Morphotaxonomical description of three new species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Bivalvulida) from edible freshwater fishes of West Bengal, India

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Abstract Three new species of *Myxobolus* are described from the mucous membrane around gill lamellae of carps of West Bengal. The new species have been named as *M. muralidharani* sp. n., *M. nilimae* sp. n. from *Labeo rohita*, and *M. harpreetae* sp. n. from *Labeo bata* respectively. Spores of *M. muralidharani* sp. n. measured 13.84 ± 0.049 (13–14) $\times$ 4.95–6 (5.35) μm , with two elongated equal sized polar capsules. Spores of *M. nilimae* sp. n. measured 15.9–17.1 (16.65) μm $\times$ 6.2–7.5 (6.9) μm , with two equal petal shaped polar capsules, 9.0–11 (10.4) μm $\times$ 3–3.5 (3.16) μm . In contrast to aforementioned two new species, *M. harpreetae* sp. n. had two unequal polar capsules, 8.7–9.2 (9.149) μm $\times$ 2.9–4.0 (3.45) μm and 7.6–8.3 (7.94) μm $\times$ 2.8–3.3 (2.9) μm in each spore, 11.4–13.8 (13.2) μm $\times$ 4.9–7.8 (6.51) μm .

Keywords *Myxobolus nilimae* sp. n. · *Myxobolus muralidharani* sp. n. · *Myxobolus harpreetae* sp. n. · *Labeo rohita* · *Labeo bata* · West Bengal · India

Introduction

Myxozoans are multicellular parasites, which cause disease in a large variety of edible fishes. More than 2200 taxa, have been described from fishes in different geographic areas (Landsberg and Lom 1991; Lom and Dykova 1992). Research on myxosporean fish parasites

is a fast developing field of ichthyoparasitology. Numerous myxosporeans, among them highly pathogenic species, are known from freshwater and marine fishes. In terms of freshwater fishes, the majority of studies both in freshwater and culture systems deal with fishes of the northern hemisphere (Eurasia and North America), while studies on myxosporeans of marine fishes have mostly been done worldwide on cage-cultured species. Among myxosporeans, the genus *Myxobolus* Bütschli 1882 is considered as the largest group of the phylum Myxozoa Grasse 1970. In a synopsis of this genus, Eiras et al. (2005) reported seven hundred fifty one valid species, while Lom and Dykova (2006) counted 792 known species as an important pathogen in freshwater and marine fishes. Genus *Myxobolus* was first established by Bütschli (1882) as bivalvulid myxosporeans with or without an iodophilous vacuole. Myxosporeans infecting fishes cause loss of production and death. Indian authors like Chakravarty (1939), Tripathi (1952), Lalitha-Kumari (1965, 1969), Seenappa and Manohar (1981), Sarkar (1985a, b, 1986), Basu and Haldar (2003, 2004), Banerjee et al. (2011), Kaur and Singh (2012), and Thounaojam et al. (2013) contributed a lot on myxosporean parasites of the tropical and subtropical countries. Data on myxosporean infections were also presented from different countries. Kalavati and Nandi (2007) reported the distribution of one hundred myxosporean species infecting fresh water as well as marine fish from India. During a survey of protozoan parasites of fish, three undescribed *Myxobolus* spp. have been recorded. Based on spore morphology three new species from carps in West Bengal, India, namely, *M. muralidharani* sp. n. and *M. nilimae* sp. n. from *Labeo rohita*, and *M. harpreetae* sp. n. from *Labeo bata* have been described here.

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Materials and methods

Fish samples and parasitological examination

Carp examined in the present study, *Labeo rohita* (n = 51) and *Labeo bata* (n = 21) were collected during the 8 month period between December 2012 and August 2013, covering all three seasons starting from winter then summer to monsoon, from ponds and markets in two districts of West Bengal, India, i.e. Hanskhali and Tehata of Nadia district and Sonarpur of South 24 Parganas district. The small-sized fishes, young in age, were then brought alive to the parasitology laboratory of University of Kalyani, Kalyani for parasitological examinations. When whitish spots were found in gill lamellae, impression preparations of gills were made on clean glass slides with a drop of 0.9 % NaCl solution, covered with cover slips, and examined for the presence of myxospores. Some of the impression preparations were stained with Lugol's iodine solution to observe iodophilous vacuoles in myxospores, and others were treated with 8 % KOH for polar filament extrusion from myxospores. For permanent specimens, the impression preparations were fixed in absolute methanol for 2–8 min and then stained with Giemsa solution for about 25–30 min, then washed in tap water and dried. Permanent slides were prepared with DPX mountant. Sporogonic plasmodia, when found, were carefully removed with sterile needles, put on clean slides with drops of distilled water, covered with cover slips and sealed with DPX mountant for examination of fresh spores under an oil immersion lens of Olympus KH phase contrast microscope. Photomicrographs of fresh and stained spores were taken at 1000× magnification. Drawings were made from stained materials with the aid of camera lucida. Measurements based on 20 fresh spores (stained with both Lugol's Iodine and Geimsa) were done with a calibrated ocular micrometer according to Lom and Arthur (1989) and Lom and Dykova (1992). All measurements are expressed in micrometer (μm) as mean ± standard deviation (SD) followed by the range in parentheses. The following abbreviations are used in the present study: LS, length of spore; LPC, length of polar capsule; PF, polar filament; WS, width of spore; and WPC, width of polar capsule.

