

## Observations on two new species of *Myxobolus* Butschli, 1882 from minor carps of Tripura, India

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**Abstract** Tripura is a north-eastern state of India with a total geographical area of 10,491 sq.km. *Labeo bata*, *L. calbasu*, *L. gonius* of the genus *Labeo* Cuvier and *Cirrhinus reba* of genus *Cirrhinus* Oken are the most popular and commercially important native minor carps of the state. During a detailed survey on the protozoan parasites of the minor carps of Tripura, two new species of myxozoan (Myxozoa: Bivalvulida) parasites *Myxobolus tripurensis* sp.n. and *Myxobolus potularis* sp.n. were encountered on the gill filaments of the host fishes. Distinctive characteristics of the plasmodia and the spores have been described.

**Keywords** Minor carps · *Myxobolus tripurensis* sp.n. · *Myxobolus potularis* sp.n. · Parasite

### Introduction

Tripura by virtue of its location in the sub-Himalayan belt has a rich and diversified fish fauna. The Myxosporeans are the most destructive pathogens within the protozoan phyla, causing large scale mortalities in natural and culture conditions (Shulman 1966). During the course of present investigations on the protozoan parasites of minor carps of the natural and cultured water bodies of the four different districts of Tripura (latitude 22°51'–24°32'N and longitude

90°10'–92°21'E), two new myxosporidian species *Myxobolus tripurensis* sp.n. and *Myxobolus potularis* sp.n. were encountered on the gill filaments and gill rakers of the host fishes. The description of the new species was done based on the guidelines of Lom and Dykova (1992).

### Materials and methods

During the survey, fish samples were collected from the natural habitat and the aquaculture ponds of all the four districts of Tripura. The host fishes were identified as per Jhingran (1975), Talwar and Jhingran (1991) and Jayaram and Dhas (2000). The sporogonic plasmodia encountered were dissected out carefully with sterile forceps and smeared on grease-free glass slides with 0.5 % NaCl solution drops and covered with cover slips and examined under the transmission light microscope (TLM). Fresh spores were treated with Lugol's iodine solution for detection of iodophilous vacuole. The Indian ink method of Lom and Vavra (1963) was used for the detection of any external mucous coating around the spores.

For making the permanent preparations air-dried smears were Giemsa stained after fixation in acetone-free absolute methanol. The measurements were done with help of a calibrated ocular micrometre on basis of 20 fresh spores treated with Lugol's iodine. The measurements of all the parameters presented are in  $\mu\text{m}$  as mean  $\pm$  SD followed in parenthesis by the range. Drawings were made on fresh or stained material with the help of a mirror type camera lucida and the computer programme Corel Draw Version 14. The abbreviations used in this paper are as follows: length of the spore (LS), width of spore (WS), length of large polar capsule (LLPC), width of large polar capsule (WLPC), length of small polar capsule (LSPC), width of

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small polar capsule (WSPC), length of polar capsule (LPC), width of polar capsule (WPC), diameter of iodophilous vacuole (DIV).

## Results and discussion

*Myxobolus tripurensis* sp.n. (Fig. 1; Plate 1)

### Plasmodia

Creamy yellow oval shaped cysts found attached on the gill filaments and on the gill rakers of the host fishes.

### Spores

The spores are small sized and round to oval in valvular view and is lenticular in sutural view. The two shell valves are thick-walled and symmetrical to each other. At the posterior portion of the spore there are suture markings. Mature spores are of size 8.1–9.4 ( $8.9 \pm 0.41$ )  $\mu\text{m}$  in length and 6.5–7.0 ( $6.7 \pm 0.19$ )  $\mu\text{m}$  in breadth.

