodophilous vacuole within their sporoplasm.

Spore index:

LS: WS = 1: 0.643

LP: WP = 1: 0.477

LS: LP = 1: 0.524

WS: WP = 1: 0.389

Intraspecific variability: general structures of fresh and stained specimens of *Myxobolus chinsurahensis* sp. n. obtained from *Anabas testudineus* (Bloch) are quite identical. However, some minor variations can be noticed in the spore morphology. Some spores have a pyriform shape with slightly elongated anterior necks. The polar capsules are, accordingly, have a rather elongated pyriform shape. In a few instances sporoplasmic distribution is limited just to the posterior part of the polar capsules and not throughout the extracapsular space of the spore cavity.

Taxonomic affinities: according to Tripathi's (1952) grouping of the genus *Myxobolus*, the present species is placed in the first group, as it has polar capsules of equal size and an intercapsular ridge. It resembles *M. mrigalae* Chakravarty, 1939 reported from scales of *Cirrhinus mrigala*; *M. bengalensis* Chakravarty et Basu, 1948 reported from branchiae of *Catla catla*; *M. sphericum* Tripathi, 1952 reported from inner sides of the scales of *Cirrhinus mrigala*; *M. potaili* Lalitha Kumari, 1969 reported from the branchiae of *Labeo potaili*; *M. (=Myxosoma) magauddi* Bajpai, Kundu et Haldar, 1981 from brain of *Trichogaster fasciatus*; *M. rohita*

Haldar, Das et Sharma, 1983 reported from the scale of *Labeo rohita* and *M. mola* Sarkar, 1993 reported from the Kidney of *Amblypharyngodon mola* in shape as well as in morphometry of the spores. However, the spores of *M. mrigalae* and *M. (=M.) magauddi* possess a pair of distinctly unequal polar capsules and hence are different from the myxozoan species under study. The spores of *M. potaili* have an anterior knob and striated shell valves, which are not seen in the present species. *M. bengalensis* although possesses spores having a similar shape and length but the slightly narrower spores (breadth of spore of *M. bengalensis* is 6.4-6.8), a pair of larger and narrower polar capsules (dimensions of polar capsule in *M. bengalensis* is 4.2-5.4 x 2.5-3.2) and prominent small intercapsular ridge (which is absent in *M. bengalensis*) make the present species very distinct from the former one. Moreover, the oval spores of *M. mola* with larger dimension [SP: 8.5-9.5 (9.0) x 7.0-8.5 (7.4); PC: 5.0-6.5 (6.0) x 3.8-4.5 (4.0)] possess a small spherical iodophilous vacuole, which is not observed in the much smaller, and tear shaped forms of the present species under study. The spores of *M. sphericum* and *M. rohita* resemble the present myxozoan by their site of infection. But shape of the spores in former two species are different (spores of *M. sphericum* is spherical and that of *M. rohita* are ovoid to rounded in vulvular view) from the present one under consideration as it was tear shaped in structure.

This species also resembles, either in size or in shape, some myxobolid species reported from other countries. These are *M. dispar* Thélohan, 1895; *M. exiguus* Thélohan, 1895; *M. musculi* Keysselit, 1908; *M. lobatus* Dogel, 1932; *M. amurensis* Akhmerov, 1960 and *M. (=Myxosoma) acuta* Fujita, 1912. Of these, the spores of *M. dispar*; *M. musculi* and *M. (=M.) acuta* have a pair of highly unequal polar capsules and hence differ from the present species. *M. exiguus* has larger spores with a few thickenings in the shell valves and its anterior ends are narrow and round which are not found in the spores of the present species. *M. lobatus* shows very close resemblance in the morphometry of the spore and polar capsules. However, the nearly parallel polar capsules and very indistinct intercapsular spine are different from the strongly convergent polar capsules and prominent intercapsular ridge of the myxozoan under consideration. Finally, *M. amurensis* has larger spores than those of the present form. Moreover, the pair of the polar capsules of *M. amurensis* are broadly pyriform and their openings are distantly placed at the

perfectly round anterior end of the spore while the spores of the present myxozoan have bluntly pointed anterior ends and a pair of strongly convergent pyriform polar capsules having very closely set openings.