Results

Three myxobolid species were observed in a form of microscopic cysts attached to the mucous membrane of gill lamellae (Table 1). Based on morphological parameters, all three myxobolid species, found in the gill of carps

correspond to definition of the genus *Myxobolus* Bütschli 1882, but none of them are known species.

Description

Myxobolus muralidharani sp. n. Fig. 1a–d

Plasmodia

Rounded Small in size (2–3 mm in diameter). Attached to the mucous membrane of gill lamellae. Creamy white in colour.

Spore (based on 20 spores from fully developed plasmodia)

In valvular view spores remarkably elongated, lancet-shaped, measuring 13.84 ± 0.049 (13–14) × 4.95–6 (5.35). The surfaces of shell valve smooth. Two slender and elongated polar capsules equal in shape occupying the anterior portions of spores and measuring 6.9–8.0 (7.38) μm × 1.9–2.9 (2.56) μm. Filamentous coils have not be observed in the capsules. Sporoplasm with round iodophilous vacuole.

Spore index

LS:WS = 1:0.385

LPC:WPC = 1:0.347

Taxonomic summary

Type host *Labeo rohita* (Hamilton 1822).

Type locality Hanskhali (latitude: 23°43'0"N and longitude 88°32'0"E) of Nadia, West Bengal, India.

Type specimens Slide No. MB/PA/KU/07-08 containing the holotype and four slides MB/PAKU/09-12 containing paratypes have been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India.

Symbiotype The host specimen bearing no.CM/PARA/01-07 deposited in the museum of the Parasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India.

Site of infection Gill lamellae.

Prevalence of infection 8 out of 21 hosts examined (38.095 %).

Specific epithet The specific epithet “*muralidharani*” is given after the name of Professor N. Muralidharan,

Table 1 Fish hosts examined and myxospores found

Host	Locality	Date of collection	Parasite	Plasmodium	Location	Prevalance
<i>Labso rohita</i>	Hanskali, Nadia, West Bengal	Dec. 2012–Aug. 2013	<i>Myxobolus muralidharani</i>	Histozoic and polysporic	Gill lamellae	8/21 (38.1 %)
<i>Labso rohita</i>	Tehattai, Nadia, West Bengal	Dec. 2012–Aug. 2013	<i>Myxobolus nilimae</i> sp.n.	Histozoic and polysporic	Gill lamellae	11/30 (36.7 %)
<i>Labeo bata</i>	Sonapur, South 24 Parganas, West Bengal	Dec. 2012–Aug. 2013	<i>Myxobolus harpreetae</i> sp. n.	Histozoic and polysporic	Gill lamellae	2/21 (9.5 %)

Fig. 1 **a, b** Light microscopic Photomicrographs of the spores of *Myxobolus muralidharani* sp. n. stained with Giemsa (unextruded) (Scale bar: 10 µm); **c, d** Camera lucida drawing of the spore of *Myxobolus muralidharani* sp. n. (Scale bar: 10 µm)

Professor of Zoology (Retd), University of Delhi, Delhi for his outstanding contribution in the field of life science.

Remarks (Table 2)

Myxobolus muralidharani sp. n

The present species can be distinguished from its closely related species by its shape, measurement of the spore, polar capsules and geographical location. The organism possesses two equal polar capsules and can therefore be placed in the group of species with equal polar capsules (Tripathi 1952).

The shape of the spore of the present species is elongated which resembles closely with *M. attui*, but the spores of *M. attui* are much wider than that of the present form. Besides the polar capsules, the present form shows difference by having an average dimension much larger than *M. attui*, thus leaving marked differences between their spore indices. While comparing the present form its other closely related species in respect of shape and measurement of spores found to be smaller than that of *M. ticto*, *M. dasgupti*, *M. sphaeralis* and *M. maguri* but larger than *M. nobeli*.