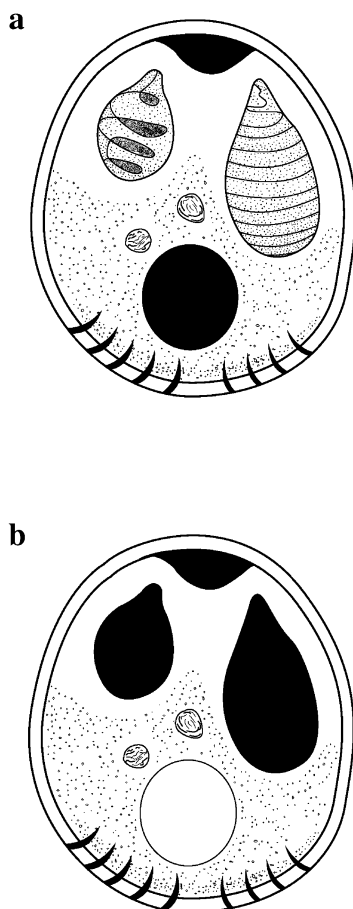
The two polar capsules are distinctly different sized. The larger polar capsule measuring 4.0–4.5 ( $4.2 \pm 0.18$ )  $\mu\text{m}$  in length and 2.0–2.6 ( $2.3 \pm 0.21$ )  $\mu\text{m}$  in breadth is elongated and oval with eight to nine closely packed polar filaments. The smaller capsule measuring 2.5–2.8 ( $2.6 \pm 0.10$ )  $\mu\text{m}$  in length and 1.6–1.9 ( $1.7 \pm 0.12$ )  $\mu\text{m}$  in breadth is oval in shape with three to four loosely coiled polar filaments. An intercapsular projection is present between the two polar capsules.

The crescent shaped granular protoplasm extends into the intercapsular space medially. An iodophilous vacuole, 2.2–2.6 ( $2.5 \pm 0.15$ )  $\mu\text{m}$  in diameter was observed on treatment with Lugol's iodine. No mucous envelope was seen around the spores on processing with Indian ink preparation.

The species under description shows very close affinity to *Myxobolus lalithae* Gupta and Khera (1988) described from the gills of *Labeo calbasu*; *M. undasuturiae*, Sarkar (1994) reported from the urinary bladder of *Amblypharyngodon mola*; *M. labeosus*, Sarkar (1995) from the mesentery of spleen of *Labeo fimbriatus* and *M. mrigalhitae*, Basu and Haldar (2003) from gills of mrigal-rohu hybrid.

*Myxobolus lalithae* shows resemblance to the present species by the presence of unequal capsules and the presence of an intercapsular ridge. However, the detailed morphometric studies of the present species revealed that the spores and the polar capsules were much smaller compared to the spores of *M. lalithae* (LS: 10.0; WS: 8.4; LLPC: 5.8; WLPC: 3.0; LSPC: 4.8; WSPC: 2.8). *M. undasuturiae* (LS: 10.6; WS: 5.7; LLPC: 6.1; WLPC: 2.3; LSPC: 4.8; WSPC: 2.8) shows apparent resemblance to the present species; however, the absence of inter capsular ridge and the elongated pyriform shape of the polar capsules differs from the present species.

The present species resembles *M. mrigalhitae* (LS: 10.8; WS: 7.9; LLPC: 4.8; WLPC: 2.9; LSPC: 3.0; WSPC: 2.1) by the presence of two unequal capsules and iodophilous vacuole as well as the parietal folds in the posterior extremities of the spore. However, the pyriform shape and the larger dimensions of the spore differ from the present species. *M. labeosus* (LS: 9.2; WS: 7.6; LLPC: 6.1; WLPC: 2.7; LSPC: 4.0; WSPC: 2.3) differs from the present species in the absence of iodophilous vacuole, morphometry and the orientation of the polar capsules.