Considering these differences with the other related species, the present myxosporidan species is proposed as a new species and named *Myxobolus chinsurahensis* sp. n.

Type host: *Anabas testudineus* (Bloch).

Type locality: Chinsurah, Hooghly, West Bengal, India.

Type specimens: paratypes are spores stained in Giemsa, in the collection of H.W.M. Laboratory of Parasitology, U. S. A., No. HWML 16708.

Prevalence and intensity of infection: 43/279 (15.41 %).

Etymology: the species is named after its type locality Chinsurah, in West Bengal.

***Myxobolus mrigalhita* sp. n. (Figs 9-12)**

Plasmodia: fully developed plasmodia are round in shape and attached with the gill filaments of infested host fishes. The earlier stages, uni- and binucleate pansporoblasts are not present but a few multinucleate pansporoblasts are observed (Fig. 9). The multinucleate pansporoblasts are irregular in shape. These have 12 nuclei, which segregate into two equal groups and form 2 sporoblasts. So the pansporoblast is disporous. The nuclei are of different shapes and nature. The capsulogenous nuclei are elongate, valvular nuclei are large and irregular and sporoplasmic nuclei appear oval.

Spores: immature spores are round to spherical in shape. Two polar capsules occupy most part of the spore cavity. Fully developed or mature spores are pyriform in front view (Figs 10, 12) and are 10.8 ± 0.32 (10.2-11.3) x 7.9 ± 0.18 (7.6-8.1) in dimensions. The posterior end is broad and rounded whereas the anterior end is narrow and blunt. The spore valves are thick at the posterior end, but thin at the anterior one. The sutural ridge is broad with straight median sutural line (Fig. 11). In the posterior extremity of the spore there lie 2-5 parietal folds (Figs 10, 12). The inner wall of the spore valve forms a thickening of intercapsular appendix (Figs 10, 12).

Two polar capsules are unequal. The larger one is 4.8 ± 0.30 (4.3-5.2) x 2.9 ± 0.16 (2.7-3.2), pyriform with broader posterior and bluntly pointed anterior ends. The smaller polar capsule is tear-shaped and 3.0 ± 0.09 (2.9-3.2) x 2.1 ± 0.06 (2.0-3.2) in measurement with slightly curved anterior end. The polar filament makes

5-6 and 3-4 spiral coils inside larger and smaller polar capsules respectively (Fig. 10). Polar filament from the larger polar capsule extrudes through the anterior end of the spore but that from the smaller one extrudes through the anterolateral end, i.e., there lie two separate openings for the extrusion of two polar filaments (Fig. 12).

The sporoplasm is granular, homogenous and fills the extracapsular space. Two karyosomatic sporoplasmic nuclei of 1.1 ± 0.08 (1.0-1.2) diameter and a spherical glycogen containing iodophilous vacuole having a diameter of 3.0 ± 0.09 (2.9-3.3) are present in the posterior part of the sporoplasm (Fig. 10).

Spore index:

LS: WS = 1: 0.731

LLPC: WLPC = 1: 0.604

LSPC: WSPC = 1: 0.7

LLPC: LSPC = 1: 0.625

WLPC: WSPC = 1: 0.724

Intraspecific variability: the variability in the spore and polar capsule morphology among the individuals of *Myxobolus mrigalhitae* sp. n. obtained from the gill filaments of hybrid between Mrigal and Rohu carp during the study period was not very significant. However, spore with slightly acuminate anterior end and larger polar capsule with 7-8 turns of polar filament were noticed in very few forms.

Taxonomic affinities: the present species with a pair of unequal polar capsule and an intercapsular ridge well satisfies the generic character of *Myxobolus* Bütschli, 1882 and can well be placed in the last fourth group of Tripathi's (1952) grouping of the genus *Myxobolus*.