The polar capsules of the present species have short neck, less extended spore body with pointed anterior and rounded posterior end and situated anteriorly just behind the apex of the spore, running parallel to each other. But in case of *M. ticto* and *M. dasgupti* the converging polar capsule with long distinct neck extended spore body differ from the present form. It is larger in *M. sphaeralis* and wider in *M. nobeli* in comparison to the present form.

Comparative study on inter-capsular process shows that among all closely related species in case of *M. nobeli* the polar capsule occupies almost three fourth of the spore body, whereas the species under discussion occupies less than half of the spore body and they are placed anteriorly, distended from each other leaving a vacuolar space in the lower part of the spore.

The present species when compared to *M. sphaeralis* and *M. ticto* in regard to the inter-capsular process, it is much wider in *M. sphaeralis* whereas in *M. ticto* it is absent but it is prominent in the present form.

The species under discussion has also been compared with two proposed new species namely, *M. nilimae* sp. n. and *M. harpreetae* sp. n. for morphological variations. The spore and polar capsules of *M. nilimae* sp.n., are much larger than that of the species under discussion. Moreover, the spore is petaloid in *M. nilimae* sp. but it is elongated and thinner in the present form. Besides, the spore index of LS: WS of both the species differs marginally.

While in comparison to *M. harpreetae* sp. n., the present species differs by having equal sized polar capsules than that of different sized polar capsules of *M. harpreetae* sp. n.

Table 2 Showing the comparative account of the *Myxobolus muralidharani* sp.n. with morphologically similar species (measurements are micrometer)

M. maguriiName of the species	Host	Site of infection	Length of spore	Width of spore	Length of polar capsule	Width of polar capsule	Author/(s)
<i>M. magurii</i>	<i>Clarias magur</i> L.	Accessory respirator organ	13–15 (14.13)	6.5–8 (7.75)	7–8 (7.53)	2–3 (2.34)	Sarkar (1993)
<i>M. dasgupti</i>	<i>Mugil tade</i> Forsskal	Gills, body muscle	11.4–19.5 (14.37)	4.9–8.1 (6.4)	7.3–11.4 (9.11)	1.6–4 (2.6)	Haldar et al. (1996)
<i>M. nobeli</i>	<i>Ophiocephalus striatus</i> Bloch	Gall bladder	11.5–14.5 (12.27)	8.3–10.5 (9.5)	5.8–8.5 (7.0)	3–4.5 (3.7)	(Sarkar 1982) Gupta and Khera (1988), Sarkar (1982)
<i>M. sphaeralis</i>	<i>Liza macrolepis</i> Smith	Gill filaments	13–15 (14.3)	6.5–8 (7.75)	7–8 (7.53)	2–3 (2.34)	Padma Dorothy and Kalavati (1992)
<i>M. ticto</i>	<i>Puntitus ticto punctatus</i> Day	Intestine, liver, muscles, Gills	12.75–15 (14.55)	6.75–9 (7.8)	4.5–7.5 (6.63)	2.5–3 (2.92)	Sheeja and Janardanan (2006)
<i>M. attui</i>	Wallago attu Bleeker	gut	12.8–15.2 (13.89)	5–9.6 (8.53)	4.8–7.2 (5.92)	2.4–4 (3.0)	Sarkar (1985a, b)
<i>Myxobolus muralidharani</i> sp.n.	<i>Labeo rohita</i>	Gill lamellae	13–14 (13.84)	4.9–6 (5.35)	6.9–8 (7.78)	1.9–2.9 (2.56)	Present study

Besides, the larger polar capsules of *M. harpreetae* sp. n are much bigger than that of the present form. Thus both the species showed variation in the spore index. In view of the above differences, the present species under discussion is considered to be new to science and hence *M. muralidharani* sp. n. is being proposed in the communication.

***Myxobolus nilimae* sp. n Fig. 2(a, b, c, d)**

Plasmodia

Small, macroscopic (1–2 mm), attached to the mucous membrane around gill lamellae. 5–10 spores present.

Spore (Measurements based on 8–9 spores in frontal view)

Spores petal shaped. Long cylindrical have narrow blunt anterior end and rounded posterior end. Two equal polar capsules with bluntly pointed anterior end and rounded posterior end, placed posteriorly from the tip of the spore and are parallel to each other. Mature spores measure of 16.6 ± 0.38 (15.9–17.0) $\times$ 6.90 ± 0.48 (6.2–7.0). Polar capsules measure 10.40 ± 0.49 (9.0–11.0) $\times$ 3.16 ± 0.6 (3.0–3.5). Polar filament closely coiled, aligned perpendicularly to the longitudinal axis of the capsules with 10–12 coils. Inter-capsular space narrow Sporoplasm occupy small portion of the spore.