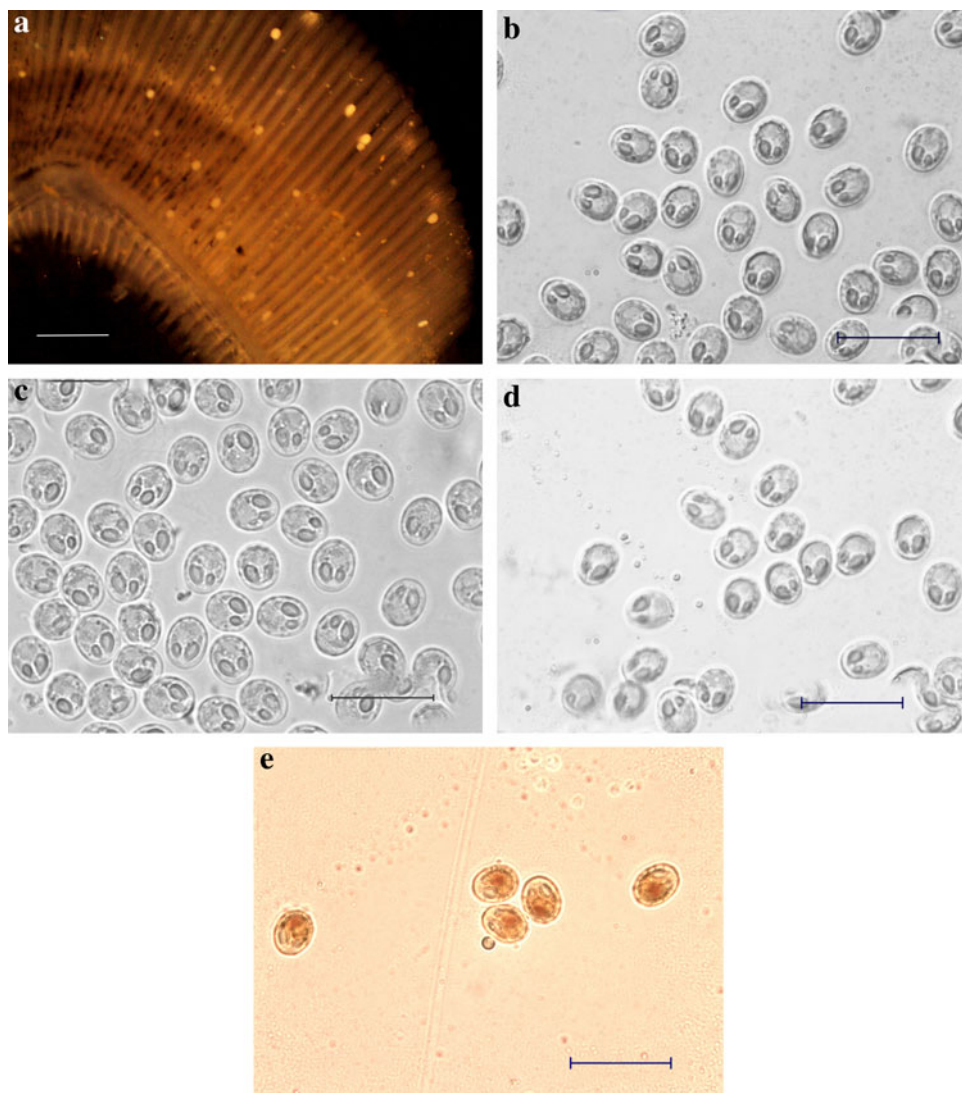
Camera lucida drawings-  
a - Fresh mature spore in valvular view revealing iodophilous vacuole- Lugol's iodine stained

b - Fixed spore in valvular view- Giemsa stained

Scale bar - 5  $\mu\text{m}$

**Fig. 1** *Myxobolus tripurensis* sp.n.

**Plate 1** *Myxobolus tripurensis* sp.n. **a** Photomicrograph of plasmodia or cyst attached on the gill filament of *Labeo calbasu*. **b–d** Photomicrographs of spores showing variations in appearances. **e** Spores stained with Lugol's iodine solution. Scale bars: **a** 700  $\mu$ m, **b–e** 20  $\mu$ m



In view of all the cited differences with the closely related species the present species is designated as a new species and named hereby as *Myxobolus tripurensis* sp.n. (Table 1).

#### Taxonomic summary

*Type host*: *Labeo calbasu* (Hamilton 1822), *Labeo bata* (Hamilton 1822) and *Cirrhinus reba* (Hamilton 1822).

*Type locality*: Sonamura, West Tripura.

*Type specimen*: Holotype on slide MX/MB/003 and paratype on slide MX/MD/003/PR, collection of Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.

*Prevalence*: 1.7

*Mean intensity*: 1.05 %.

*Infection loci*: Gills.

*Etymology*: The specific name *tripurensis* represents the State of Tripura from where the host fishes were collected.

*Myxobolus potularis* sp.n. (Fig. 2, Plate 2)

#### Plasmodia

Fully developed plasmodia are elongated and oval in shape and are conspicuous on lifting the gill operculum of the host fishes. The cysts are comparatively large-sized enveloping the gill filament tips. They are creamy yellow in colour and observed mostly as one or two cysts parasitising a single gill.