The present species shows similarities either in shape or in morphometry with *M. toyami* Kudo, 1915 reported from the connective tissue of gill lamellae of *Cyprinus carpio haematopterus*; *M. mrigalae* Chakravarty, 1939 reported from the scales of *Cirrhinus mrigala*; *M. indicum* Tripathi, 1952 reported from the scales, liver and intestinal wall of *Cirrhinus mrigala*; *M. anisocapsularis* Shulman, 1962 reported from the gill lamellae of *Hemibarbus labeo* and *H. maculatus*; *M. chondrostomi* Donec, 1962 reported from the muscles of *Chondrostoma nasus*; *M. koli* Lalitha Kumari, 1969 reported from the liver and intestine of *Barbus punjaubensis*; *M. bhadrensis* Seenappa et Manohar, 1981 reported from the muscle of *Labeo rohita* and *M. vedavatiensis* Seenappa et Manohar, 1981 reported from the gills of *Cirrhinus mrigala*.

Of these above mentioned species, the spores of *M. toyami* (LS: 14.0-15.0); *M. anisocapsularis* (LS: 15.0-15.5); *M. chondrostomi* (LS: 13.5-17.0) are larger than that in the present species. The spores of *M. mrigalae* (SP: 7.2 x 8.2, LPC: 5.1 x 3.0, SPC: 3.0 x 2.0); *M. koli* (SP: 8.4 x 6.0, LPC: 4.3 x 2.8, SPC: 2.0 x 1.2) and *M. osmaniae* (SP: 12.0-15.0 x 7.1-10.0, LPC: 5.0-7.1 x 2.1-3.6, SPC: 2.9-3.9 x 1.4-2.9) show much similarity with the present species. However, spherical to oval spore of *M. mrigalae*, oval spore of *M. koli* and somewhat pyriform spore of *M. osmaniae* are distinctly different from the pyriform spore of the species under study. Similarly beside the shape, the spores of *M. bhadrensis* (SP: 9.5 x 7.1, LPC: 3.5 x 3.2, SPC: 2.5 x 1.7) and *M. vedavatiensis* (SP: 13.8 x 9.2, LPC: 6.0-7.0 x 3.0-4.0, SPC: 3.0-5.0 x 2.0-3.0) are morphometrically different from the spores of present myxozoan. The present species also differs from the oval (SP: 9.5-10.8 x 7.5-8.2) and much smaller polar capsules (LPC: 2.7-3.6 x 1.8, SPC: 1.8 x 1.0) of *M. indicum*.

Besides, the present myxozoan is also compared with a very recent species of the genus *Myxobolus* - *M. ophthalmusculata* Basu et Haldar, 2002 reported from the eye muscles of *Cirrhinus mrigala*. But the pyriform spore with smooth shell valves and slightly undulating sutural ridge of the latter species is larger (SP: 13.1 x 8.0) in dimensions from the pyriform spore with 2-5 parietal folds and straight median suture of the present species under consideration. Furthermore, the host of the present species is a hybrid between Mrigal (*Cirrhinus mrigala*) and Rohu (*Labeo rohita*) carp from which no such species are described earlier.

Considering the above facts, it is evident that the present species is a new myxozoan and hence it has been proposed as *Myxobolus mrigalhitae* sp. n. in this dissertation.

Type host: hybrid between Mrigal (*Cirrhinus mrigala*) and Rohu (*Labeo rohita*) carp.

Type locality: Kalna, Burdwan, West Bengal, India.

Type specimens: paratypes are spores stained in Giemsa, in the collection of H.W.M. Laboratory of Parasitology, U. S. A., No. HWML 16706.

Prevalence and intensity of infection: 241/372 (64.72%).

Etymology: the species is named after its type host hybrid between Mrigal (*Cirrhinus mrigala*) and Rohu (*Labeo rohita*) carp.

DISCUSSION

In this communication three new species of *Myxobolus* Bütschli, 1882 have been reported among which two viz. *Myxobolus calcariferum* sp. n., *Myxobolus chinsurahensis* sp. n., although have no iodophilous vacuole are placed in the genus *Myxobolus* Bütschli, 1882 based on the spore characters given by Kudo (1920, 1933).

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