Fig. 2 a, b Light microscopic Photomicrographs of the spores of *Myxobolus nilimae* sp. n. stained with Giemsa (unextruded) (Scale bar: 10 µm); c, d Camera lucida drawing of the spore of *Myxobolus nilimae* sp. n. (Scale bar: 10 µm)

Spore index

LS: WS = 1: 0.4156

LPC: WPC = 1:0.3038

Taxonomic summary

Type host *Labeo rohita* (Hamilton 1822).

Type locality Tehatta (latitude: 23°43'0"N and longitude 88°32'0"E), Nadia, West Bengal, India.

Type specimens Slide No. MB/PA/KU/3-5 containing the holotype and other slides MB/PA/KU/6-8 containing paratype have been deposited at the Prasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India.

Symbiotype The host specimen bearing no.CM/PARA/8-10 deposited at the museum of the Parasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India.

Site of infection Gill lamellae.

Prevalence of infection 11 out of 30 hosts examined (36.7 %).

Specific epithet The specific epithet “*nilimae*” has been given after the name of Prof. Neelima Gupta, Professor of Animal Science, MJP Rohilkhand University, Uttar Pradesh, India who have immensely contributed in the field of Protozoology.

Remarks (Table 3)

According to Tripathi (1952) grouping of the genus *Myxobolus*, the species under report can be placed in the second group as the spores possesses equal polar capsules.

The species under discussion is petaloid with narrow blunt anterior end and rounded posterior end with much wider surface area but no distinct opening found at the anterior end. The present species superficially resembles *M. karnatacae*, *M. rocatlae*, *M. saraswati* and *M. scatophagi* in respect to spore length. In comparison to *M. karnatacae*, *M. rocatlae* and *M. saraswati*, the spores are oval to spherical with rounded anterior and posterior extremities and without any parital folds. While comparing the size, the species under discussion is smaller than *M. karnatacae*, *M. rocatlae* and *M. saraswati* and larger than *M. scatophagi*. But the species under discussion differs in respect to length and width of spore and polar capsules from all other compared species. In *M. scatophagi*, the tip of the anterior region of the spores is thickened to a notch, whereas, in the present form no distinct opening has been found at the anterior end. But both the species resemble with each other in not having distinct inter-capsular ridge but differ from each other in having different geographic locations and site of infection as well as the host species.

When the present form is further compared with the other two new species namely, *Myxobolus murlalidherani* sp. n and *M. harpreetae* sp. n., it has been observed that spores and polar capsules of the present species are much larger than that of the *M. murlalidherani* sp. n. The species under discussion also differs in its appearance with *M. murlalidherani* sp. n. as it is somewhat petal shaped whereas *M. murlalidherani* sp. n. is elongated and much narrower. Its comparison to *M. harpreetae* sp. n. it shows difference in polar capsules. In *M. harpreetae* sp. n. the polar capsules are unequal whereas, they are equal in the present form. The spore index of both the species are significantly different.

Further, comparative study revealed that 10–12 filamentous coils are clearly present in the present form while the filamentous coils are invisible in case of *M.*

Table 3 Showing the comparative account of the *Myxobolus nilimae* sp. n. with morphologically similar species (measurements are in micrometer)

Name of the species	Host	Site of infection	Length of spore	Width of spore	Length of polar capsule	Width of polar capsule	Author/(s)
<i>M. karnatacae</i>	<i>Barbus chola</i> Ham. and Buch.	Caudal muscle	16.3–19.04 (17.58)	10.88–13.6 (11.1)	7.3–10.88 (11.11)	3.58–5.44 (4.78)	(Hagargi and Amoji 1981) and Landsberg and Lom (1991)
<i>M. rocatlae</i>	<i>Catla catla</i> Ham.x <i>Labeo rohita</i> Ham (hybrid)	Gills, guts	16.9–19.3 (18.3)	5.6–6.9 (6.0)	10.4–13.7 (12.6)	2.2–3.1 (2.8)	Basu and Halde (2002a)
<i>M. saraswati</i>	<i>Clarias batrachus</i> L.	Kidneys	10–20	7.8–11	3.5–9.5	2–3.5	Gupta and Saraswat (1993)
<i>M. scatophagi</i>	<i>Scatophagus argus</i> L.	Gill arch	11.4–17.9 (14.0)	4.0–8.1	5.6–9.8 (7.13)	1.6–3.2 (2.36)	Haldar et al. (1996)
<i>Myxobolus nilimae</i> sp.n.	<i>Labeo rohita</i>	Gill lamellae	15.9–17.0 (16.65)	6.2–7.0 (6.89)	9.0–11.0 (10.4)	3–3.5 (3.16)	Present study

muralidharani sp. n. while in *M. harpreetae* sp. n. the coils are present but their appearance is not much clear.