#### Spores

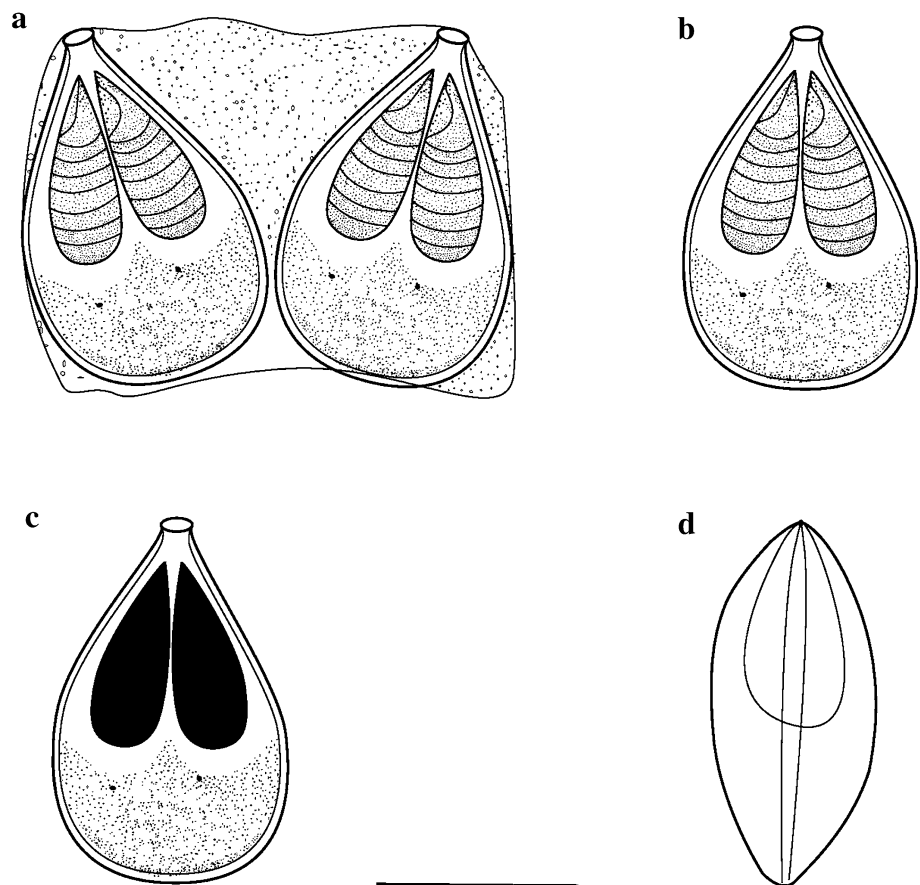
In front view, the mature spores are tear-shaped to oval with the anterior end bluntly pointed and posterior end greatly rounded and pot-like. Towards the anterior portion the spores take a nearly bottle-neck shape with a small knob which is clearly evident in fresh as well as stained

**Table 1** Statistical measurements of morphometric parameters of *Myxobolus tripurensis* sp.n.

Morphometric parameters	Range	X	SD	SE	CV (%)
LS	8.1–9.4	8.9	0.41	0.09	4.61
WS	6.5–7.0	6.7	0.19	0.04	2.84
LLPC	4.0–4.5	4.2	0.18	0.04	4.28
WLPC	2.0–2.6	2.3	0.21	0.05	9.13
LSPC	2.5–2.8	2.6	0.10	0.02	3.85
WSPC	1.6–1.9	1.7	0.12	0.03	7.06
DIV	2.2–2.6	2.5	0.15	0.03	6.00

preparations. The shell valves are completely devoid of any shell markings, suture ridge is present but the suture line is not clear. Mature spores measure 8.3–9.3 ( $9.0 \pm 0.29$ )  $\mu\text{m}$  in length and 5.4–6.3 ( $6.0 \pm 0.21$ )  $\mu\text{m}$  in breadth.

The two polar capsules are equal in size and is pyriform with greatly rounded or curved posterior end and is pointed towards the anterior proximity. The polar filaments are evidently visible in fresh as well as in stained condition. There are 7–8 tightly packed coils inside each capsule. The polar capsules measure 5.0–5.6 ( $5.4 \pm 0.19$ )  $\mu\text{m}$  in length and 1.6–2.5 ( $2.2 \pm 0.29$ )  $\mu\text{m}$  in breadth.

**Fig. 2** *Myxobolus potularis* sp.n.

Camera lucida drawings-

a - Late developmental stages-Disporous pansporoblasts- Lugol's iodine stained

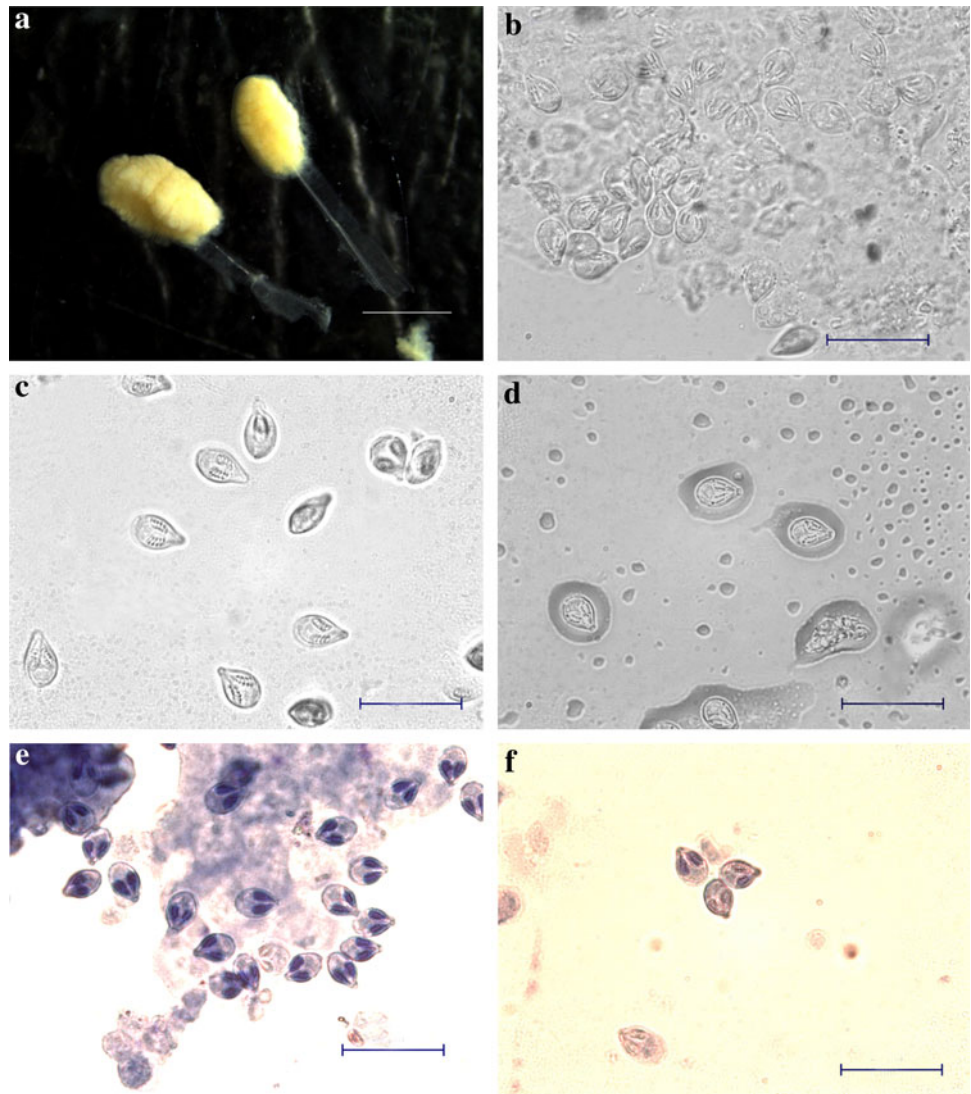
b - Fresh Mature spore in valvular view- Lugol's iodine stained

c - Fixed spore in valvular view- Giemsa stained

d - Fresh spore in sutural view- Lugol's iodine stained

Scale bar - 5  $\mu\text{m}$

**Plate 2** *Myxobolus potularis* sp.n. **a** Photomicrograph of plasmodia or cyst attached on the tip of a gill filament of *Cirrhinus reba*. **b–d** Photomicrographs of spores showing variations. **e, f** Giemsa stained spores. Scale bars: **a** 120  $\mu$ m, **b–f** 20  $\mu$ m



Granular and homogenous mass of cytoplasm fills the extra capsular region of the spore cavity and there are very minute cytoplasmic nuclei. Spores lack mucous envelope surrounding the cyst wall and no iodophilous vacuole was detected on treatment with Lugol's iodine solution.