It is clear from the above discussion that the present species is new to science; therefore it is given a separate taxonomic status and designated as *Myxobolus nilmae* sp. n.

***Myxobolus harpreetae* sp. n. Fig. 3(a, b, c)**

Plasmodia

Plasmodia small (1–2 mm), attached with the mucous membrane around gill lamellae. Colour creamy white, shape rounded.

Fig. 3 **a** Light microscopic Photomicrographs of the spores of *Myxobolus harpreetae* sp. n. stained with Giemsa (unextruded) (Scale bar: 10 μm); **b** and **c** Camera lucida drawing of the spore of *Myxobolus harpreetae* sp. n. (Scale bar: 10 μm)

Spore (Measurements based on 8–9 spores in frontal view)

The spores are ellipsoidal in frontal view. Length of the spores 12.7 ± 0.51 (range 12–13.4) μm, width 7.4 ± 0.53 (range 6.7–8.3) μm, Polar capsules elongated, different in size, slightly converging anteriorly. Smaller polar capsule oval and larger one irregularly oval shaped. Mature spores measure 13.2 ± 0.86 (11.4–13.8) × 6.51 ± 1.003 (4.9–7.8). Anterior end of the spore rounded and posterior end blunt semicircular. Larger and smaller polar capsules measure 9.149 ± 0.13 (8.7–9.2) × 3.45 ± 0.26 (2.90–4.0) and 7.94 ± 0.26 (7.6–8.3) × 2.93 ± 0.2 (2.7–3.0) respectively. The polar capsules elongated with anterior portion slightly pointed and blunt posterior end. Intercapsular space absent sporoplasm with round iodophilous vacuole which was asynchronous in shape.

Spore index

LS: WS = 1:0.4931
 LLPC: WLPC = 1:0.3770
 LSPC: WSPC = 1:0.3690
 LLPC: LSPC = 1:0.8678
 WLPC: WSPC = 1:0.9345

Taxonomic summary

Type host *Labeo bata* (Hamilton 1822).

Type locality Sonarpur, (22.4200°N, longitude: 88.4200°E) South 24 Parganas West Bengal, India.

Type specimens Slide No. MB/PA/KU/15-19 containing Holotype and other slides MB/PAKU/09-12 containing Paratype have been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India.

Symbiotype The host specimen bearing no. CM/PA/ICU/10-12 deposited at the museum of the Parasitology Laboratory, Department of Zoology, University of Kalyani, West Bengal, India.

Site of infection Gill lamellae.

Prevalence of infection 2 out of 21 hosts examined (9.52 %).

Specific epithet The specific epithet “*harpreetae*” has been given after the name of Prof. Harpreet Kaur, University Department of Zoology, Punjabi University, Patiala, India for her contributions on myxosporean parasites of fish.

Remarks (Table 4)***Myxobolus harpreetae* sp. n.**

In having unequal polar capsules, the myxozoan from the gills of *Labeo bata*, reported in this communication can be segregated from those species of the genus *Myxobolus* having equal polar capsules. The *Myxobolus* species described here is unique in measurement of spore body,

polar capsule, host fish and habitat from its closely related species with unequal polar capsules.

While comparing the present form with its closely related species, it has been found that the length of the spore of the present form is smaller in comparison to the spore of *M. attui*, but larger in case of *M. naini*. The shape of the spore also varies. It is oval and somewhat elongated having one small and another double sized irregularly cylindrical polar capsule. The study of the polar capsule

Table 4 Showing the comparative account of the *Myxobolus harpreetae* sp. n. with morphologically similar species (measurements are in micrometer)

Name of the species	Host	Site of infection	Length of spore	Width of spore	Length of larger polar capsule	Width of larger polar capsule	Length of smaller polar capsule	Width of smaller polar capsule	Author(s)
<i>M. altgarhensis</i> <i>M. ophicephali</i> <i>M. punctatus</i>	<i>Ophiocephalus punctatus</i> Bloch	Accessory respiratory membrane	12–14	6–7.5	6.5–8	2–2.5	6–7	2.5	Bhatt and Siddiqui (1964), Raychaudhuri and Chakravarty (1970)
<i>M. analfinus</i>	<i>Heteropneustes fossilis</i> Bloch	Anal fin	12.3	4.1	8.6	2.5	6.2	1.8	Basu et al. (2009)
<i>M. attui</i>	<i>Wallago attu</i> Bleeker	Gut	12.8–15.2 (13.89)	5–9.6	10–11 (10.6)	3.5–4.5 (3.97)	8–9 (8.7)	2.5–3 (2.72)	Sarkar (1985a, b)
<i>M. barbi</i>	<i>Barbus ticto</i> Ham.	Skin	2.6–13.5	9.0	9.6–11.2	4.0–4.8	7.2–8.8	1.6	Tripathi (1952)
<i>M. chakravarty</i>	<i>Catla catla</i> Ham.	Internal eye musculature	12.1–14.3 (12.3)	7.7–10.5	5.5–6.6	3.3–5	4.4–5	2.2–4.4	Haldar et al. (1983)
<i>M. mahendrae</i>	<i>catla catla</i> Ham	Gill arch epithelium buccopharyngeal cavity	11.52–13.96 (12.7)	9.77–10.47 (9.2)	6.28–7.33 (6.98)	3.49–4.19 (3.73)	4.19–6.98 (5.44)	3.14–3.49 (3.42)	Sarkar (1986)
<i>M. nainit</i>	<i>Cirrhinna mrigala</i> Ham.	Gills	12.9	8.2	4.9	3.1	3.33	1.63	Kaur and Singh (2008)
<i>M. ophthalmusculata</i>	<i>Cirrhinna mrigala</i> Ham.	Eye muscles	12.4–13.97 (13.13)	7.45–9.18 (8.04)	4.9–6.12 (5.47)	2.65–3.37 (3.06)	2.35–3.37 (3.03)	1.842.14 (1.99)	Basu and Haldar (2002a, 2002b)
<i>Myxobolus inaequalis</i>	South American fresh water fish	Musculature	5.5 µm	3.3 µm					Gurley (1893)
<i>Myxobolus inaequus</i>	South American fresh water fish	Musculature	19.8 µm	8.6 µm					Kent and Hoffman (1948)
<i>M. bilongi</i>	<i>Labeo</i> sp.	Gills, fins	14–17	11.5–14.5	6.5–8	4–6	4–6.5	3–4	Fomena and Bouix (1994)
<i>Myxobolus harpreetae</i> sp. n.	<i>Labeo bata</i>	Gill lamellae	11.4–13.8 (13.2)	4.9–7.8 (6.51)	8.7–9.2 (9.149)	2.9–3.4 (3.45)	7.6–8.3 (7.94)	2.7–3.0 (2.9)	Present study

shows that the posterior end of the larger polar capsule is very much elongated with rounded lower tip and somewhat pointed anterior tip. The larger polar capsule occupies more than half while the smaller one occupies less than one third of the spore. Further study shows that the polar capsule of the present form is comparatively smaller while compared to *M. mahendrae* and *M. opthalmusculata*, whereas the smaller polar capsule of the present form is comparatively larger in comparison to *M. opthalmusculata*, *M. mahendrae*, *M. naini*, *M. chakravarti* but smaller when compared to *M. attui*, *M. barbi*, *M. analfinus* and *M. aligarhensis*.

The polar filament was not clearly visible during the time of description, but filamentous coil has been noticed which is situated perpendicular to the polar capsular axis. Sporoplasm occupies the extra-capsular space behind it. The shape is much larger in the present form when compared to the other described forms.

Moreover, the study of the spore of the present form shows its close resemblance with muscle dwelling species of cyprinae group namely, *M. musculi* and *M. pseudodispar*. But compared to both, on the basis of the polar capsule they differ from each other by having elongated, anteriorly converging polar capsules.

Comparison of present species with other *Myxobolus* spp. found parasitizing South American and other freshwater fishes show that in the present form the polar capsules are unequal in size with asymmetric shape of the valves in frontal view while of the South American species only *M. inaequalis* and *M. inaequus*, polar capsules are also unequal in size. But the spores of *M. inaequalis* are much smaller ($5.5\ \mu\text{m} \times 3.3\ \mu\text{m}$) than that of the species under discussion while in *M. inaequus*, the spores are larger than the present form and are more elongated. Of the *Myxobolus* sp of the other regions of the world which have spores with polar capsules of unequal size, namely *M. bilongi*, have dimensions similar to the present form, but the anterior end of the spores is slightly truncated (Table 4).