The present species resembles *Myxobolus nodularis* Southwell and Prasad 1918 observed from the muscles of

*Rasbora daniconius* (Southwell and Prasad 1918); *M. mrigalae*, Chakravarty (1939) reported from the scales of *Cirrhinus cirrhosus*; *M. bengalensis*, Chakravarty and Basu (1948) from the branchiae of *Catla catla*; *M. potaili*, Lalithakumari (1969) described from the branchiae of *Labeo potaili*; *M. dujardini*, Lom and Noble 1984 reported from the fins, gills and the visceral organs of *Rutilus* spp., *Leuciscus cephalus*, *Abramis* spp., etc. *M. anili*, Sarkar (1989) found in the mesentery of *Rhinomugil corsula*; *M. jiroveci*, Lom et al. (1989) from the brain of *Cottus gobio* and *M. mola*, Sarkar 1993 reported from the kidney of *Amblypharyngodon mola*.

Though the spores of *M. bengalensis* (LS: 8.5–9.3; WS: 6.4–6.8; LPC: 4.2–5.4; WPC: 2.5–3.2) show remarkable similarity with the spores of the present species in the general appearance and the presence of the anterior protuberance, the presence of a spherical iodophilous vacuole which is lacking in the present species and the wider polar

**Table 2** Statistical measurements of morphometric parameters of *Myxobolus potularis* sp.n.

Morphometric parameters	Range	X	SD	SE	CV (%)
LS	8.3–9.3	9.0	0.29	0.06	3.22
WS	5.4–6.3	6.0	0.21	0.05	3.50
LPC	5.0–5.6	5.4	0.19	0.04	3.52
WPC	1.6–2.5	2.2	0.29	0.06	13.18

capsule of *M. bengalensis* diverts from the present species which is much narrower; thus the species under discussion distinguishes itself from *M. bengalensis*.

The present species perhaps shows the maximum morphological affinity with the spores of *M. dujardini* described by Lom and Noble (1984). Any how, on correlating the morphometry of the spores it was observed that *M. dujardini* (LS: 11.0–13.0; WS: 5.0–8.0) has much longer and wider spores than the present species, thus making it distinct. In spite of the presence of an anterior knob in the spores of *M. potaili* (LS: 7.2; WS: 5.4; LPC: 3.3; WPC: 2.0), it differs from the species under consideration by having striations in the shell valves which is absent in the present case. Though the spores of *M. mrigalae* (LS: 7.2; WS: 8.2; LLPC: 5.1; WLPC: 3.0; LSPC: 3.0; WSPC: 2.0) exhibit some superficial similarity in shape, they differ from the present case distinctly by the presence of two unequal polar capsules.

Spores of *M. jiroveci* (LS: 13.9; WS: 9.7; LPC: 6.2; WPC: 2.9) though has striking resemblance in the spore morphology with the present case, it differs in two significant aspects that is the presence of a distinct inter capsular appendix and very prominent markings along the sutural edge of the spore except in its anterior third, these two features lacking in the present case thus making the latter a different species. The spores of *M. anili* (LS: 10.7; WS: 8.6; LPC: 4.4; WPC: 2.0) are much longer and wider than the spores of the present species making it different from the latter whereas the polar capsules of *M. nodularis* (LS: 9.0; WS: 7.2; LPC: 3.0–4.0) are much smaller in length than the species under consideration. Further though superficially relating to *M. mola* (LS: 9.0; WS: 7.4; LPC: 6.0; WPC: 4.0), the detailed morphological comparison revealing a small round iodophilous vacuole and the oval-shaped spore leads to the conclusion that the tear-shaped spores of the present species devoid of iodophilous vacuole is distinct from *M. mola*.

All the above cited disparities with the closely related species justify the designation of this myxozoan as a new species and is hereby christened as *Myxobolus potularis* sp.n. (Table 2).

#### Taxonomic summary

*Type host*: *Labeo calbasu* (Hamilton 1822), *Labeo bata* (Hamilton 1822), *Labeo gonius* (Hamilton 1822) and *Cirrhinus reba* (Hamilton 1822).

*Type locality*: Udaipur, South Tripura.

*Type specimen*: Holotype on slide MX/MC/005 and paratype on slide MX/MC/008, collection of Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.

*Prevalence*: 1.7

*Mean Intensity*: 1.05 %.

*Infection loci*: Gill filaments.

*Etymology*: The specific epithet *potularis* symbolises the peculiar pot shaped structure of the spore of the parasite.

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