In addition, the present form has been further compared with the other two proposed new species namely, *M. nilimae* n. sp. and *M. muralidharani* n. sp. The appearance of the present form is ellipsoidal while in *M. muralidharani* n. sp it is elongated and thinner and *M. nilimae* n. sp petaloid shaped. The study of spore shows the spore of the present form is much smaller in comparison to *M. nilimae* n. sp. The major difference lies within the polar capsules because they are unequal in the present form while they are equal in other two described forms. The spore index of both the species are significantly different. The comparative study on filamentous coil shows that in present form it is less distinctly visible while in *M. nilimae* n.sp only 10–12 filamentous coils are present.

Conclusion

The paper deals with three noble species under the phylum Myxozoa Grasse, 1970, of the genus *Myxobolus* Butschli, 1881. These species have been isolated from the fresh water food fishes of West Bengal, India. Among the three new species, two have been collected from the ponds adjoining to Jalangi river of Tehatta and Churni river near Hanskhali of the Nadia district of West Bengal, India. The species, *M. harpreetae* sp. n. has been collected from the Ganga river of Sonarpur of South 24 Paraganas district, West Bengal, India. After thorough comparison with their respectively closely related species and with each other presented here it may confirmly stated that the species are new to science and very much different from each other in respect of morphometry and geographic locations. Thus in this communication all the data regarding their morphometric, habitats and hosts have been explored. Therefore an extensive survey and maintaining all aspects of taxonomy, dealing with the morphological and geographical descriptions of species concern, these three species being presented as new to science.

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References

- Banerjee S, Bandyopadhyay PK, Göçmen B, Mitra AK (2011) *Myxobolus lalbaghensis* sp. n. from a freshwater fish of West Bengal, India. North West J Zool 7(1):1–4
- Basu S, Haldar DP (2002a) *Myxobolous opthalmusculata*, a new species of histozoic myxozoan (Myxozoa: Bivalvulida) from *Cirrhinus mrigala* (Hamilton). Proc Zool Society. 55:43–48
- Basu S, Haldar DP (2002b) Observations on three new species of *Myxobolus* Butschli, 1882 from hybrid carps of West Bengal, India. Indian J Environ Ecoplan 6:629–640
- Basu S, Haldar DP (2003) Three new species of *Myxobolus* Butschli, 1882 from different food fishes of West Bengal, India. Acta Protozool 42:245–251
- Basu S, Haldar DP (2004) Description of three new myxosporean species (Myxozoa: Myxosporea: Bivalvulida) of the genera *Myxobilatus* Davis, 1944 and *Myxobolus* Butschli, 1882. Acta Protozool 43:337–343
- Basu S, Modak BK, Haldar DP (2009) Two new species of *Myxobolus* Butschli, 1882 (Myxozoa: Mysporea: Bivalvulida) from food fishes of West Bengal, India: a light and scanning electron microscopy study. Acta Protozool 48:83–89
- Bhatt VS, Siddiqui WA (1964) Four new species of Myxosporidia from the Indian fresh water fish *Ophiocephalus punctatus* Bloch. J Protozool 11:314–316
- Bütschli O (1882) Myxosporidia. In: Bronn HG (ed) Klassen und Ordnungen des Thier-Reichs 1(1). Winter, Leipzig, pp 590–603
- Chakravarty M (1939) Studies on Myxosporidia from the fishes of Bengal, with a note on myxosporidian infection in aquaria fishes. Arch Protistenkd 92:169–178

- Eiras JC, Molnar K, Lu YS (2005) Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Bivalbulida). *Syst Parasitol* 61(1):1–46
- Fomena A, Bouix G (1994) New Myxosporidea species (Myxozoa) from freshwater teleosts in Southern Cameroon (Central Africa). *J Afr Zool* 108:481–491
- Gupta S, Khera S (1988) Review of the genus *Myxobolus* Bütschli, 1882. *Res Bull (Sci) Panjab Univ* 39:45–48
- Gupta N, Saraswat H (1993) Haemoprotozoosis in *Clarias batrachus* (Linn) from freshwater ponds of Rohilkhand region, India. *Himal J Environ Zool* 7:47–52
- Gurley RR (1893) On the classification of the myxosporidia, a group of protozoan parasites infesting fishes. *Bull US Fishery Comm* 11:407–420
- Hargari SS, Amoji SD (1981) *Myxosoma karnatakae* n. sp. (Protozoa: Myxosporidia) from the caudal muscles of freshwater water teleost fish, *Barbus chola* (Ham.et.Buch.). *ArchProtistenkd* 124:385–390
- Haldar DP, Das MK, Sharma BK (1983) Studies on protozoan parasites from fishes Four new species of the genera *Henneguya* Thélohan, 1892 *Thelohanellus* Kudo, 1933 and *Myxobolus* Bütschli, 1882. *Arch Protistenk* 127:283–296
- Haldar DP, Samal KK, Mukhopadhyaya D (1996) Studies on protozoan parasites of fishes in Orissa: eight species of *Myxobolus* Bütschli (Myxozoa: Bivalbulida). *J Beng Nat Hist Soc* 16:3–24
- Kalavati C, Nandi NC (2007) Handbook on myxosporidian parasites of Indian fishes. ZSI, Kolkata India. *Zoos Print J* 22:2527–2530
- Kaur H, Singh R (2008) Observations on one new species, of genus *Myxobolus*—*M. naini* and redescription of *M. magaudii* recorded from freshwater fishes of Kanjali Wetland of Punjab, India. In: *Proceedings of 20th National Congress Parasitology*, pp. 75–79
- Kaur H, Singh R (2012) A synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Bivalbulida) parasitizing Indian fishes and a revised dichotomous key to myxosporean genera. *Syst Parasitol* 81:17–37
- Kent M L, Margolis L, Corliss JO (1994) The demise of a class of protists—taxonomic and nomenclatural revisions proposed for the protist phylum Myxozoa Grassé, 1970. *Can J Zool* 72:932–937
- Lalitha-Kumari PS (1965) On a new species of *Henneguya* (Protozoa: Myxosporidia) from Indian freshwater fish, *Ophiocephalus gachua*. *Riv Parassitol* 26:79–84
- Lalitha-Kumari PS (1969) Studies on parasitic protozoa (Myxosporidia) of freshwater fishes of Andhra Pradesh, India. *Riv Parassitol* 30:154–225
- Landsberg JH, Lom J (1991) Taxonomy of the genera of the *Myxobolus/Myxosoma* group (Myxobolidae: Myxosporea), current listing of species and revision of synonyms. *Syst Parasitol* 18:165–186
- Lom J, Arthur JR (1989) A guideline for the preparation of species descriptions in Myxosporea. *J Fish Dis* 12:151–156
- Lom J, Dykova I (1992) Protozoan parasites of fishes. Development in aquaculture and fisheries Sciences, vol 26. Elsevier, Amsterdam
- Lom J, Dykova I (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol* 53:1–36
- Padma Dorothy K, Kalavati C (1992) Description of four species of *Myxobolus* Bütschli, 1882 from the mullet, *Lizamacrolepis*, in the backwater regions of Visakhapatnam, east coast of India. *Proc Zool Society Cal* 45:197–204
- Raychaudhuri S, Chakravarty MM (1970) Studies on Myxosporidia (Protozoa, Sporozoa) from the food fishes of Bengal. I. Three new species from *Ophicephalus punctatus* Bloch. *Acta Protozool* 8:167–175
- Sarkar NK (1982) On three new myxosporidian parasites (Myxozoa) of the ophicephalid fishes of West Bengal, India. *Acta Protozool* 21:239–244
- Sarkar NK (1985a) Some coelozoic Myxosporidia (Myxozoa: Myxosporea) from a freshwater water teleost fish of River Padma. *Acta Protozool* 24:47–53
- Sarkar NK (1985b) Myxosporidian *Henneguya mystusia* sp. n. (Myxozoa: Myxosporea) from the gills of a freshwater teleost fish *Mystus* sp. *Acta Protozool* 24:55–58
- Sarkar NK (1986) On two new species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea) from the fresh water fishes of West Bengal, India. *Acta Protozool* 25:235–239
- Sarkar NK (1993) On two new species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea) from some freshwater fish of West Bengal, India. *Proc Zool Society Cal* 46:61–66
- Seenappa D, Manohar L (1981) Five new species of *Myxobolus* (Myxosporea, Protozoa), parasitic in *Cirrhinia mrigala* (Hamilton) and *Labeo rohita* (Hamilton), with a note on anew host record for *Myxosporea curmucae* Seenappa and Manohar. *J Protozool* 28:358–360
- Sheeja PK, Janardanan KP (2006) Two new myxosporean parasites (Myxozoa; Myxobolidae) from the freshwater fish *Puntius ticto punctatus* Day in Kerala. *Syst Parasitol* 61:1–46
- Thounaojam H, Mohilal N, Bandyopadhyay PK, Mitra AK, Güreli G (2013) *Myxobolus leafa* sp. nov. (Myxozoa: Bivalbulida) from the gill filament of *Labeo bata* (Hamilton) from Manipur, India. *Türkiye Parazit Derg* 37:40–43
- Tripathi YR (1952) Studies on the parasites of Indian fishes. I. Protozoa. Myxosporidia together with a checklist of parasitic protozoa described from Indian fishes. *Rec Ind Museum* 50:63–88